Exploring the Role of the Macrophage Migration Inhibitory Factor Polymorphism in Asthma Pathogenesis and Innate Immune Training: Implications for Mesenchymal Stromal Cell Therapy

A thesis submitted to Maynooth University for the degree of

Doctor of Philosophy

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Declaration of Authorship

I, Hazel Dunbar, declare that this thesis has not been previously submitted in whole or in part to this or any other university for any other degree. This thesis is the sole work of the author, with the exception of the development of the preclinical mouse model which was a combined effort with Ian Hawthorne, a joint member of this project. All aspects of the project that are shared with Ian Hawthorne have been declared in the relevant figure legends by an asterisk (*).

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Abstract

The incidence of asthma is rising, posing a significant economic burden worldwide. Despite treatment advancements, some asthmatics remain unresponsive, highlighting the need for a deeper understanding of asthma pathogenesis. Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine with a central role in the innate immune system and asthma development. A functional 'CATT' polymorphism controls MIF expression in the human population. The role of a high expressing $CATT_7$ allele in asthma is currently unknown. Repetitive inhalation of airborne allergens such as house dust mite (HDM), damages the airway epithelium and trains the innate immune system over time, inducing epigenetic changes. The influence of the MIF polymorphism on innate immune training remains unexplored. Mesenchymal stromal cells (MSCs) can respond and communicate with their surrounding microenvironment. The impact of endogenous human MIF on MSC licensing and its functional role in MSC therapeutic efficacy is undocumented. Using novel humanised MIF mice, this study is the first to elucidate the role of the human MIF $CATT_7$ allelic variant in a model of allergic airway inflammation. High expression of human MIF exacerbated HDM-induced asthma pathophysiology in $CATT_7$ mice, identified by measuring changes in histology, Th2 cytokines and respiratory mechanics. MIF-licensed MSCs exhibited enhanced cytoprotective function, promoting airway epithelial wound closure in a VEGF-dependent manner. Moreover, this thesis illustrates the first evidence of innate training in macrophages from high human MIF expressing $CATT_7$ mice, which was suppressed with MSC co-culture using transwell inserts. This research is of upmost clinical and translational importance, as the generation of this robust humanised MIF mouse model can facilitate the future development of treatments for MIF-dependent diseases, like asthma. Furthermore, this cutting-edge research makes a substantial contribution to the field of cellular therapy, paving the way for MSC application in high MIF-expressing patients, where these cells can modulate an over-active immune response, suppressing excessive, pathogenic inflammation.

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MSCs Reduce Allergic Airway Inflammation in Humanised MIF Mice. *MIF Virtual Seminar Series 2021, Structure, Biology and Disease of MIF Proteins. Oct, 2021.*

MIF inhibitor ISO-1 attenuates house dust mite induced allergic airway inflammation in a humanised MIF expressing mouse model. *Maynooth University Seminar Series. Sept, 2021.*

The role of MIF in Mesenchymal Stromal Cell Licensing. *Maynooth University Seminar Series. Oct, 2019.*

Influence of MIF on Mesenchymal Stromal Cell Therapeutic Efficacy. *Maynooth University Seminar Series. Jan, 2019.*

Posters:

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Dunbar, H., Hawthorne, I. J., Armstrong, M., Donnelly, S., English, K. MIF enhances mesenchymal stromal cell efficacy in allergic airway inflammation. *British Society of Immunology Annual Meeting. Dec, 2022.*

Dunbar, H., Hawthorne, I. J., Donnelly, S., English, K. MIF polymorphism alters MSC efficacy in asthma. *Maynooth University Research Day. May, 2022.*

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Abbreviations

AD	Adipose
AHR	Airway Hyperresponsiveness
AIRE	Asthma Insights and Reality in Europe
ARDS	Acute Respiratory Distress Syndrome
ARG1	Arginase 1
A549	Adenocarcinomic Human Alveolar Basal Epithelial Cells
BALF	Bronchoalveolar Lavage Fluid
BCG	Bacillus Calmette-Guérin
BCL-2	B Cell Lymphoma 2
BEAS-2B	Bronchial Airway Epithelial cells
BM	Bone Marrow
BMDM	Bone Marrow Derived Macrophages
CD	Cluster of Differentiation
cDNA	Complementary Deoxyribonucleic Acid
CHI3l3	Chitinase Like 3
CM	Conditioned Media
CO_2	Carbon Dioxide
COX	Cyclo-oxygenase
CV	Crystal Violet
CXCR	Chemokine Receptor
DC	Dendritic Cell
D-DT	D-Dopachrome Tautomerase
DEP	Diesel Exhaust Particle
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
DPX	Dibutylphthalate Polystyrene Xylene
ºC	Degrees Celsius
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
EV	Extracellular Vesicle
FACS	Fluorescence-Activated Cell Sorting
GAN	Global Asthma Network
GINA	Global Initiative for Asthma
GMM	Genetically Modified Microorganisms
GMO	Genetically Modified Organisms
GvHD	Graft versus Host Disease
hBM-MSC	Human Bone Marrow-derived Mesenchymal Stromal Cell
HDM	House Dust Mite
H&E	Haemotoxylin and Eosin
HPRA	Health Products Regulatory Authority
HPRT	Hypoxanthine Phosphoribosyltransferase

HSPC	Hematopoietic Stem and Progenitor Cells
ICAM	Intercellular Adhesion Molecule
IDO	Indoleamine 2,3-Dioxygenase
IFNγ	Interferon gamma
IgE	Immunoglobulin E
IL	Interleukin
ILC	Innate Lymphoid Cells
IN	Intranasal
INCA	Inhaler Compliance Assessment
iNOS	Inducible Nitric Oxide Synthase
IP	Intraperitoneal
IPF	Idiopathic Pulmonary Fibrosis
IV	Intravenous
kDa	Kilodalton
KDR	Kinase Insert Domain Receptor
Kg	Kilogram
KYN	Kynurenine
LN	Lymphnode
LPS	Lipopolysaccharide
mAb	Monoclonal Antibody
MCh	Methacholine
M-CSF	Macrophage-Colony Stimulating Factor
Mg	Milligram
MIF	Macrophage Migration Inhibitory Factor
miRNA	Micro Ribonucleic Acid
Ml	Milliliter
mRNA	Messenger Ribonucleic Acid
MSC	Mesenchymal Stromal Cells
MT	Masson's Trichrome
MTA	Methylthioadenosine
NBF	Neutral Buffered Formalin
Ng	Nanograms
NMBA	Neuromuscular Blocking Agent
NOS2	Nitric Oxide Synthase 2
NS	Not Statistically Significant
NV	Nanovesicles
OVA	Ovalbumin
O_2	Oxygen
PAS	Periodic Acid Schiff
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PD-L1	Programmed Cell Death-Ligand 1
Pg	Picograms
PGE_2	Prostaglandin E2

RETNLA	Resistin-Like Molecule Alpha
PRR	Pattern Recognition Receptor
RBC	Red Blood Cell
RETNLA	Resistin-Like Alpha
rhMIF	Recombinant Human Macrophage Migration Inhibitory Factor
RORγt	RAR-related Orphan Receptor Gamma
RPMI	Roswell Park Memorial Institute
RT-PCR	Real Time-Polymerase Chain Reaction
SABA	Short Acting Beta Agonists
SEM	Standard Error of the Mean
SNP	Single Nucleotide Polymorphism
STAT3	Signal Transducers and Activators of Transcription 3
TGF-β	Transforming Growth Factor Beta
Th	T Helper
TLR	Toll-Like Receptor
TNFα	Tumour Necrosis Factor Alpha
TREG	T Regulatory
TSG-6	Tumor Necrosis Factor-stimulated Gene-6
TSLP	Thymic Stromal Lymphopoietin
TUNEL	terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling
UC	Umbilical Cord
UM	Micromolar
VEGF	Vascular Endothelial Growth Factor
WGP	Whole Glucan Particle
WT	Wildtype
XIAP	X-Linked Inhibitor of Apoptosis Protein

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Chapter 1:

Introduction

1.1 ASTHMA

1.1.1 Epidemiology

Asthma is an atopic disease rapidly increasing in prevalence worldwide. The term 'asthma', which is a Greek word meaning "wind" or "to blow", was first coined by Hippocrates to describe respiratory distress dating as far back as 400BC. Asthma is a chronic condition of the upper respiratory tract, classified by an inflammatory response triggered by the inhalation of environmental allergens or pollutants (Porsbjerg et al., 2023). This disease is comprised of a variety of subtypes, depending on the age of onset (i.e., child-onset or adult-onset), the causative agent and the associated-inflammatory response. Thus, asthma can be classified as exercise-induced, allergic, occupational, or seasonal, making this condition heterogeneous in nature (Mäkikyrö et al., 2023). Furthermore, there may be an influential genetic component to the development of this airway disease, making it hereditary. Atopy is defined as a predisposition to an overexaggerated IgE-mediated allergic immune response to otherwise non-harmful triggers. Those that are diagnosed with atopic asthma are also known to be susceptible to other atopic co-morbidities, such as atopic dermatitis, allergic rhinitis, and food allergies (Hammad & Lambrecht, 2021; Yaneva & Darlenski, 2021).

1.1.2 Incidence and Economic Burden

The incidence of asthma is increasing with the continued industrialisation of the western world. Asthma affects over 339 million people worldwide (European Respiratory Society, accessed 3rd May 2023), with approximately 450,000 asthmatics in Ireland (National Clinical Programme for Asthma, HSE, 2020). In recent years, the increased frequency of asthma, along with its economic consequences have been extensively documented in many

countries, including Spain (Gall et al., 2023; Valero et al., 2023), Italy (Maio et al., 2023), Greece (Bakakos et al., 2023), Denmark (Al-Shuweli et al., 2023), Austria (Renner et al., 2023), United Kingdom (Creese et al., 2022; Hall et al., 2022; Pavord et al., 2023; Simms-Williams et al., 2022), Finland (Kantomaa et al., 2023) and Portugal (Camarinha et al., 2023). Studies investigating the incidence of this disease in Europe have arisen over the past two decades, such as the 'Asthma Insight and Reality in Europe' (AIRE) study that took place in the early 2000s, the 'Recognise Asthma and Link to Symptoms and Experience' (REALISE) study in 2014 (Price et al., 2014) and the more recent RECOGNISE study in 2018 (Kanniess et al., 2021), emphasising not only the severity of this condition, but also the ineffectiveness of existing treatments to control disease progression.

According to the 2022 Global Asthma Report prepared by the Global Asthma Network (GAN), over 1000 people die from asthma per day, with most cases being preventable if the required, optimal treatment had been provided ("The Global Asthma Report 2022," 2022). Moreover, the increase in asthma prevalence and incidence is associated with a heavy economic burden. The global initiative for Asthma (GINA) which took place in 2019 illustrated the extent of the asthma-associated economic burden in Spain, where the overuse of short-acting β-agonists (SABA) resulted in a mean cost per patient ranging from €2231-4243 per annum. Furthermore, this SABA overuse is demonstrated where some patients are being prescribed more than 12 cannisters a year, with the recommended dose being less than 2 per annum (Valero et al., 2023). SABA overuse has also been documented in Europe in the SABINA study (SABA use IN Asthma), with 38-51% of asthma patients over-using this treatment in the UK (de las Vecillas & Quirce, 2023). In Ireland, asthma contributed to an economic burden of 472 million euro in 2019 with 8,000 hospital admissions with an average 2.68 day stay. Lastly, over 1.4 million workdays were lost due to asthma in 2016 at an estimated cost of 200 million euro,

illustrating the impact this respiratory condition has on both the economy and the quality of life of those affected ('Easing the Economic Burden of Asthma' Report, Asthma.ie, accessed on 3rd May 2023).

1.1.3 Diagnosis

As stated, the onset of asthma can occur in childhood or adulthood, which can be diagnosed using a multitude of tests. These may include a physical exam, involving the measurement of lung function using spirometry devices that can quantify peak air flow in response to methacholine challenge, a chemical bronchoconstrictor which can facilitate the measuring of asthma-related airway constriction. Examinations such as chest x-rays, nitric oxide tests or eosinophil counts in sputum may be used in conjunction to spirometry to further facilitate an asthma diagnosis. Furthermore, these tests may be performed directly after exercise or the inhalation of cold air, helping to differentiate the cause of symptoms, or the classification of subtype of asthma present. Since 2018, the diagnostic aim is to break down asthma characteristics into 'treatable traits', with categories such as symptoms (coughing, wheezing), modifiable triggers (allergens), functional (airflow obstruction or bronchial hyperresponsiveness), radiological (airway thickening), biological (eosinophilia, elevated IgE) and pathological (airway remodelling) (Pavord et al., 2018). Furthermore, these criteria also take extra-pulmonary traits such as sleep apnoea, and behavioural traits like poor medication adherence into account (Pavord et al., 2018). Once diagnosed, asthma can be classified as mild intermittent, mild persistent, moderate persistent or severe persistent, depending on the frequency of exacerbations.

1.1.4 Aetiology

A healthy lung displays both physical and chemical attributes which together act as a defence mechanism against external pathogens and thus, the development of disease. The mucosal component of the respiratory system is one of the first lines of defence of the immune system (Altorki et al., 2019; Jakwerth et al., 2022). However in chronic inflammatory disease such as in allergic asthma, these defence mechanisms may be imbalanced or compromised, making the lung susceptible to invasion by environmental particulates capable of mounting an immunological response. Additionally, if the lung is exposed to low-grade allergen challenges on a continuing basis, this could train innate cell populations, resulting in an over-zealous immune system that is prone to future exacerbations (Thiriou et al., 2017). Agents that can trigger this response can range from allergens (mold, dust, animal dander, pollen), irritants (cigarette smoke, pollution, aerosols), medicines (aspirin) or even changes in weather (Gautier & Charpin, 2017). Furthermore, the development of this chronic airway condition is multifactorial, with inherited genetics (e.g., genetic polymorphisms), living conditions, socioeconomic background and birth order all contributing to the likelihood of developing asthma (von Mutius, 2009). A recent association has been made between perinatal characteristics, the home environment and lower income homes with asthma prevalence, also bringing to light the impact of socioeconomics on asthma development in early life (Creese et al., 2022). In addition to the pooling of epigenetic modifications in populations, environmental variation induced by urbanisation (Tuazon et al., 2022) and climate change can not only increase the incidence of asthma, but also increase the presence of one of the main causative aeroallergens, house dust mite (Acevedo et al., 2019).

1.1.5 House Dust Mite Allergenicity

Most asthma cases are atopic in nature, with 50-85% of asthmatics being allergic to house dust mite (Gregory & Lloyd, 2011; Nelson et al., 1996), making allergic asthma the most prominent subtype of this condition. House dust mite (HDM) is an indoor source of aeroallergen, with the most common species of mite being *Dermatophagoides pteronyssinus* and *Dermatophagoides farina*e, both belonging to the Acari taxonomical subclass and commonly found in temperate regions (Chapman et al., 2007; Jacquet, 2013). However, the difference between these subtypes of dust mite is that *D. pteronyssinus* is commonly found across Europe, whilst *D. farinae* is predominately found in the United States (Sarwar, 2020). Along with the chitin-derived exoskeleton and faecal matter from the mite itself, HDM is associated with a plethora of additional components which collectively induce an allergic immune response. These include pattern recognition receptor (PRR) ligands associated with HDM, such as lipopolysaccharide (LPS) and β-glucan, both known to play a role in the development of innate immunity. Moreover, the protease content of these mites is central to their allergenicity (Soh et al., 2023), which include group 1 der p 1/der f 1 and group 2 der p 2/der f 2. HDM-associated proteolytic enzymes, specifically the group 1 cysteine and serine proteases (e.g., trypsin and chymotrypsin) which account for 75% of proteases (Stewart et al., 1994), act on epithelial tight junction proteins such as occludin and zonula occludens-1 (Wan et al., 1999, 2001), enhancing their ability to reach the submucosa and antigen-presenting cells (Figure 1). Importantly, the make-up of different types of HDM-proteases such as cysteine and serine may account for variability between batches of commercially available HDM allergen. In addition to their protease activity, the endotoxin content may also facilitate this batch-to-batch variability, as allergen extracts contain varying levels of LPS from colonising gram-negative *Bartonella* bacteria (Gregory & Lloyd, 2011; Valerio et al., 2005), which activate

TLR4 and TLR2 on both bronchial and dendritic cells, driving the immunological response (Hammad et al., 2009; Phipps et al., 2009). Thus, moving forward, transparency in the endotoxin content of the HDM batch used should be documented, facilitating the generation of robust models of acute HDM-induced airway inflammation (Pascoe et al., 2020).

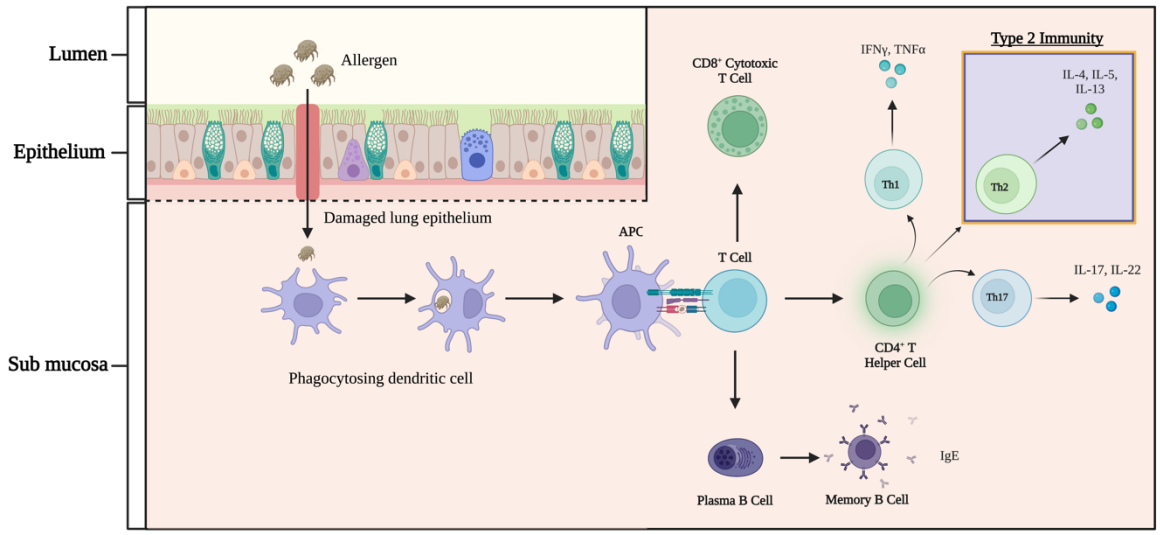


Figure 1. Schematic depicting the immunological process after house dust mite sensitisation. Following inhalation, HDM enters through a dysfunctional, leaky epithelial membrane barrier. Antigen presenting cells such as dendritic cells engulf HDM particles, displaying them on their surface to naïve T cells. In HDM-induced type 2 inflammation, T cells differentiate into type 2 helper T cells, producing cytokines such as IL-4, IL-5 and IL-13 which drive further allergic airway inflammation. Plasma B cells recognise HDM and proliferate memory B cells, secreting HDM-specific IgE.

1.1.6 Clinical Pathophysiology

Defined as a heterogeneous disease, there is a plethora of symptoms associated with asthma. With 'airway hyperresponsiveness' being an umbrella term for the pathophysiology associated this condition, histological and cellular inflammation comprise the basis of the development of symptoms. Clinical representation of this disease includes shortness of breath, wheezing cough and tightening of the chest, varying in intensity over time as a result of numerous inflammatory processes, classically type 2, such as the production of cytokine mediators, overproduction of mucus, airway broncho-restriction and histological changes within lung architecture (Figure 2).

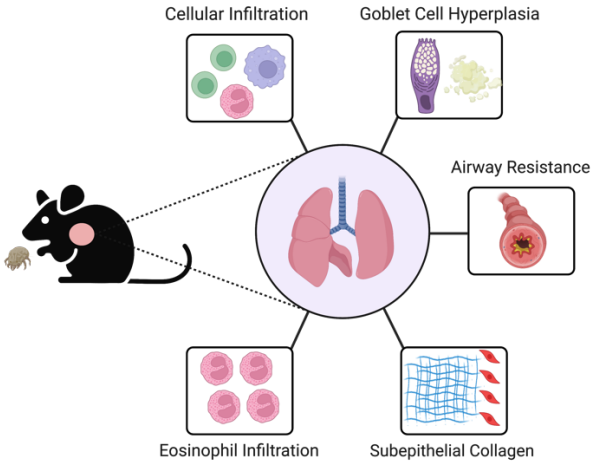


Figure 2. Cardinal features of allergic airway inflammation after house dust mite challenge. A HDM-induced model of allergic airway inflammation can investigate pathological hallmarks of asthma, such as levels of cellular infiltration, goblet cell hyperplasia, eosinophil infiltration, subepithelial collagen and airway resistance.

Allergens, specifically those of the *Dermatophagoides* species, act to penetrate the damaged epithelial barrier upon inhalation (Jakwerth et al., 2022), activating the key antigen presenting dendritic cells, triggering an influx of innate lymphoid cells (ILC2) (Fonseca et al., 2019; Li et al., 2019; Wolterink et al., 2012), type 2 T helper cells and Th2 cytokines; IL-4, IL-

5 and IL-13 (Lambrecht et al., 2019). Moreover, HDM is now thought to bind to the LMAN1 receptor on dendritic and airway epithelial cells (Miller et al., 2023). Upon the release of Th2 cytokines, a downstream allergic immunological process occurs with IL-4 activating the class switch of IgG to IgE, IL-5 mobilising and recruiting eosinophils from the bone marrow and IL-13, which cooperates with IL-4 to drive aspects pivotal to airway remodelling, such as goblet cell hyperplasia. Furthermore, activated antigen-presenting dendritic cells (DCs), eosinophils and basophils migrate to the mediastinal lymph nodes in a TLR4/MyD88-dependent manner (Hammad et al., 2010). Whilst a hallmark of allergic asthma is the increased production of IL-4, IL-5 and IL-13 by mast cells, basophils and Th2 helper cells, which can be measured in the blood or bronchoalveolar lavage fluid of asthmatic patients to determine a type 2-high (eosinophilic) or -low (non-eosinophilic) signature (Fahy, 2015), histological changes within the lung architecture are also indicative of asthma pathophysiology (Hammad & Lambrecht, 2021). Cellular inflammation, mucus overproduction and subepithelial collagen are histological hallmarks which collectively facilitate the increased airway hyperresponsiveness (AHR) and airway remodelling which occur after repeated exacerbations (Boonpiyathad et al., 2019). Cellular inflammation occurs as a result of an influx of predominantly eosinophils, but also macrophages and lymphocytes into the airways and surrounding parenchyma. Together, this results in the thickening of the airway and peri-bronchial regions, facilitating airway obstruction. Furthermore, inhalation of asthmatic triggers, such as HDM, increases goblet cell hyperplasia, leading to enhanced production of mucus (Dunican et al., 2018). Lastly, repeated exacerbations in asthma creates a vicious cycle of airway damage and repair, resulting in cyclical scar formation, enhancing the remodelling of the airways and narrowing of the bronchi. These histological modifications cooperatively alter the architecture of the lung microenvironment, decline lung function and worsen symptoms.

1.1.7 Asthma, Macrophages, and Innate Immune Training

The innate immune system is at the forefront of asthma pathogenesis, where inhaled triggers immediately interact with innate immune effector cells, such as eosinophils and macrophages (Thiriou et al., 2017). Innate immune training is classified as the induction of immunological memory, training these innate cells at an epigenetic level to remember previous pathogen encounters, and thus heightening their inflammatory response to future challenges.

It is suspected that trained immunity occurs in asthma, as key components of the clinically relevant aeroallergen house dust mite (HDM), such as endotoxin and β-glucan (Gregory & Lloyd, 2011; Hammad et al., 2009), are known to be capable of training the cells of the innate immune system over time (Lechner et al., 2022). Evidence has shown that T helper 2 cells can also be 'trained', where Th2 memory cells were found to reside in the lung for more than 2 years after the induction of experimental asthma, maintaining a level of 'allergic memory' (Bošnjak et al., 2019). New evidence has also demonstrated that the use of an anti-CD3 antibody was efficacious in reducing lung-localised effector memory T cells, abrogating subsequent asthma exacerbations after HDM exposure, reiterating the pathogenic role of innate immunity in asthma (Sethi et al., 2023). Furthermore, eosinophils may also be involved in innate immunity, as when activated, they have demonstrated efficacy in providing anti-viral immunity in mice (Percopo et al., 2014; Sabogal Piñeros et al., 2019; Samarasinghe et al., 2017). Although T cells and eosinophils can exhibit aspects of trained immunity, phagocytic macrophages are known drivers of innate immunity. Macrophages are a population of myeloid-derived cells characterised by their plasticity. Upon encountering a pathogen (i.e., HDM), these cells are essential in the uptake, processing, and presentation of inhaled substances to alert the adaptive immune system, making them a key cell in asthma pathogenesis and innate immunity.

Macrophage activation is dependent on their surrounding environment, where they can be classically activated to an M1 pro-inflammatory phenotype detected by increased TNFα, IL-6, IL-1β and iNOS, or alternatively to an M2 anti-inflammatory phenotype detected by TGF-β, Arginase-1, Retnla (FIZZ1) or Chi3l3 (YM1). Macrophages are known to be predominately M2 in asthma due to increased levels of IL-4 present, where these cells drive airway remodelling and wound healing within the lung (Abdelaziz et al., 2020). However mounting evidence indicates that macrophage activation is not a clear-cut process, with the M1-M2 axis being an ever-changing spectrum (Lambrecht & Hammad, 2017). An over-activated, trained immune response can be detrimental in the context of asthma, as the primed innate immune system is hyperresponsive to inhaled allergens, irritants, or environmental changes, resulting in frequent exacerbations.

Thus, macrophages, eosinophils, and Th2-mediated cytokines (Scott et al., 2023) play important functional roles in asthma pathogenesis, making these suitable therapeutic targets for the development of biologics to treat this allergic airway disease.

1.1.8 Current Treatments

There is an abundance of medicines that can be prescribed to manage the symptoms of allergic asthma. These can be divided based on their mechanism of action, such as the quick-acting resolution or long-term maintenance of symptoms. Quick-acting treatments work to resolve an immediate influx of inflammation, where they dilate narrowed airways. These treatments range from short-acting beta agonists (SABA), anti-cholinergic agents or in severe cases, oral or intravenous corticosteroids which are often associated with many adverse effects. Treatments targeted at the long-term maintenance of asthma act to reduce the overall level of inflammation, preventing the risk of future asthma attacks. These include combination inhalers

(controllers), theophylline, leukotriene receptor antagonists and inhaled corticosteroids, which importantly have less associated adverse effects than those administered orally or intravenously. As allergic asthma is often associated with increases of IgE and histamine, allergy medications may be an additional route of treatment. However, there are many negatives associated with these treatment options, as these treatments only act to control or mask the symptoms associated with this condition, meaning there is an unmet need for the development of treatments to tackle this condition at a physiological level. Furthermore, the prevalence of asthma is documented to be increased in low socio-economic areas, meaning a high percentage of asthma cases go uncontrolled as long-term treatments are out of financial reach. Specifically in Ireland, out of 480,000 documented cases of asthma, approximately 240,000 are classified as having uncontrolled asthma (National Clinical Programme for Asthma, HSE, 2020). In a worldwide study covering 25 countries, the percentage of those with partially controlled asthma were 30.7% in children, 22.3% in adolescents and 22.9% in adults, and uncontrolled asthma were 25.3% in children, 22.3% in adolescents and 16% in adults (Figure 3) (García-Marcos et al., 2022). Thus, this demonstrates clearly that approximately half of the asthmatic population have uncontrolled symptoms. In the AIRE study, in Portugal, 327 active asthmatic patients were contacted and asked to fill a form, where 35.2% had uncontrolled asthma and 64.8% partially controlled, with none of the individuals having total control of asthma upon assessment (Camarinha et al., 2023). Furthermore, asthma was sub-optimally controlled in Spain (Gall et al., 2023), Europe (de las Vecillas & Quirce, 2023), and the UK, where even when patients were put on higher doses of inhaled corticosteroids, this was still not effective at preventing further exacerbations (Pavord et al., 2023). Along with high levels of non-adherence to medical protocols and with the use of short-acting bronchodilators alone no longer being a recommended treatment for adults with asthma (Reddel et al., 2019), there is a pressing need for the development of new biologics that can control asthma, by not only

targeting the physiological processes involved in the pathogenesis of this airway disease, but also to improve rates of medical non-compliance and the overuse of current treatment options.

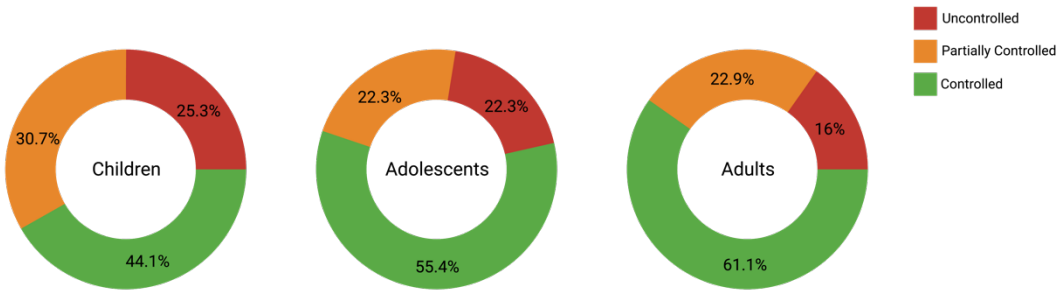


Figure 3. Percentage of asthma control across 25 countries. Uncontrolled (red), partially controlled (orange) and controlled (green). Adapted from Global Asthma Report 2022, (García-Marcos et al., 2022).

Current licensed biologics that are being used to treat this chronic condition include Dupilumab (IL-4Rα/IL-13Rα), Lebrikizumab (IL-13), Reslizumab (IL-5), Benralizumab (IL-5), Mepolizumab (IL-5), Omazilumab and Tezepelumab (Ramonell et al., 2020; Sun et al., 2023). Dupilumab (DUPIXENT®), an anti-IL-4 receptor antibody, acts by targeting a subunit of IL-4 and IL-13 receptor complexes, preventing these receptors from forming a heterodimer on the cell surface. Thus, this makes Dupilumab efficacious in the prevention of HDM-induced pathology, improving lung function (Agache et al., 2020; Castro et al., 2018; Le Floc'h et al., 2020). In a similar fashion, Lebrikizumab, a monoclonal antibody (mAb) targeted at the soluble Th2 cytokine IL-13, is another option for severe uncontrolled asthma (Szefler et al., 2022), whilst also being used as a monotherapy for atopic dermatitis (Blauvelt et al., 2023; Butala et al., 2023; Silverberg et al., 2023).

Prior to the development of these biologics, 3-10% of asthmatics had their symptoms classified as uncontrolled (Hekking et al., 2015). To better control this respiratory condition, along with patient choice or even ease of administration, switches between types of IL-5 targeted therapy have been published. For example, in an Irish study examining patients previously on the IL-5 targeting mAb Reslizumab (Cinqair® (US), Cinqaero® (EU)), which can only be administered intravenously, were documented to have favourable outcomes when switched to Benralizumab (FASENRA®) (Busse et al., 2019) or Mepolizumab (Walsh et al., 2023). Incredible research progress has been made surrounding Mepolizumab (NUCALA®), an anti-IL5 antibody designed to target uncontrolled severe eosinophilic asthma where its administration reduces eosinophil activity, measured through blood eosinophil counts and eosinophil cationic protein (ECP) levels (Bush, 2023; Chupp et al., 2023; Kroes et al., 2023; Laorden et al., 2023; Loli-Ausejo et al., 2023; Pavord et al., 2023; Pavord et al., 2012; Rodrigues et al., 2023).

Additional to biologics that target Th2 cytokines (Pelaia et al., 2022), Omalizumab (ZOLAIR®) acts by binding allergen-specific IgE in the blood stream of moderate to severe asthmatics (Bourgoin-Heck et al., 2018; Casale et al., 2019; Humbert et al., 2018). Thus, this monoclonal antibody is also utilised as a treatment for other atopic conditions, as it can prevent allergic responses associated to those involved with nasal polyps or even chronic spontaneous urticaria (CSU).

Another therapeutic route is to target other aspects of this airway disease such as TSLP or the IL-33/ST2 pathway. Thymic stromal lymphopoietin (TSLP), found to be upregulated in the bronchoalveolar lavage fluid (BALF) of severe asthmatics (Lee et al., 2017; Liu et al., 2018), is an inflammatory mediator produced and secreted by epithelial cells that drives allergic

asthma by activating ILC2s and facilitating the differentiation of Th2 $CD4^+$ T cells (Li et al., 2018). Tezepelumab (Tezspire®), a human monoclonal antibody targeting epithelial cell-derived cytokine TSLP has demonstrated efficacy in the management of asthma previously defined as being uncontrolled by long-acting beta-agonists or inhaled glucocorticoid steroids (Bel, 2017; Corren et al., 2017; Menzies-Gow et al., 2023). Moreover, anti-ST2 biologics such as Asteogolimab which are developed against the IL-33 receptor ST2, facilitate an alternative therapy for those that are classified as eosinophil 'low' as opposed to 'high' (Kelsen et al., 2021). In addition to demonstrating efficacy in asthma, Asteogolimab has also been used in cases of chronic obstructive pulmonary disease (Yousuf et al., 2022) and COVID-19-induced pneumonia (Waters et al., 2023).

Whilst targeting specific immune checkpoints that play a role in the pathogenesis of asthma is beneficial, it is important to broaden the horizons in treatment options for asthma, striving for a treatment which can control and modulate the pathophysiology associated with severe uncontrolled asthma (Kanannejad et al., 2023).

1.1.9 Personalised Medicine through Patient Stratification

With the future looking not so bright for the treatment of respiratory diseases, especially asthma, with the increase of environment toxins, global warming with enhanced CO_2 levels, increased numbers of live stocks and overall increases in autoimmunity, other avenues need to be explored to find treatments capable of controlling asthma whilst tackling this disease at a physiological level. By stratifying patients utilising biomarkers of disease such as sputum eosinophil counts, levels of allergen-specific IgE or even investigating the genetic background of patients, treatments could be tailored to target a specific mechanism of pathogenesis (Drake

et al., 2019). Additional to creating personalised medical treatment, patient stratification may also facilitate the prediction of therapeutic success prior to administration, preventing worries linked to a decline in asthma management after switching medications (Mukherjee et al., 2020). Furthermore, the development of a new digital device known as the Inhaler Compliance Assessment (INCA) can now differentiate patients with severe asthma and those with difficult-to-treat asthma by identifying non-adherence or improper use of medical protocols (Hale et al., 2023). Most importantly, the utilisation of cell-based therapies such as mesenchymal stromal cells, which can interact with the host immune microenvironment and act accordingly (Dunbar et al., 2021), would be extremely beneficial as these responsive, living cells can calm the immune response and thus, decrease cases of uncontrolled asthma.

1.2 MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF)

1.2.1 Introduction

Macrophage migration inhibitory factor (MIF), is a pro-inflammatory innate cytokine originally discovered in the 1960s, identified by its ability to inhibit the random movement and migration of macrophages (George & Vaughan, 1962). In 1966, MIF was recognised as a protein that was secreted into the conditioned media of activated T lymphocytes (David, 1966). Once the genetic sequence was determined in 1989 (Weiser et al., 1989), pituitary-derived recombinant MIF became available in 1993 (Bernhagen et al., 1993). Importantly, MIF is found pre-formed in intracellular pools. Thus, this pro-inflammatory cytokine is only released upon stimulation, as otherwise its highly ubiquitous expression may disrupt bodily homeostasis (Calandra & Roger, 2003). Although this cytokine is constitutively expressed by several cell types, cells involved in the development of asthma, such as eosinophils and macrophages, are

known to be important sources of MIF (Calandra et al., 1994; Calandra & Roger, 2003; Hart et al., 1998; Rossi et al., 1998).

1.2.2 Genetics and Polymorphisms

The MIF gene is located on chromosome 22 (22q11.2) in humans. A homolog of the MIF gene has been identified as D-Dopachrome Tautomerase (DDT) (Esumi et al., 1998; Merk et al., 2011; Sugimoto et al., 1997) also located on the same chromosome. Although, it is believed that these homologs may have evolved to have different functions (Calandra & Roger, 2003). Two polymorphisms are associated with the MIF gene, both playing a role in human disease. The first polymorphism, associated with the onset of juvenile arthritis, was the first identified SNP in the MIF gene, involving a single nucleotide sequence change, a G to C transition at position -173 (Donn et al., 2001). The second polymorphism is of particular importance for this research, involving 5 to 8 repeats of a microsatellite CATT tetranucleotide sequence at position 794 downstream of the promotor region (−794 $CATT_{5–8}$, rs5844572) (Baugh et al., 2002). This functional promotor polymorphism influences disease progression, as with an increase in the number of tetranucleotide repeats, this ($CATT_n$) sequence correlates with disease severity, due to enhanced MIF promotor activity, and thus MIF expression (Awandare et al., 2009; Du et al., 2020; Plant et al., 2005). In an Irish population, there are four variations of this allele, classified as 5-, 6-, 7- or 8-CATT polymorphisms, with the 5-CATT allele having the lowest MIF promotor activity (Plant et al., 2005). Moreover, the 5- and 7-CATT haplotypes are associated with atopy, with 5-CATT being linked to lower risk of atopy, again demonstrating the possible role of MIF in asthma susceptibility (Hizawa et al., 2004). In rheumatoid arthritis, 5 repeats of this CATT sequence, known as $CATT_5$, has been linked to a milder phenotype of this disease (Baugh et al., 2002). Similarly in cystic fibrosis, the 5-repeat

allele was associated with a milder disease phenotype (Melotti et al., 2014). In asthma, the impact of the high expressing $CATT_7$ allele remains to be definitively proven.

1.2.3 MIF Receptors, Signalling and Function

The homotrimer three-dimensional crystal structure of macrophage migration inhibitory factor, 37.5kDa in size, was identified in 1996 (Leng & Bucala, 2006; Sugimoto et al., 1996; Sun et al., 1996). MIF is known to signal through the phosphorylation of p44/p42 extracellular-signal-regulated kinases (ERK-1/2), members of the MAP kinase family (Fingerle-Rowson et al., 2003; Leng & Bucala, 2006; Mitchell et al., 1999). Through this analysis, the MIF cell surface receptor CD74 was identified, where MIF activated ERK signalling in $CD74^{+/+}$ macrophages, but not $CD74^{-/-}$ macrophages (Leng et al., 2003). Moreover, it is thought that MIF does not signal through CD74 alone, but that an interaction between the transmembrane domain CD74 and a signal transduction molecule CD44 is required for this pro-inflammatory cytokine to exert its function (Kang & Bucala, 2019; Leng et al., 2003; Schwartz et al., 2009; Shi et al., 2006; Yoo et al., 2016). Other subsequent pathways by which MIF signals through include protein kinase B (PI3K), NF-κB and AMP-activated protein kinase (AMPK) (Amin et al., 2006; Miller et al., 2008). Additionally, there is evidence that MIF is also a non-cognate ligand of the chemotactic receptors CXCR2 and CXCR4 (Bernhagen et al., 2007), but also CXCR7, which can form complexes with both CXCR4 and CD74 (Alampour-Rajabi et al., 2015)

1.2.4 MIF in Asthma

MIF's implication in chronic lung disease has been thoroughly documented (Florez-Sampedro, Soto-Gamez, et al., 2020; Jalce & Guignabert, 2020), where this pro-inflammatory cytokine has implications in chronic obstructive pulmonary disease (Husebø et al., 2016; Russell et al., 2016; Sauler et al., 2014; Zhang et al., 2019), sarcoidosis (Plant et al., 2006), cystic fibrosis (Adamali et al., 2012; Melotti et al., 2014; Plant et al., 2005; Tynan et al., 2017), pulmonary idiopathic fibrosis (Bargagli et al., 2009; Günther et al., 2018; Olivieri et al., 2016; Tanino et al., 2002), influenza (Smith et al., 2019), COVID-19 (Aksakal et al., 2021; Donnelly, 2023; Shin et al., 2023), lung cancer (Arenberg et al., 2010; Coleman et al., 2008; D'Amato-Brito et al., 2016; Guo et al., 2013; Li et al., 2017; Mawhinney et al., 2014; Rendon et al., 2007) and most importantly, asthma.

A potential role of MIF in asthma was first introduced in 1998, where eosinophils, an important innate cell involved in asthma pathogenesis, were found to secrete this pro-inflammatory cytokine (Rossi et al., 1998). In 2000, increased levels of MIF were identified in the serum and sputum of asthmatic patients, being even further increased in those with symptoms, compared to those who were asymptomatic (Yamaguchi et al., 2000). The field of MIF polymorphisms in asthma progressed in 2005, when bronchoalveolar lavage fluid and sputum from asthmatic patients had elevated levels of MIF compared to non-atopic healthy volunteers (Mizue et al., 2005). Under the Global Initiative for Asthma (GINA), 315 human DNA samples were genotyped for this CATT polymorphism, with 151 of these samples being from asthma patients. Through this genetic analysis, a correlation was found between the 5-CATT allele and milder forms of asthma (Mizue et al., 2005). Although the impact of higher

repeats of this human MIF allele have been recently portrayed in the context of COVID-19 (Shin et al., 2023), their role in asthma severity has not yet been elucidated.

Mounting evidence has indicated a role of murine MIF in asthma, through the use of ovalbumin (OVA) and HDM preclinical models, as when mice deficient in MIF were sensitised with OVA, they exhibited lower levels of Th2 cytokines and allergen-specific IgE (Mizue et al., 2005). Evidence of an increase in the presence of MIF in the bronchial epithelium of rats has been indicated after OVA-challenge (Kobayashi, 2006). Interestingly, MIF was found to drive airway remodelling in an OVA model by increasing autophagy in the airway smooth muscle in a CD74-dependent manner (Li et al., 2021). In addition to asthma models induced by synthetic OVA sensitisation, a role for MIF in HDM-induced allergic asthma has been recently demonstrated, proving it is essential for asthma development. $MIF^{-/-}$ mice illustrated decreased levels of eosinophil infiltration, mucus secretion, subepithelial fibrosis, Th2 cytokines and overall airway hyperresponsiveness after HDM challenge (Lintomen et al., 2023). In a model of epicutaneous OVA sensitisation, draining lymph nodes from OVA-treated $MIF^{-/-}$ mice had decreased levels of Th2 cytokines compared to OVA-treated WT control mice, demonstrating MIF may be crucial in both the sensitisation and elicitation phases of Th2-mediated allergic disease (Das et al., 2011). Importantly, lower levels of eosinophils and their associated lipid bodies were recorded in $MIF^{-/-}$ balb/c OVA sensitised mice. Thus, it's hypothesised that MIF plays a role in eosinophil activation and therefore lipid release, synthesised by leukotriene C4, making MIF a pivotal cytokine in exacerbation of allergic asthma (Vieira-de-Abreu et al., 2011). This suggests that MIF may be a suitable therapeutic target to control allergic inflammation, as this pro-inflammatory cytokine is found to be key in the pathological progression of this allergic airway disease.

1.2.5 MIF Inhibitors

As the biological activity of this pleiotropic cytokine plays a role in processes pivotal to asthma development, MIF could be an optimal therapeutic target. MIF biological activity can be blocked using small molecular weight inhibitors or anti-MIF antibodies. Although there is evidence of anti-MIF antibodies such as Imalumab being used in clinical trials, the negative side of using these biologics to target MIF is that they do not effectively target intracellular pools of this cytokine (Mahalingam et al., 2020). Thus, the development of small molecular weight inhibitors which bind to the active site of MIF and block its biological activity may be superior. There are many different categories of these MIF inhibitors, however the most studied include the isooxazoline MIF inhibitor ISO-1 ((S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester) (Al-Abed et al., 2005) and the novel isocoumarin MIF inhibitor SCD-19 (3-(2-methylphenyl)-1H-isochromen-1-one) (Doroudian et al., 2020; Kok et al., 2018; Mawhinney et al., 2014; Tynan et al., 2017). Notably, the active site of this cytokine is thought to play a role in MIF's tautomerase enzymatic activity, however this aspect of MIF's biological activity is still under investigation (Adamali et al., 2012; Bendrat et al., 1997; Dios et al., 2002; Lubetsky et al., 1999; Senter et al., 2002).

The use of these inhibitors has been documented in both OVA and HDM-induced models of allergic asthma. The blockade of MIF facilitated a decrease in eosinophil maturation within the bone marrow niche, thus lowering the migration or chemotaxis of these cells to inflamed sites of injury such as the lung (de Souza et al., 2015; Magalhaes et al., 2009). Moreover, MIF can aid T cell activation (Bacher et al., 1996), and utilising a MIF antagonist can improve asthma pathogenesis (Chen et al., 2010; Dunbar and Hawthorne et al., 2023; Lan et al., 2020; R. Li et al., 2021; Lintomen et al., 2023). However, it is important to note that

when a neutralising anti-MIF antibody was utilised in an OVA model of allergic asthma, airway hyperresponsiveness and inflammation were abrogated, but no effect was seen on Th2 cytokines. Thus, this demonstrates that MIF is important for asthma, but is not essential for Th2 differentiation (Magalhães et al., 2007). Similarly when an anti-MIF antibody was used in balb/c mice sensitised with OVA, no change was noted in BALF cytokine levels or serum IgE, perhaps due to the inability of these antibodies to inhibit intracellular MIF (Amano et al., 2007). Furthermore, the use of an anti-MIF antibody demonstrated a reduction in the histological hallmarks of HDM-induced asthma, such as goblet cell hyperplasia and subepithelial fibrosis (Lintomen et al., 2023). To further elucidate the role of MIF in airway inflammation and epithelial barrier dysfunction after HDM challenge, an inhibitor of MIF biological activity, ISO-1 was utilised. ISO-1 had the ability to reduce HDM-associated airway inflammation, whilst also improving the dysregulated arrangement of E-cadherin, identifying the role of this pro-inflammatory cytokine in epithelial barrier disruption (Lan et al., 2020). When investigating the impact of MIF on the synthesis of TGF-β, a key mediator in airway remodelling, ISO-1 significantly reduced TGF-β at the mRNA and protein level in lung tissue after OVA sensitisation, perhaps describing a mechanism by which MIF accelerates airway inflammation and remodelling (Chen et al., 2010).

Although recent literature has demonstrated the role of murine MIF in allergic asthma, the generation of a robust model of acute allergic HDM-induced airway inflammation elucidating the role of the human MIF ‘CATT’ polymorphism has not yet been investigated.

1.2.6 MIF and Autoimmune Disease

As previously discussed, the clinically relevant HDM allergen has demonstrated capacity to induce innate immune training (Thiriou et al., 2017). This section will discuss the interplay between macrophage migration inhibitor factor and the innate immune system, and how it may facilitate the training of the immune response.

MIF is at the forefront of the regulation of innate immunity. At the beginning of neonatal life, this innate cytokine is increased 10-fold compared to adults, illustrating the important functionality of this cytokine in protecting against infection (Roger et al., 2016). However, as a result, elevated levels of MIF may also result in an unwarranted and over-exacerbated inflammatory response, doing more damage than good. MIF has demonstrated important functions in the innate system in the past decade, with this cytokine known to upregulate Toll-Like receptor 4 (TLR4) (Roger, David, et al., 2001; Roger et al., 2003), crucial for the detection of microbial pathogens and the subsequent activation of cells of the innate immune system, such as macrophages. Thus, MIF exerts several indirect pro-inflammatory functions, including the release of pro-inflammatory mediators such as TNFα, IL-1 and nitric oxide (NO) from macrophages (Bernhagen, Mitchell, et al., 1994; Calandra & Roger, 2003). MIF has been implicated to have a functional role in the protection against a variety of infections such as those caused by gram negative bacteria (Roger, Glauser, et al., 2001), *Mycobacterium tuberculosis* (Das et al., 2013), *Leishmania major* (Jüttner et al., 1998; Satoskar et al., 2001), *Taenia crassiceps* (Rodríguez-Sosa et al., 2003) and *Salmonella typhimurium* (Koebernick et al., 2002), where MIF-deficient mice failed to control infection. Moreover, macrophages have been documented to release MIF after the ingestion of *Plasmodium chabaudi*-infected erythrocytes (Martiney et al., 2000), perhaps demonstrating the possibility

of a MIF positive feedback loop. In the context of asthma, a chronic airway disease which is commonly treated by glucocorticoid steroids, MIF has been identified to over-ride the action of these steroids, perhaps even promoting resistance to this form of treatment and thus driving airway inflammation (Allam et al., 2022; Calandra et al., 1995).

A level of homeostasis is of upmost importance within the immune system, to prevent the development of autoimmunity and a plethora of autoimmune-related diseases such as diabetes (Cvetkovic et al., 2005; Korf et al., 2017; Sánchez-Zamora & Rodriguez-Sosa, 2014; Stosic-Grujicic et al., 2008), rheumatoid arthritis (Bilsborrow et al., 2019; Kim & Kim, 2016; Morand et al., 2006) and systemic lupus erythematosus (Greven et al., 2010; Lang et al., 2015; Tu et al., 2019). In the presence of enhanced levels of inflammation or a high MIF expression allele (e.g. $CATT_7$ polymorphism), increased levels of MIF reduces macrophage and monocyte apoptosis by inhibiting p53. As a result, this sustains the accumulation of pro-inflammatory macrophages, exacerbating inflammation and driving autoimmunity (Mitchell et al., 2002).

Innate immune training is classified as the re-programming of macrophages and their pre-cursors by a primary stimulus, which primes these cells to mount an enhanced pro-inflammatory response upon encountering a secondary, often heterologous stimulus (Dagenais et al., 2023; Domínguez-Andrés et al., 2023; Hartung & Esser-von Bieren, 2022; Li et al., 2022; Mulder et al., 2019; Netea et al., 2020). Stimuli that are known to initiate this innate immune training include HDM (Lechner et al., 2022), β-glucan (Bekkering et al., 2016; Brichkina & Simon, 2023), LPS (Zahalka et al., 2022) or cathelicidin analogs (van Dijk et al., 2022). In contrast to pro-inflammatory innate training, stimuli such as helminth infection can induce anti-inflammatory innate immunity (Finlay et al., 2016; Quinn et al., 2019). However new evidence has suggested that after receiving a primary stimulus of whole beta-glucan particles (WGP),

macrophages can react to a secondary stimuli of MIF, produced by tumour cells (Ding et al., 2023). This fast-acting, memory-inducing training effect can be identified by hallmark changes in the epigenetic signature of cells, such as the methylation of their DNA by methyltransferase enzymes, or through the measurement of their production of mediators such as TNFα, IL-6 (Ochando et al., 2023) or IL-1β, a cytokine known to play a role in MIF-facilitated NLRP3 inflammasome activation (Harris et al., 2019; Lang et al., 2018; Li et al., 2022). Literature has documented the complex ability of this innate cytokine to polarise macrophages to both a pro-inflammatory M1 phenotype where they play a role in host defence against pathogens (Castro et al., 2017; Cotzomi-Ortega et al., 2021; Feng et al., 2021; Li et al., 2022), but also an anti-inflammatory M2 phenotype where they aid tissue repair and wound healing (Falcone et al., 2001; Filbey et al., 2019; Prieto-Lafuente et al., 2009). Thus, MIF can have dichotomous effects on macrophage polarisation depending on the concentration of MIF available, the presence of other cytokines, and the tissue microenvironment. Although MIF is known to have a crucial role in driving autoimmunity as previously discussed, the knowledge on MIF's function in innate immune training is limited. Moreover, there is a gap in the literature concerning the impact of the human $CATT_7$ MIF polymorphism and trained immunity, especially in an allergic asthma lung microenvironment induced by the HDM aeroallergen.

1.3 MESENCHYMAL STROMAL CELLS (MSCS)

1.3.1 Introduction

Mesenchymal stromal cells (MSCs) have gained popularity for their therapeutic potential in treating inflammatory diseases, owing to their robust immunomodulatory and cytoprotective abilities (Hoang et al., 2022). Intrinsic variability is documented between MSCs

sourced from different tissues, including the bone marrow (BM), adipose tissue (AD) or umbilical cord (UC), in addition to donor-to-donor variability (Ankrum, Ong, et al., 2014; Costa et al., 2021). Extrinsic differences are introduced through the culturing methods of these cells, the route of administration and overall cell viability (Giri & Galipeau, 2020; Matthay et al., 2019). Thus, although MSCs are one of the most extensively studied cell-based therapies, their heterogeneity remains the primary concern leading to discrepancies in their clinical therapeutic efficacy. To improve the consistency and efficacy of MSC-based therapy for inflammatory diseases, pre-licensing these cells by optimising or modifying the conditions in which they grow, may harness their ability to regulate an over-zealous immune response and consequently boost their clinical success. First tested in humans in 1995 (Lazarus et al., 1995), MSC therapeutic intervention is well documented, especially in field of chronic pulmonary disease (Doherty et al., 2023) where their mechanisms of action have been studied in acute respiratory distress syndrome (ARDS) (Gorman et al., 2021; Liu et al., 2022; Wick et al., 2021; Zhang et al., 2022), idiopathic pulmonary fibrosis (IPF) (Atanasova et al., 2022; Li et al., 2018; Samarelli et al., 2021; Shi, Han, et al., 2021; Yang et al., 2021), COVID-19 (Rossello-Gelabert et al., 2022; Shi, Wang, et al., 2021; Xu et al., 2022; Yao et al., 2022) and importantly asthma (Choi et al., 2022; Cruz et al., 2015; Cruz et al., 2018; de Castro et al., 2017; Kim et al., 2022; Mo, Kang, et al., 2022).

1.3.2 Mechanism of Action

Mesenchymal stromal cells can elicit their immunomodulatory and cytoprotective effects by mechanisms both dependent and independent of cell-cell contact. *In vivo*, MSCs interact with their surrounding microenvironment. When first reaching a site of inflammation or injury, such as in the lung after intravenous administration, MSCs become either pro-

inflammatory or anti-inflammatory depending on the signals received from their environment (Dunbar et al., 2021). In a pro-inflammatory manner, MSCs come in contact with and activate effector cells, such as T cells, macrophages and neutrophils to assist in pathogen clearance. Conversely, MSCs can modulate an over-active immune response by communicating with tolerogenic dendritic cells, T regulatory cells or M2 anti-inflammatory macrophages (Carty et al., 2017). Furthermore, our group has thoroughly demonstrated MSCs ability to suppress T cell proliferation (Carty et al., 2018; Corbett et al., 2021; English et al., 2007, 2013; Tobin et al., 2013).

Additional to their contact-dependent functions, MSCs mediate the majority of their immunosuppressive and cytoprotective effects through their secretome (Műzes & Sipos, 2022; Su et al., 2023; Szabłowska-Gadomska et al., 2023; Xia et al., 2019). The secretome is a collection of soluble mediators secreted by MSCs, perhaps accounting for their distal effects *in vivo* (Cahill et al., 2016; Carty et al., 2021; Corbett et al., 2021; English et al., 2007; Kennelly et al., 2016). Along with the modulation of the immune response through paracrine mechanisms, MSCs can also modulate surrounding cells through the release of exosomes (Biswas et al., 2019; de Araujo Farias et al., 2018; Reza et al., 2016; Shang et al., 2019), or even through mitochondrial transfer (Li et al., 2019; Mohammadalipour et al., 2020; Yuan et al., 2021). A recent study demonstrated that mitochondrial DNA from MSCs could downregulate Th1 differentiation via the master Th1 transcription factor T-bet, perhaps illustrating another mechanism by which MSCs can calm the immune response (Akhter et al., 2023). Furthermore, MSCs are known for their expression of cyclooxygenase (COX) enzymes, which facilitate the production of prostaglandin H2 from arachidonic acid and its conversion to the lipid mediator prostaglandin E2 (Burand et al., 2020; Duffy et al., 2011; English, 2013; English et al., 2007), which can bind to macrophages through EP2 and EP4 receptors.

Additionally, indoleamine 2,3-dioxygenase (IDO) is another well-known enzyme involved in MSC function, where this enzyme acts to catabolise tryptophan into kynurenine, modulating T cell proliferation (Boland et al., 2022; Carty et al., 2021; Chinnadurai et al., 2014; English et al., 2013; English & Wood, 2013).

Along with MSCs being able to modulate the immune response, they can re-educate the resident population of phagocytic macrophages present. As previously discussed, macrophages can be polarised to an M1 or M2 phenotype by the inflammatory milieu in their vicinity. It is hypothesised that MSCs can re-programme macrophages through the secretion of pro-inflammatory cytokines such as IL-6 and IL-8, or anti-inflammatory mediators like IDO, PGE_2, TGF-β and even VEGF.

1.3.3 MSC and Macrophage Cross-Talk

Along with the inflammatory lung microenvironment being a key mediator in MSC licensing (Dunbar et al., 2021), the recipient's resident macrophage population can also play a role in MSCs' mechanisms of action. As MSCs are known to become apoptotic shortly after their administration *in vivo* (Carty et al., 2021; Eggenhofer et al., 2012), increasing evidence has demonstrated the importance of their phagocytosis by macrophages, eliciting their immunomodulatory effects in an indirect paracrine manner (Braza et al., 2016; Galleu et al., 2017; Pang et al., 2021; Stevens et al., 2020).

MSC therapeutic efficacy requires the presence of macrophages (Galipeau, 2021; Mathias et al., 2013), where a bi-directional, complex cross-talk between these cell populations can determine the inflammatory fate of their resident microenvironment. Elegantly described

by Carty et al., (2017), MSCs can modulate macrophages through active (secretion of TSG-6 and PGE_2) and passive (being phagocytosed, secreting miRNA containing exosomes and mitochondrial transfer) means. Moreover, MSCs are primarily known to polarise macrophages to an M2, anti-inflammatory phenotype (Biswas et al., 2019; Braza et al., 2016; François et al., 2012; Kim & Hematti, 2009; Selleri et al., 2016; Xie et al., 2016; Yuan et al., 2021), which in return, triggers macrophages to secrete mediators which can act to license MSCs and further boost their immunomodulatory efficacy (Carty et al., 2017). Conversely, there is evidence that MSCs can also polarise macrophages to an M1 phenotype in response to parasitic *Leishmania major* infection (Dameshghi et al., 2016) or in *E.coli* pneumonia-induced sepsis (Rabani et al., 2018).

During allergic inflammation, such as after the inhalation of the aeroallergen HDM, blood monocytes are recruited to the lung where they differentiate into macrophages depending on the cytokine milieu present (Poston et al., 1992; Tomita et al., 1995). In asthma, there is an increase in Th2 cytokines which alternatively activate macrophages to an M2 type which evidently contributes to airway remodelling. In HDM-challenged mice that over-expressed IL-13, hUC-MSCs decrease the level of monocyte infiltration into the lung, specifically $LyC6^-$ monocytes (Mo, Kim, et al., 2022). In an additional study investigating the mechanism by which Liporxstatin-1 licensed MSCs decreased asthma pathology, MSCs were noted to attenuate $LyC6^+$ M2 macrophages (Kim et al., 2022). Furthermore, MSCs have been identified to reduce Th2 allergic inflammation by modulating lung macrophages in a paracrine manner (Mo, Kang, et al., 2022). *In vitro*, murine MSC (BM, AD, lung) co-cultured with RAW 264.7 macrophages decreased the mRNA expression of iNOS, demonstrating MSCs ability to decrease pro-inflammatory M1 macrophages (Abreu et al., 2017). After OVA sensitisation, small extracellular vesicles derived from MSCs (MSC-sEV) significantly decreased the

recruitment and polarisation of monocyte-derived M2 alveolar macrophages, thus facilitating a decrease in allergic airway inflammation (Fang et al., 2020).

Whilst these studies have demonstrated the ability of MSCs to polarise macrophages to an anti-inflammatory phenotype, perhaps in a cell-contact dependent manner through the expression of PD-L1 (Chinnadurai et al., 2014), ICAM (Espagnolle et al., 2017) or even mitochondrial transfer (Jackson et al., 2016), the role of the MSC secretome containing kynurenine (KYN) or prostaglandin E2 (PGE_2) is also important (Chiossone et al., 2016; François et al., 2012; Galipeau, 2021). MSC production of PGE_2 has been demonstrated to calm macrophages through EP2 and EP4 receptors (Burand et al., 2020; Kubo et al., 2004; Németh et al., 2009; Shinomiya et al., 2001).

As MSCs can effectively modulate macrophages in the context of different inflammatory diseases, perhaps they could be used to therapeutically target macrophages, re-wiring their inflammatory response and thus reducing trained immunity (Mulder et al., 2019).

1.3.4 MSC licensing

The phenomenon of MSC licensing or activation has been studied extensively in recent years (Boland et al., 2018; Carty et al., 2021; Carvalho et al., 2019; Corbett et al., 2021; English, 2013; Hackel et al., 2023; López-García & Castro-Manrreza, 2021; Noronha et al., 2019), particularly in the context of the asthmatic microenvironment (Abreu, Rolandsson Enes, et al., 2019; Abreu, Xisto, et al., 2019; Rolandsson Enes et al., 2021). Early reports of MSC activation can be traced back to the 1960s, where activated MSCs improved bone remodelling (Hattner & Frost, 1963). However, MSC activation now refers to the process of inducing a biological

response in these cells, typically to enhance their therapeutic properties. Mesenchymal stromal cells (MSCs) require activation to exert their full therapeutic potential, which can be carried out through licensing these cells *in vitro* with stimuli or *in vivo* in an inflammatory disease microenvironment. This can be achieved through various methods, such as exposure to pro-inflammatory stimuli (Boland et al., 2018; Carty et al., 2021; Carvalho et al., 2019; Corbett et al., 2021; López-García & Castro-Manrreza, 2021; Lynch et al., 2020), including injury, inflammation, and disease (Abreu, Rolandsson Enes, et al., 2019; Abreu, Xisto, et al., 2019; Dunbar et al., 2021; Rolandsson Enes et al., 2021). MSC licensing can lead to changes in the expression of genes, cell surface markers, and secreted molecules, subsequently influencing the behaviour of these cells *in vivo*, optimising their regenerative, immunomodulatory, or anti-inflammatory effects for a given application. Additionally to pre-conditioning cells *in vitro*, MSCs can also be activated *in vivo* after administration to the body. The pro-inflammatory lung microenvironment is known to contain adequate threshold levels of stimuli to facilitate this licensing of MSCs *in vivo* (Dunbar et al., 2021). Importantly, unlike other routes of administration of these cells, when MSCs are administered intravenously (I.V.) they are known to first migrate to the lung through the pulmonary circulatory system, termed "first passe" (Galipeau & Sensébé, 2018; Sensebé & Fleury-Cappellesso, 2013), making the lung an optimal site for *in vivo* licensing. Furthermore, due to the large size of these stromal cells (ranging from 16–53 μm) (Furlani et al., 2009), the majority of MSCs administered I.V. may get trapped within the lung architecture, with only small subsets of this population reaching distal organs (Carty et al., 2021; Ferrini et al., 2021; Leibacher & Henschler, 2016; Németh et al., 2009). Although the viability of MSCs in the lung is short-lived with these cells becoming apoptotic after 24hrs (Eggenhofer et al., 2012; Galleu et al., 2017; Giri & Galipeau, 2020; Pang et al., 2021), pre-licensing MSCs may facilitate increased retention in target organs (Carty et al., 2021; Guo et al., 2018; Liesveld et al., 2020; Ullah et al., 2019).

As discussed, macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine involved in the pathogenesis of many inflammatory diseases. Increasing bodies of evidence are outlining the possibility of this cytokine to facilitate MSC licensing both *in vitro* and *in vivo*. The impact of MIF on MSC function has previously been studied *in vitro* (Xia, Zhang, et al., 2015), where MSCs have been transfected (Liu et al., 2020; Zhang, Zhu, et al., 2019; Zhu et al., 2021) or licensed with this cytokine (Abdul-Aziz et al., 2017; Chen et al., 2020; Xia, Xie, et al., 2015; Zhuang et al., 2020). MIF is involved in the upregulation of survival pathways in many cell types (Gore et al., 2008; Lue et al., 2007; Ohta et al., 2012). Thus, it may not be surprising that MIF licensing can improve MSCs survival *in vitro*.

After licensing with 100ng/ml of recombinant MIF, rat bone marrow MSCs demonstrated a level of protection against hypoxia and serum-deprivation induced apoptosis. MIF appeared to stimulate c-Met in an CD74-dependent manner, decreasing oxidative stress through PI3K/Akt-FOXO3a signalling (Xia, Xie, et al., 2015). In hypoxic conditions, human bone marrow MSCs demonstrated an increase in endogenous MIF production which led to the activation of the Akt pathway and subsequently decreased mRNA markers of cell senescence. Keeping in mind the body of knowledge illustrating the ability of MIF to license MSCs, perhaps this is presenting a positive feedback mechanism by which MIF-derived from MSCs can act to boost their own survival in hypoxic conditions (Palumbo et al., 2014). A similar positive feedback loop with MIF and the MSC production of MIF has also been demonstrated in a cancer environment (Lourenco et al., 2014; Zhang et al., 2022). Adding to this hypoxic theme, the over-expression of MIF in aged MSCs (isolated from bone marrow of humans aged 72-80) was seen to reduce cellular senescence by activating autophagy in a 3-methyladenine-dependent manner. When aged MSCs that overexpressed MIF were transferred into a rat model of myocardial infarction, these MSCs increased angiogenesis and improved cell survival

compared to aged-MSC controls (Zhang, Zhu, et al., 2019). Furthermore, rat bone marrow MSCs licensed with 100ng/ml of recombinant MIF were rescued from doxorubicin-induced senescence through activation of PI3K-Akt signalling (Xia & Hou, 2017), similar to Xia, Xie, et al., (2015). Lastly, in yet another model of hypoxia and serum deprivation-induced MSC apoptosis, MIF licensing protected murine bone marrow MSCs by interacting with lincRNA-p21 and the activation of the Wnt/B-catenin pathway (Zhuang et al., 2018).

Although literature has elucidated the positive impact of pre-conditioning MSCs with recombinant MIF, improving their viability after hypoxia exposure, the area of MSCs licensed with this pro-inflammatory cytokine is still lacking robust, reproducible studies, especially *in vivo*. MIF is known to be upregulated in the bronchoalveolar lavage fluid and sputum of asthma patients (Mizue et al., 2005), and thus it is imperative to investigate MSC efficacy in a MIF-mediated inflammatory microenvironment (Hawthorne, Dunbar et al., in reveiw) With the increase of questions surrounding the robustness of commercially available recombinant MIF and the difficulty of cloning and purifying MIF from Jurkat T cells (Bernhagen, Mitchell, et al., 1994), new ways of investigating the endogenous role of this cytokine on MSC efficacy are required. Lastly, whilst the demonstration of increased MSC viability and migration after MIF licensing is important, more focus on the functionality of MIF-licensed MSCs would be extremely beneficial, especially as there is evidence supporting the ability of MIF to upregulate COX-2 activity and PGE_2 release (Sampey et al., 2001; Zhang, Zhou, et al., 2019), both of which are pivotal aspects of MSC's immunomodulatory function.

1.3.5 MSCs in Asthma

The impact of the inflammatory lung environment present in chronic respiratory disease on MSC function has been thoroughly documented, where MSCs can respond to their surrounding environment with their therapeutic function being influenced by the threshold levels of stimuli present, creating a unique cell-host interplay (Abreu, Rolandsson Enes, et al., 2019; Abreu, Xisto, et al., 2019; Cereta et al., 2021; Dunbar et al., 2021; Rolandsson Enes et al., 2021). Over the past decade, there has been an exponential increase in the therapeutic use of MSCs in preclinical models of asthma (Figure 4).

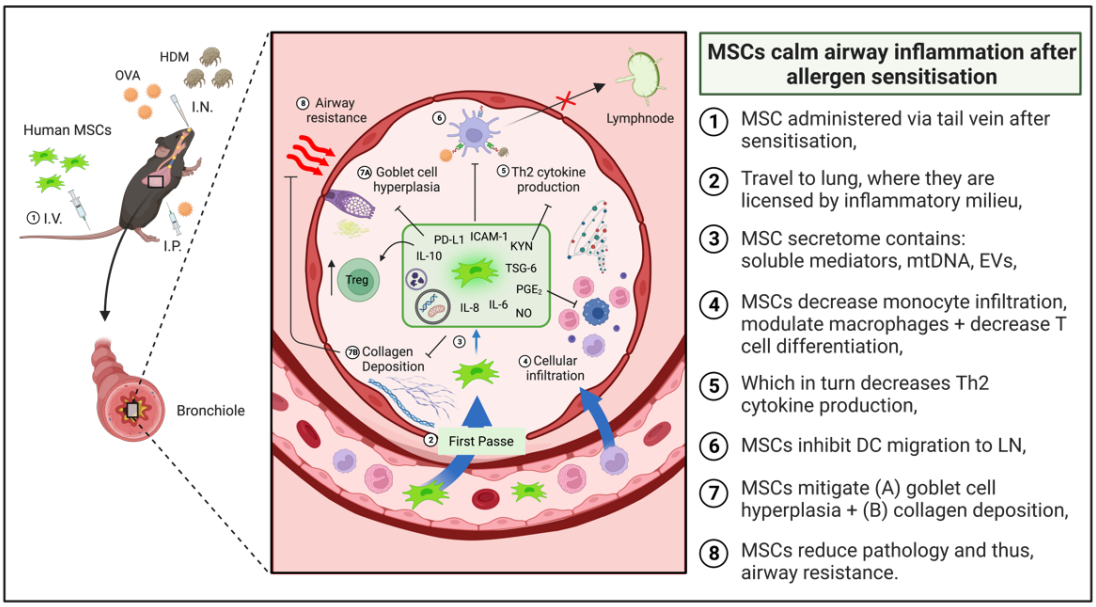


Figure 4. Mesenchymal stromal cells reduce allergic airway inflammation. Mice are sensitised with HDM or OVA to induce allergic airway inflammation. MSCs administered through the tail vein travel through the pulmonary circulatory system and reach the lung. The inflammatory cytokine milieu present in the lung acts to license MSCs. Licensed MSCs have increased expression of COX-2 (Prostaglandin E_2), IDO (Kynurenine), ICAM and PD-L1. Thus, these activated MSCs also have the enhanced secretion of mediators (KYN, TSG-6, PGE_2, NO) and cytokines (IL-6, IL-8, IL-10), along with extracellular vesicles containing mitochondrial DNA and proteins which facilitate their therapeutic efficacy. MSCs modulate the lung microenvironment by decreasing the level of monocyte infiltration, re-educating alveolar macrophages and decreasing T cell differentiation, reducing Th2 cytokines IL-4,

IL-5 and IL-13. Furthermore, MSCs inhibit the migration of HDM and OVA presenting dendritic cells to lymphnodes. Collectively, MSCs can reduce hallmarks of asthma pathology, including goblet cell hyperplasia and collagen deposition, thus abrogating airway resistance.

Abbreviations: House Dust Mite (HDM), Ovalbumin (OVA), Intravenous (I.V.), Intraperitoneal (I.P.), Intranasal (I.N.), Cyclooxygenase-2 (COX-2), Prostaglandin E_2 (PGE_2), Indoleamine 2,3-Dioxygenase (IDO), Kynurenine (KYN), Programmed Death-Ligand 1 (PD-L1), Intracellular adhesion molecule-1 (ICAM-1), Tumor Necrosis Factor-Inducible Gene 6 (TSG-6), Mitochondrial DNA (mtDNA), Extracellular Vesicles (EVs), Dendritic Cells (DCs), Lymph nodes (LN).

In mouse models of *Alternaria alternata*-induced and house dust mite (HDM)/diesel exhaust particle (DEP)-induced asthma, umbilical cord-derived MSCs could modulate Th2 and innate lymphoid (ILC2) effector cells by decreasing their production of IL-5 and IL-13, cytokines known to have a key role in driving allergic airway inflammation (Shin et al., 2021). In balb/c OVA sensitised mice, bone marrow-derived MSCs reduced airway hyperresponsiveness but adipose MSCs did not, illustrating the variability seen between MSCs isolated from different tissue sources (Choi et al., 2022). Moreover, the differential therapeutic effects from bone marrow, adipose tissue and lung isolated MSCs were demonstrated in another OVA model, where only BM-MSCs could improve the lung mechanics and function of these mice (Abreu et al., 2017). Adipose MSCs and their secreted extracellular vesicles (EV) act differently in C57/BL6 mice sensitised with OVA, where MSC-EVs decreased T lymphocytes and IL-5 in BALF whilst whole MSCs did not (de Castro et al., 2017). In a mixed Th2/Th17 *Aspergillus* Hyphal Extract-induced model of asthma, systemically administered conditioned media and extracellular vesicles from human MSCs significantly reduced airway hyperresponsiveness, inflammation and the Th2/Th17 phenotype (Cruz et al., 2015). A novel study administering conditioned media from MSCs through the tail vein of OVA sensitised mice, demonstrated that the secretome was as efficacious as the administration of the MSC in decreasing Th2 cytokines IL-4, IL-5 and IL-13, reiterating the important alternative of using

the cell-free MSC secretome as a therapeutic intervention (Ma et al., 2022; Műzes & Sipos, 2022; Xia et al., 2019). In a model of OVA and LPS-induced Th17 neutrophilic asthma, the intravenous administration of MSCs attenuated the level of Th17 inflammation, IL-17A, phospho-STAT3, GATA3 and RORγt, demonstrating the suppressive effect of these stromal cells on T helper cell differentiation (Fang et al., 2018). Importantly, MSCs have also illustrated their ability to modulate the allergic Th2 immune response induced by the pertinent, more clinically relevant HDM allergen (Abreu et al., 2018; Abreu, Xisto, et al., 2019; Castro et al., 2020; Duong et al., 2015; Mariñas-Pardo et al., 2014). Lastly, and most recently, the first use of MSC EV-mimetic nanovesicles (NV) were found to effectively reduce airway inflammation, cytokine production and eosinophil infiltration after OVA sensitisation, regardless of the route of administration (Bandeira et al., 2023). With further evaluation of the safety of these MSC-nanovesicles as a treatment for asthma, they may serve as another string to our MSC bow.

1.3.6 Limitations of MSCs in Asthma

It has been established that human mesenchymal stromal cells (hMSC) are more efficacious (Cruz et al., 2015) and safer than murine (mMSCs) (Miura et al., 2006) and rat (rMSCs) MSCs (Foudah et al., 2009), in addition to the translational relevance of using human MSCs for the treatment of human inflammatory diseases. Unfortunately, the success of human MSCs in clinical trials is still hindered by their heterogeneity, which arises through donor-to-donor variability, disparity in MSC efficacy between isolation sites, the immunological status of the recipient at the time of receiving this cell therapy and also the logistics of their utilisation in the clinic (Cruz et al., 2015; Wiese et al., 2022). Moreover, concerns about their aggregation after administration resulting in a decrease in therapeutic efficacy (Burand et al., 2020), along with the risk of their contribution to the formation of thromboembolisms after intravenous

administration (Moll et al., 2022) are areas requiring more research in order to translate these cells from bench to bedside.

Alas, in addition to the rise of strict regulations and criteria regarding MSC use (Moll et al., 2022; Wilson et al., 2021; Wright et al., 2021), pre-conditioning these cells to tailor their destined therapeutic function might be a solution, along with the possibility of using cell-free MSC derivatives, such as their secretome, exosomes or even nanovesicles. Furthermore, patient stratification can assist in predicting the therapeutic outcome after MSC administration, as the MSC environment is known to have an impact on their efficacy *in vivo* (Abreu, Rolandsson Enes, et al., 2019; Abreu, Xisto, et al., 2019; Dunbar et al., 2021; Rolandsson Enes et al., 2021). To interpret how MSCs will function in a particular setting, the immunological status of the recipient can be investigated prior to administration. In a mouse model of HDM-induced asthma, serum from HDM challenge mice was used to license MSCs prior to their intratracheal administration 24 hours after last challenge (Abreu, Xisto, et al., 2019). MSCs licensed with asthmatic serum had increased therapeutic efficacy, with an ability to decrease Th2 cytokines in the BALF, along with improving lung function compared to unlicensed MSCs (Abreu, Xisto, et al., 2019). This opens the possibility of predicting MSC therapeutic efficacy based on the inflammatory status of the recipient's microenvironment at the time of MSC infusion (Galipeau & Sensébé, 2018). In humans, MSCs' efficacy in graft versus host disease (GvHD) was investigated, where it was observed that $CD8^+$ T cells and $CD56^+$ natural killer cells were responsible for killing MSCs *in vivo*, enhancing their immunomodulatory activity (Galleu et al., 2017). Therefore to differentiate responders to MSC administration from non-responders, the cytotoxicity of the host's immune system was analysed, with patients who's $CD8^+$ T cells and $CD56^+$ natural killer cells could efficiently kill MSCs *in vitro*, responding well to MSC treatment *in vivo* (Galleu et al., 2017).

Genetic stratification may be another possible route of investigating suitable candidates for MSC therapy. As previously discussed, the functional polymorphism containing the 7-repeat tetranucleotide sequence at position 794 downstream of the promotor region of the human MIF gene, increases the promoter activity and thus expression of MIF (Baugh et al., 2002; Plant et al., 2005). Furthermore, MIF is known to have a role in the exacerbation of asthma pathophysiology (Mizue et al., 2005). As MSCs are sentinel cells, with the ability to perceive their surrounding microenvironment and adapt their therapeutic response, genotyping candidates for the presence of the CATT MIF polymorphism could predict the success of this cellular therapy prior to their administration *in vivo*.

To conclude, uncontrolled severe asthma is still an evident problem from both a physiological and an economical point of view. Human macrophage migration inhibitory factor plays a fundamental role in the development of many inflammatory disorders, particularly asthma. Mesenchymal stromal cells can be licensed or activated by pro-inflammatory cytokines such as MIF, boosting their therapeutic ability to calm and re-programme the innate immune system. Using novel transgenic mice expressing the human $CATT_7$ MIF polymorphism, this thesis sets out to elucidate the impact of high human MIF expression on HDM-induced allergic airway inflammation using a potent MIF antagonist SCD-19. Moreover, this research sought to investigate the licensing effect of MIF on human bone marrow-derived MSC cytoprotective functions and the ability of MSCs to modulate macrophage polarisation, and to re-educate the hematopoietic stem and progenitor cells from humanised $CATT_7$ MIF mice.

1.4 Hypothesis:

High expression of human MIF under the $CATT_7$ promotor polymorphism exacerbates HDM-induced allergic asthma. Human MIF acts to license MSCs, boosting their ability to release cytoprotective mediators into their secretome and calm pro-inflammatory macrophages trained by HDM exposure.

Aims:

1. To characterise novel transgenic human MIF expressing mice and generate a robust physiological scale of HDM-induced airway inflammation, investigating the specific role of MIF using MIF inhibitors.

2. To investigate the ability of human MIF to license MSCs and improve their ability to promote epithelial wound healing *in vitro.*

3. To elucidate the role of human MIF in macrophage polarisation under basal conditions.

4. To establish if human MIF drives innate immune training in HDM-treated macrophages.

5. To identify if MSCs can calm the macrophage pro-inflammatory response by re-programming progenitor cells from the bone marrow of human MIF expressing $CATT_7$ mice.

Chapter 2:

The biological role of macrophage migration inhibitory factor in novel humanised MIF mice in a model of allergic airway inflammation

2.1 Abstract

Macrophage migration inhibitory factor (MIF) expression is controlled by a functional promotor polymorphism, where the number of tetranucleotide repeats ($CATT_n$) regulates MIF expression. To examine the role of this polymorphism in a pre-clinical model of allergic airway inflammation, novel humanised MIF transgenic mice expressing the $CATT_7$ allele were used to investigate the impact of high human MIF expression in airway inflammation following house dust mite challenge. Under basal and diseased conditions, significantly elevated levels of MIF were measured in the bronchoalveolar lavage fluid, splenocytes, bone marrow-derived macrophages and lung tissue from $CATT_7$ mice compared to WT mice. After HDM challenge, $CATT_7$ mice, which express high levels of human MIF, illustrated significantly elevated levels of immune cell infiltration, production of inflammatory mediators, goblet cell hyperplasia, subepithelial collagen deposition and airway resistance compared to WT controls. To fully elucidate the implications of high basal levels of MIF in airway inflammation, a potent MIF inhibitor SCD-19 was used to block the biological activity of this pro-inflammatory cytokine. SCD-19 significantly mitigated the pathophysiology observed in $CATT_7$ mice after HDM challenge, demonstrating the fundamental role of endogenous human MIF expression in the severity of airway inflammation *in vivo*.

2.2 Introduction

There is an urgent unmet need for novel therapeutics for the management and treatment of allergic asthma. Clinically characterised by airway remodelling and hyperresponsiveness, this chronic lung condition accounts for a substantial economic burden worldwide (Attar-Zadeh et al., 2023; Faverio et al., 2023; Harada et al., 2023; López-Tiro et al., 2022). There is currently no cure for asthma. As the majority of current treatments act to manage symptoms associated with lung inflammation, there is still a pursuit to develop biologics that efficiently tackle airway remodelling and thus, the persistence of severe, uncontrolled asthma.

50-85% of asthmatics are allergic to house dust mite (HDM) (Dullaers et al., 2017), making this a clinically relevant aeroallergen, in comparison to its demoded counterpart, ovalbumin (OVA). OVA requires an adjuvant upon administration via the non-physiological intraperitoneal route in order to elicit a Th2 response equivalent to that induced by HDM (Eisenbarth et al., 2002; Tsitoura et al., 2000). HDM-driven asthma results from the inhalation of dust mite faecal pellets, or the crude extracts of the mite itself, known as *Dermatophagoides*, which acts to penetrate epithelial cells and activate the key antigen presenting dendritic cells, triggering an influx of type 2 T helper cells and Th2 cytokines; IL-4, IL-5 and IL-13. Incidence of allergic asthma is known to be a complex interplay between environmental (i.e., inhalation of allergens, pollution etc) and genetic factors (i.e., inheritance or polymorphisms) (von Mutius, 2009), along with others such as birth order or childhood infections (Gregory & Lloyd, 2011). Current treatments for asthma focus on the management of symptoms and consist of the utilisation of bronchodilators and glucocorticoid steroids to control the intensity and number of allergic exacerbations. Advances have been made with the availability of add-on maintenance biologics such as Dupilumab (DUPIXENT®) (Faverio, 2023; Thelen et al., 2023;

Rabe et al., 2022), Mepolizumab (Ortega et al., 2014) and Lebrikizumab (Austin et al., 2020; Szefler et al., 2022), which target IL-4, IL-5 and IL-13 receptors respectively. Unfortunately, the majority of therapies are still targeted at the maintenance rather than the resolution of this condition. Although these biologics target cytokines and receptors that drive airway inflammation, they do not act on a widespread physiological level to definitively reverse multifaceted histological damage inflicted by repeated exacerbations, thus not being a long term solution to this lung disease.

Macrophage migration inhibitory factor (MIF) is detected at high levels in the bronchoalveolar lavage fluid (BALF) and serum of asthmatic patients (Mizue et al., 2005). First discovered in 1966 (David, 1966) and cloned in 1989 (Weiser et al., 1989), this pro-inflammatory cytokine is known to play a pivotal role in many inflammatory diseases (Bucala, 2013) including asthma (Florez-Sampedro, Soto-Gamez, et al., 2020; Kobayashi, 2006; Rossi et al., 1998), rheumatoid arthritis (Morand et al., 2006; Sánchez-Zuno et al., 2021; Yoo et al., 2016) and sepsis (Bernhagen, Calandra, et al., 1994; Bozza et al., 2004; Tilstam et al., 2021; Toldi et al., 2021). The level of MIF expression can vary in humans due to a functional repeat polymorphism implicating a tetranucleotide sequence ($CATT_n$), found at position 794 downstream of the promotor region in the MIF gene (Baugh et al., 2002), where the higher the number of repeats, the stronger the gene promotor activity and thus higher MIF expression. Four types of allelic variations are known, classified as $CATT_5$, $CATT_6$, $CATT_7$ and $CATT_8$, with the $CATT_5$ repeat allele presenting the lowest promotor activity (Plant et al., 2006). Overall it was found that this low expressing MIF $CATT_5$ allele correlated with lower levels of inflammation and thus milder forms of disease (Baugh et al., 2002; Melotti et al., 2014; Plant et al., 2005).

MIF is pre-formed and stored within the cytoplasm of the cell, to ensure that it can be rapidly released upon stimulation, such as in response to an inflammatory stimulus (Bacher et al., 1996; Bernhagen, Mitchell, et al., 1994; Calandra et al., 1994; Calandra & Roger, 2003). If MIF were to constantly leak out of the cell, it could lead to dysregulated immune responses. Therefore, pre-formed MIF is kept in intracellular pools until it is needed. Importantly for asthmatics, MIF is known to suppress the action of glucocorticoids, reducing the efficacy of this current first line therapy for this atopic condition (Bloom et al., 2016; Calandra et al., 1994).

Studies have previously established a role for this pro-inflammatory cytokine in asthma, for example in a mouse model of OVA-induced airway inflammation, MIF deficient ($MIF^{-/-}$) mice had lower levels of pulmonary inflammation, Th2 cytokines and airway hyperresponsiveness (AHR) compared to wildtype controls (Mizue et al., 2005). To further demonstrate the role of this innate cytokine, anti-MIF antibodies and small molecular weight inhibitors have been utilised to block its activity. Administration of a MIF neutralising antibody mitigated the MIF-related induction of AHR in an OVA model in balb/c mice, but notably had no effect on the production of Th2 cytokines or IgE (Magalhães et al., 2007). The prototypical MIF antagonist ISO-1 could abrogate AHR and airway inflammation in balb/c mice challenged with HDM, along with demonstrating MIF's role in epithelial barrier dysfunction *in vitro* (Lan et al., 2020). In another model of OVA-induced allergic asthma in balb/c mice, a polyclonal anti-MIF antibody decreased the level of cellular infiltration in BALF from these mice, but failed to decrease Th2 cytokines or IgE. As MIF neutralising antibodies can induce the apoptosis of inflammatory cells, this may explain the decreased cell numbers observed in the BALF collected from these mice (Amano et al., 2007). The link between MIF and airway remodelling in C57BL/6 mice was elucidated by Li et al., (2021), where ISO-1 decreased

autophagy processes in smooth muscle cells, thus reducing the incidence of airway remodelling after OVA sensitisation. Importantly, these studies have been investigating murine sources of MIF. To further advance the translational knowledge around this clinically relevant cytokine, the role of human MIF must also be elucidated. This study is the first to use novel transgenic humanised MIF mice containing the human MIF gene controlled by the human $CATT_n$ promotor polymorphism to further investigate human MIF's role in a model of acute allergic lung inflammation.

MIF's role in many inflammatory conditions makes it an attractive candidate therapeutic target (Bilsborrow et al., 2019; Krammer et al., 2023; Luo et al., 2021; Thiele et al., 2022). Previous clinical trials have targeted MIF through the use of biologics such as anti-MIF antibodies, specifically Imalumab or BAX69 (NCT01765790) (Mahalingam et al., 2020). Moreover, small molecule inhibitors may be superior to anti-MIF antibodies, as they not only target MIF extracellularly, but can also have an inhibitory effect on intracellular MIF (Florez-Sampedro, Soto-Gamez, et al., 2020). Evidence shows that MIF's biological activity may be linked to its tautomerase activity. However, it is important to note that the precise mechanisms by which MIF exerts its biological effects are still not fully understood, and the relative contributions of its different functional domains (including the tautomerase active site) to these effects are an area of ongoing research. MIF's tautomerase activity is related to a unique catalytic site located within an N-terminal proline (Pro1) and surrounding hydrophobic pocket of MIF (Adamali et al., 2012; Bendrat et al., 1997; Lubetsky et al., 1999), making this pro-inflammatory cytokine unique. No physiological substrate for this catalytic site has been identified thus far, but it has been discovered that MIF can act as a catalyst to the tautomerisation of D-dopachrome (Sugimoto et al., 1999) and phenylpyruvate tautomerase (Rosengren et al., 1997). By binding to this active site, an area where the tautomerase active

site and the CD74 receptor functionally overlap, small molecule inhibitors induce conformational changes which indirectly inhibit MIF's function (Sinitski et al., 2019). Categories of these inhibitors include Chromen-4-one scaffolds [Orita-13 (2001)], Isoxazolines [ISO-1 (2002)], 1,2,3-triazoles [Jorgensen-3G or Jorgensen-3H (2010)], Covalent inhibitors [4-IPP (2008)], Isocoumarins [SCD-19 (2014)] or others such as those with a Benzoxazinone scaffold [NVS-2 (2008)] (Kok et al., 2018). In this study, the isocoumarin MIF inhibitor SCD-19 (3-(2-methylphenyl)-1H-isochromen-1-one) was used. The prototypical isooxazoline MIF inhibitor ISO-1 ((S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester) was also used (shown in supplementary figures).

This study is the first to use novel transgenic humanised MIF mice to investigate the role of the high expressing $CATT_7$ allele in driving allergic airway inflammation following house dust mite challenge. Using this unique model, the important role of endogenous human MIF expression on house dust mite-induced allergic airway inflammation was confirmed by utilising small molecular weight inhibitors *in vivo*.

2.3 Materials and Methods

2.3.1 Ethical Approval and HPRA Compliance

All procedures involving the use of animals were carried out by licensed personnel. Ethical approval for all work was granted by the ethics committee of Maynooth University (BRESC-2018-13). Project Authorisation was received from the HPRA (AE19124/P022), whereby the terms of the animal experiments within this project were outlined and adhered to.

2.3.2 Compliance with GMO and Safety Guidelines

All GMO/GMM work was performed according to approved standard operation procedures and recording protocols approved by the Environmental Protection Agency (Ireland). Safe working practices were employed throughout this study as documented in the Biology Department, Maynooth University Safety Manual.

2.3.3 Transgenic Humanised MIF Mice

A C57BL/6 mouse strain expressing the human high expressing MIF allele (*MIF*^{CATT7} [(C57BL/6NTac-Miftm3884.1(MIF)Tac-Tg(CAG-Flpe)2Arte] mice) was created using vector-based recombinant replacement of murine MIF by Taconic Biosciences (Rensselaer, NY). Validation of human but not murine MIF mRNA expression was verified by qPCR, and -794 CATT-length dependent stimulated MIF production was confirmed *in vivo* (Shin et al., 2022). These humanised MIF transgenic mice were kindly provided by our collaborator Prof. Seamas Donnelly (Trinity College Dublin, Ireland). All mice were housed according to the

HPRA SAP (Ireland) guidelines and used with ethical approval under the terms of AE19124/P022 project authorisation from HPRA.

2.3.4 Preclinical Model of Acute Allergic Airway Inflammation

$CATT_7$ and WT mice (6-18 weeks old) were challenged with 25µg of house dust mite allergen, *Dermatophagoides pteroyssinus*, with an endotoxin content of 9937.5 EU/vial (Greer Labs, Lenoir, USA) or PBS control intranasally (I.N.) under isoflurane anaesthesia, on days 0, 2, 4, 7, 9, 11, 14, 16, 18. After each challenge, mice were returned to their cage and monitored closely. An animal welfare score sheet was utilised throughout the study, where examining features included: weight loss, activity, general appearance and clinical signs.

2.3.5 MIF Inhibitor

SCD-19 (3-(2-methylphenyl)-1H-isochromen-1-one) (Specs.net, Netherlands), a small molecular weight inhibitor of macrophage migration inhibitory factor (MIF) biological activity was used in a house dust mite model of acute allergic asthma. 35mg/kg of SCD-19 or vehicle control (70% ethanol in PBS) was administered intraperitoneally (I.P.) twice weekly for three weeks; day 0, 4, 7, 11, 14, and 18. For dose analysis, SCD-19 was administered twice a week for one week (days 14 and 18).

2.3.6 Histological Analysis

On day 21 of the model, lungs were harvested, processed and sectioned. Tissue sections (5μm) were stained for Masson's Trichrome (Sigma-Aldrich), Periodic Acid Schiff (Abcam) and Haematoxylin and Eosin-Y (Richard Allan Scientific). Samples were air dried and a coverslip was mounted with DPX mounting media (BDH). 4X and 20X images were taken using an Olympus BX51 light microscope.

2.3.7 Histological Scoring

Following staining, slides were coded without reference to prior treatment and examined in a blind manner. For H&E, images were scored using a composite scale (1–9); comprising of infiltration or aggregation of inflammatory cells in air space or vessel wall [1 = only wall, 2 = few cells (1–5 cells) in air space, 3 = intermediate, 4 = severe (air space congested)]; interstitial congestion and hyaline membrane [formation: 1 = normal lung, 2 = moderate (<25% of lung section), 3 = intermediate (25–50% of lung section), 4 = severe (>50% of lung section)]; haemorrhage: (0 = absent, 1 = present) (Ehrentraut et al., 2013). For PAS, images were scored by counting the number of PAS positive (magenta) mucin-producing goblet cells present within the airway, relative to the diameter to the airway. For Masson's Trichrome, the percentage of subepithelial collagen was quantified using Fiji software with a Trainable Weka Segmentation plugin.

2.3.8 Bronchoalveolar lavage fluid (BALF) Retrieval

Mice were sacrificed by lethal overdose of sodium pentobarbital via I.P. injection on day 18 of the model, 4 hours after last challenge. A tracheostomy and cannulation was performed, where a 27 gauge cannula was secured in place with sutures. 1ml of cold endotoxin free PBS (Sigma) was infused into the lungs through the cannula using a 1ml syringe for 3 gentle instillations. BALF was kept on ice before being centrifuged at 300g for 5 minutes at 4°C. The supernatant was collected and 10X protease inhibitor solution (Roche) was added to prevent protein degradation. Samples were analysed immediately and were not freeze thawed.

2.3.9 BALF Cell Analysis

Cells were isolated from BALF via centrifugation and resuspended in 100µl of endotoxin-free PBS for counting. Cytospin funnels were pre-wet by spinning with 300µl of PBS onto glass slides at 600rpm for 5 minutes. $1x10^5$ cells in a volume of 300µl of PBS were spun onto fresh labelled glass slides at 600rpm for 10 minutes using a RotoFix 32 cytocentrifuge (Hettich Zentrifugen). Slides were airdried before being stained with Kwik-Diff™ Stain (Shandon, ThermoScientific); 25 seconds in fixative, 15 seconds in solution I (Eosin) and 15 seconds in solution II (Methylene blue). Slides were imaged on an Olympus BX51 light microscope until 300 cells could be counted. Cells were identified using the following morphological characteristics: neutrophils (nucleus containing 2-5 lobes), eosinophils (bi-lobed nucleus and cytoplasmic granules), macrophages (single nucleus, cell large in size) or lymphocytes (single nucleus, cell small in size). Cells populations were divided by the number of total cells counted and normalised to the volume of BALF collected.

2.3.10 Generation of L929 Conditioned media (M-CSF)

L929 cells were thawed, seeded in complete RPMI and incubated at 37°C/5% CO_2/20% O_2 for 7 days. Supernatant was collected and centrifuged at 300g for 5 minutes to remove cell debris. After being passed through a 0.2µm filter for sterilisation, conditioned media containing M-CSF was aliquoted and stored at -80°C.

2.3.11 Isolation of Bone Marrow-Derived Macrophages (BMDM)

Mice were humanely sacrificed using the cervical dislocation technique. Bone marrow was isolated from femur and tibia and centrifuged at 300g for 5 minutes. Red blood cells were lysed using 1X RBC lysis buffer (eBioscience) for 5 minutes. Cells were centrifuged at 300g for 5 minutes before being split in two T175 flasks per mouse. Cells were grown for 7 days in complete cRPMI supplemented with 20% L929 conditioned media. Supernatants were collected, centrifuged at 300g for 5 minutes and filter sterilised to remove cell debris. Aliquots were stored at -20°C

2.3.12 Isolation of Splenocytes

Mice were humanely sacrificed using the cervical dislocation technique. Spleens were collected and homogenised using a 70µM filter and a sterile syringe plunger. The filter was flushed with 10mls of cRPMI supplemented with 2mM of L-glutamine and 0.1% mercaptoethanol (Invitrogen-Gibco) and cells were centrifuged at 300g for 5 minutes. Red blood cells were lysed using 1X RBC lysis buffer (eBioscience) for 5 minutes. Cells were resuspended in cRPMI, counted and seeded at a density of $2x10^5$ cells/well of 96 well plate.

Supernatants were collected after 24hrs, centrifuged at 300g for 5 minutes and filter sterilised to remove cell debris. Aliquots were stored at -20°C.

2.3.13 Cytokine Analysis

BALF supernatants were analysed for murine Th2 cytokines IL-4 (Biolegend), IL-5 (Biolegend) and IL-13 (eBioscience) by ELISA. BALF, BMDM and splenocyte supernatants were analysed for human MIF (R&D Systems) by ELISA following the manufacturer's instructions.

2.3.14 Gene Expression

On day 18, 4 hours after last HDM challenge, a lung section was taken and snap frozen in liquid nitrogen to analyse gene expression. Tissue was homogenised with a D1000 handheld homogeniser (Sigma-Aldrich) and total RNA was extracted using TRIzol (Ambion Life Sciences) according to manufacturer's instructions. RNA concentrations were measured using a spectrophotometer (Nanodrop 2000, ThermoScientific, Wilmington DE, USA) and were equalised to 100ng/µl before cDNA synthesis. cDNA synthesis was performed by manufacturer's instructions (Quantobio cDNA synthesis kit). Real Time-Polymerase Chain Reaction (RT-PCR) was carried out using PerfeCta SYBR Green FastMix (Quantbio). Expression was quantified in relation to the housekeeper gene HPRT using the ΔCT method. The fold change in the relative gene expression was determined by calculating the $2^{-\Delta\Delta CT}$ values.

Table 1. Table containing forward and reverse primer sequences for species-specific target genes to analyse gene expression by RT-PCR

Gene name	Species	Sequence
hprt	Mouse	Fwd: AGGGATTTGAATCACGTTTG Rev: TTTACTGGCAACATCAACAG
mif	Mouse	Fwd: AATCCAGTCTTTTGATGCAG Rev: GCTACTCTCTCTAAGTCCAC
mif	Human	Fwd: CAGGGTCTACATCAACTATTAC Rev: TTATTTCTCCCCACCAGAAG

2.3.15 FlexiVent® Lung Function

Mice were anesthetised with 150mg/kg ketamine and 2mg/kg medetomidine via subcutaneous injection and the surgical plane of anaesthesia was reached. A tail vein catheter was inserted. Tracheostomy and cannulation was carried out and the subject was placed close to the FlexiVent® FX system (SCIREQ, Emka Technologies, Paris, France) and mechanical ventilation was initiated by selecting a predefined ventilation. Every 6 minutes, alfaxan and 0.5mg/kg atracurium besilate, a neuromuscular blocking agent (NMBA), was administered through the tail vail catheter to ensure passive breathing. The measurement of lung function was initiated and approximately 100µl of PBS or increasing concentrations of the bronchoconstrictor methacholine (MCh) (3.125, 12.5 and 25mg/ml) was loaded into the nebulizer. Upon completion of lung function measurements at baseline and following increasing aerosolised methacholine challenges, the ventilator was stopped and the mouse was euthanised using either I.P. injection of sodium pentobarbital or via cervical dislocation.

2.3.16 Statistical Methods

Data are presented as mean ± SEM. Results were analysed using a statistical software package (GraphPad Prism, San Diego, CA). A one-way ANOVA test was used to test for statistical significance of differences when multiple experimental groups were compared with an independent variable, whilst two-way ANOVA was used for two independent variables. A *post-hoc* Tukey's multiple comparison test was used to compare the means of different treatment groups.

2.4 Results

2.4.1 Humanised MIF mice express significantly higher levels of human MIF compared to WT control mice

Novel transgenic humanised MIF mice were generated to represent the varying levels of MIF expression under the functional promotor polymorphism within the human population. C57BL/6 mice were humanised by replacing the murine MIF gene with the human counterpart. Within this human MIF gene, 794 downstream of the promotor region, where the number of tetranucleotide repeats regulates MIF allele expression (Baugh et al., 2002), 7 repeats of this tetranucleotide sequence 'CATT' generated $CATT_7$ mice, containing the high expressing MIF allele (Figure 1A). To verify species-specific levels of MIF expression, cDNA was synthesised from lung tissue of these mice. WT mice expressed significantly higher levels of murine MIF compared to $CATT_7$ mice. Humanised $CATT_7$ MIF mice expressed significantly higher levels of human MIF compared to WT mice (Figure 1B).

To investigate the role of the human MIF polymorphism in a disease setting, a model of acute allergic airway inflammation was generated. The clinically relevant house dust mite (HDM) allergen was administered intranasally three times a week for three weeks to induce airway inflammation. To evaluate the role of human MIF in allergic asthma, a MIF antagonist SCD-19 or a vehicle control was administered intraperitoneally twice a week for three weeks (Figure 1C).

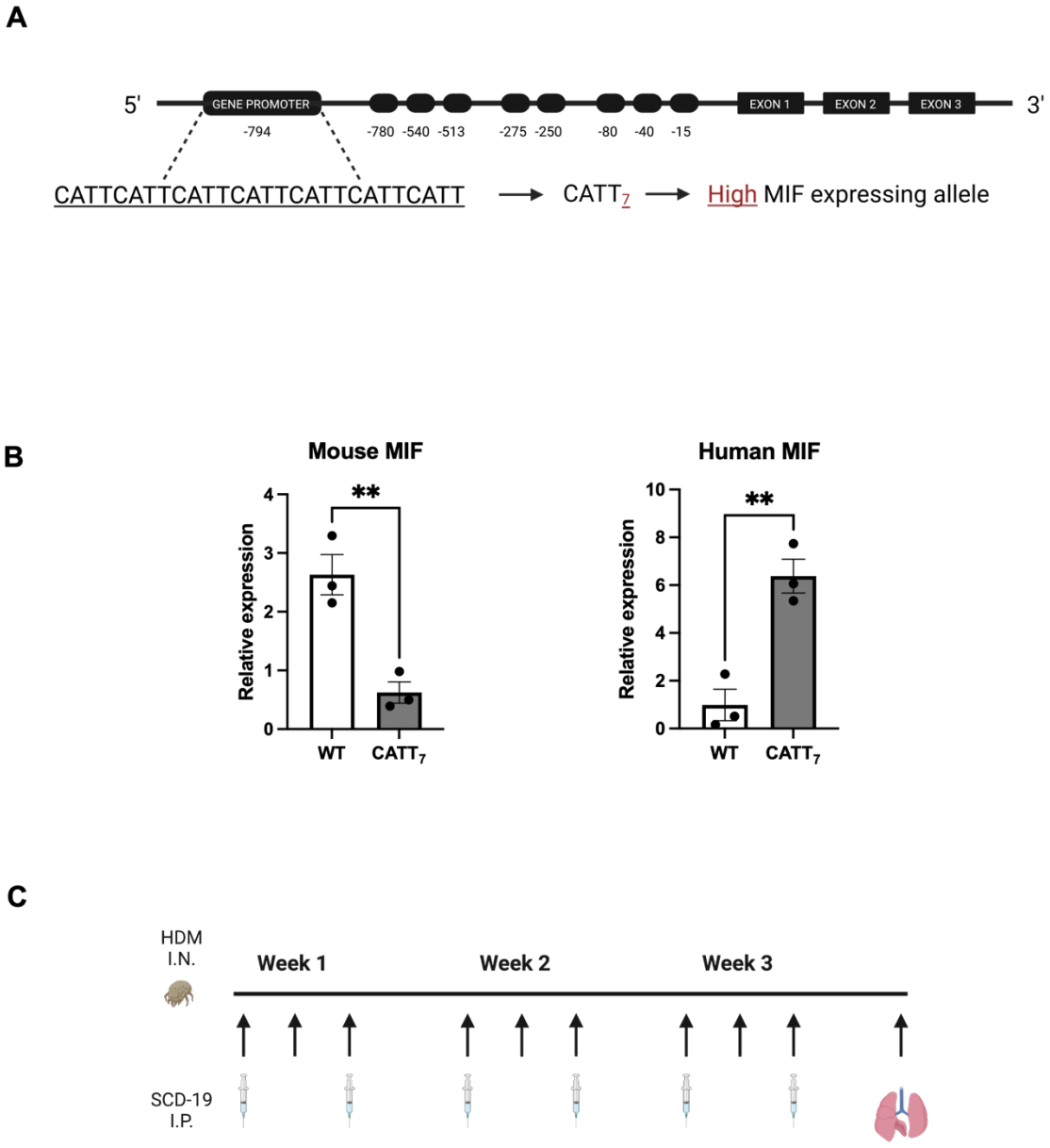


Figure 1. Schematic of polymorphism in transgenic humanised MIF mice and acute model of allergic airway inflammation. A Human MIF gene containing a functional polymorphism in position 794 downstream of the promotor region, where the number of tetranucleotide repeats controls promotor activity and MIF allele expression; 7 repeats = $CATT_7$ mice. **B** Relative expression of mouse and human MIF in bone marrow-derived macrophages from WT and $CATT_7$ mice respectively. Data are presented as mean ± SEM; N=3 per group **p<0.01. **C** Preclinical mouse model of acute allergic airway inflammation. Mice received 25µg of house dust mite or PBS control intranasally 3 times for 3 weeks. MIF inhibitor SCD-19 or vehicle control were administered intraperitoneally twice a week for full 3 weeks or final week only. BALF was collected on day 18, 4 hours after last challenge or lungs were taken for histology on day 21.

2.4.2 Functional MIF promotor polymorphism leads to high human MIF expression in basal and disease conditions

To investigate baseline levels of MIF production, bronchoalveolar lavage fluid (BALF), bone marrow-derived macrophages (BMDMs) and splenocytes were isolated from naïve WT and $CATT_7$ mice. Human MIF (hMIF) production was measured by ELISA. Naïve $CATT_7$ mice had significantly increased levels of hMIF protein in the BALF (Figure 2A), BMDMs (Figure 2B) and splenocytes (Figure 2C) compared to WT mice.

A model of acute allergic airway inflammation was generated as described (Figure 1C). On day 18, 4hrs after last challenge, BALF and the upper left lobe of lung tissue were collected to measure hMIF production under disease conditions. BALF from HDM-challenged $CATT_7$ mice had significantly increased levels of hMIF protein compared to WT mice (Figure 2D). At the mRNA level, HDM-challenged $CATT_7$ mice had significantly increased MIF gene expression, with a 45-fold increase compared WT mice (Figure 2E).

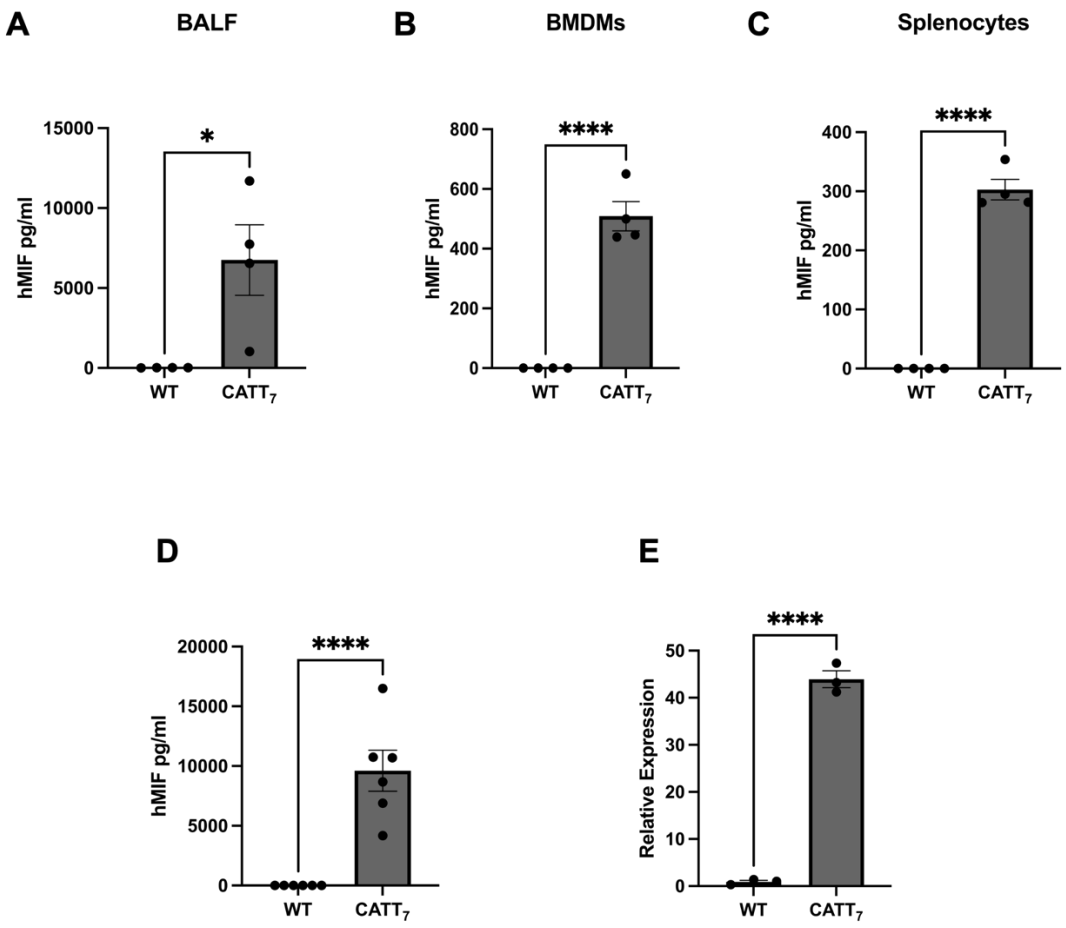


Figure 2. Functional $CATT_7$ polymorphisms leads to expression of hMIF under basal and disease conditions. A hMIF levels in bronchoalveolar lavage fluid of naïve WT and $CATT_7$ mice. **B** hMIF production from BMDMs of naïve WT and $CATT_7$ mice. **C** hMIF production by splenocytes from naïve WT and $CATT_7$ mice. **D** Levels of hMIF detected in the BAL fluid of WT and $CATT_7$ mice challenged with 25µg of HDM for 3 weeks. **E** Relative expression of hMIF detected in lung of WT and $CATT_7$ mice challenged with 25µg of HDM for 3 weeks, detected by real-time PCR. Data are presented as mean ± SEM; N=3-6 per group *p<0.05, ****p<0.0001. **(*)** *These data were generated as part of a shared project and appear in the thesis of Ian Hawthorne.*

2.4.3 Human $CATT_7$ allele significantly increases the Th2 signature in a HDM model of allergic airway inflammation

The infiltration of immune cells to the lung is a hallmark of acute airway inflammation (Lloyd, 2002; Synek et al., 1996). Thus, we first sought to measure the level of cellular infiltration in the BALF of WT and $CATT_7$ mice challenged with HDM or PBS control. BALF from HDM-challenged $CATT_7$ mice exhibited significantly elevated total cell counts compared to $CATT_7$ mice that received PBS control (Figure 3A). Notably, total cell counts from HDM-WT were not significantly elevated compared to PBS control WT mice (Figure 3A).

Our hypothesis was that the MIF functional promotor polymorphism plays a role in disease severity, specifically in a model of acute allergic airway inflammation. This was proven as BALF from HDM-challenged high MIF expressing-$CATT_7$ mice exhibited significantly elevated total cell counts compared to HDM-challenged WT mice (Figure 3A).

Allergic airway inflammation is commonly associated with a type 2 helper T cell profile, linked to eosinophilia (Lambrecht et al., 2019). Cell populations in the BALF were identified by carrying out differential cell counts and identifying infiltrated immune cells based on their morphology (Figure 3C). BALF from HDM-challenged $CATT_7$ mice exhibited significantly elevated numbers of eosinophils compared to $CATT_7$ mice that received PBS control (Figure 3B). As elevated Th2 cytokines are also a key player in allergic airway inflammation, we sought to measure IL-4, IL-5 and IL-13 in the BALF of $CATT_7$ and WT mice challenged with HDM or PBS control. As expected, BALF from HDM-$CATT_7$ mice had significantly higher levels of IL-4 (Figure 3D) and IL-13 (Figure 3F) compared to PBS controls. Levels of IL-5 in BALF from HDM-$CATT_7$ mice were not significantly different to levels detected in BALF of PBS $CATT_7$ mice (Figure 3E). MIF was a key driver of this Th2

signature, as BALF from the high MIF expressing $CATT_7$ mice had significantly higher levels of IL-4 and IL-13 compared to WT mice. No significant difference in Th2 cytokine production was noted between HDM-WT mice and WT mice that received a PBS control (Figure 3D, E, F).

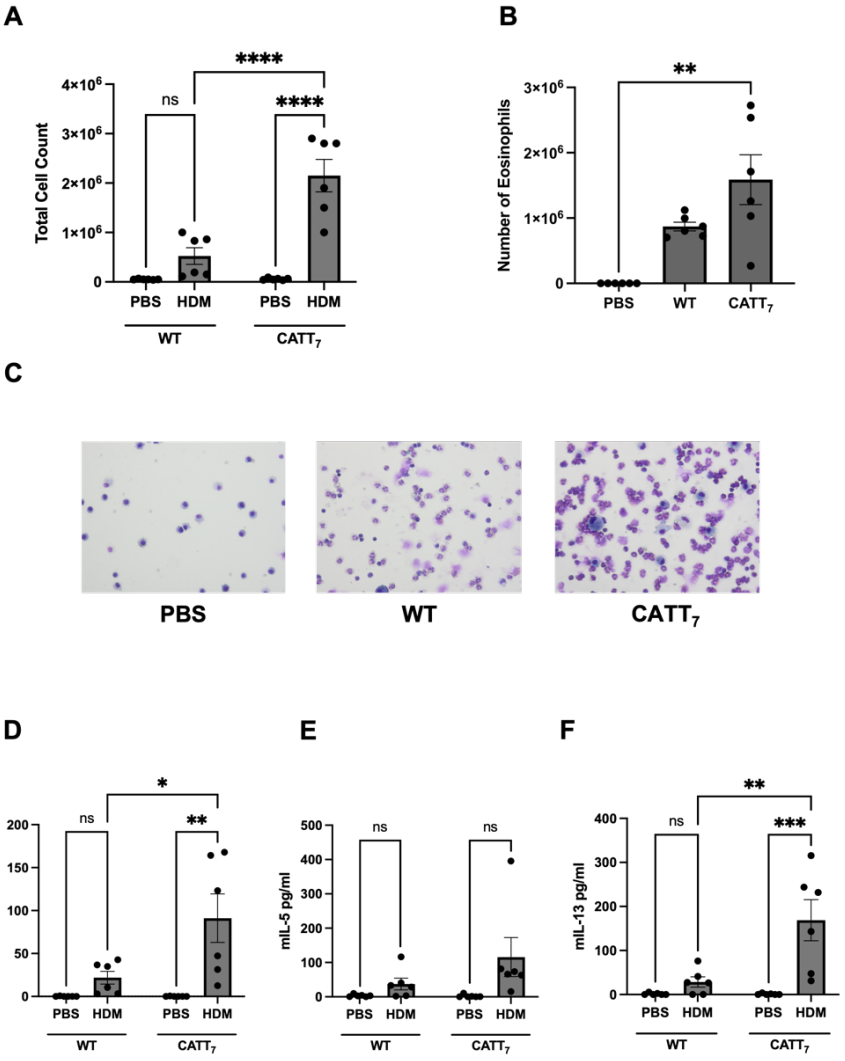


Figure 3. Human $CATT_7$ allele significantly increases the Th2 signature in a HDM model of allergic airway inflammation. A Total cell count recovered from BALF. **B** BALF eosinophil count determined by differential staining of cytospin slides. **C** 300 cells were counted and identified based on morphology. Cell numbers were normalised to the volume of BALF collected. Cytokine levels of **D** IL-4, **E** IL-5 and **F** IL-13 in the BALF determined by ELISA. Data are presented as mean ± SEM; N=6 per group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. **(*)** *Data in figures A, B, D, E and F were generated as part of a shared project and appear in the thesis of Ian Hawthorne.*

2.3.4 Human $CATT_7$ allele exacerbates airway inflammation in a house dust mite model of allergic asthma

In cases of allergic asthma, repeated exacerbations can result in a change in lung architecture, specifically characterised as an increase in goblet cell hyperplasia, subepithelial collagen and cellular infiltration, together resulting in airway remodelling (Hammad & Lambrecht, 2021).

Through histological analysis, it was found that repeated challenge of HDM in our model of airway inflammation also induced these hallmark alterations in lung structure. HDM-challenged mice expressing the human $CATT_7$ allele had significantly increased numbers of PAS positive goblet cells compared to $CATT_7$ PBS control mice (Figure 4A, B). Similarly, WT mice also had significantly increased numbers of PAS positive cells compared to their PBS counterparts (Figure 4A, B). High expression of human MIF was found to further exacerbate airway goblet cell hyperplasia, as HDM-$CATT_7$ mice had significantly increased numbers of PAS positive cells compared to WT mice (Figure 4A, B).

Subepithelial collagen deposition can be measured using Masson's Trichrome stain, an indirect marker of fibrosis. HDM challenge significantly increased the percentage of subepithelial collagen in $CATT_7$ and WT mice compared to PBS controls (Figure 4C, D). High expression of human MIF increased subepithelial collagen deposition, as HDM-$CATT_7$ mice had significantly increased percentage of subepithelial collagen compared to WT mice (Figure 4C, D).

A significant increase in cellular infiltration, specifically eosinophils was observed in the BALF of HDM-challenged $CATT_7$ mice compared to PBS controls (Figure 3A, B). Utilising H&E staining, this cellular infiltration was confirmed at a histological level. HDM-challenged $CATT_7$ and WT mice had a significantly higher H&E score compared to PBS controls (Figure 4E, F). High expression of human MIF elevated cellular lung infiltration, as HDM-$CATT_7$ mice had significantly elevated H&E scores when compared to WT mice (Figure 4E, F). Together the histological analysis for goblet cell hyperplasia, subepithelial collagen deposition and cellular infiltration highlight the pivotal role high human MIF expression has in driving disease severity in the context of allergic airway inflammation.

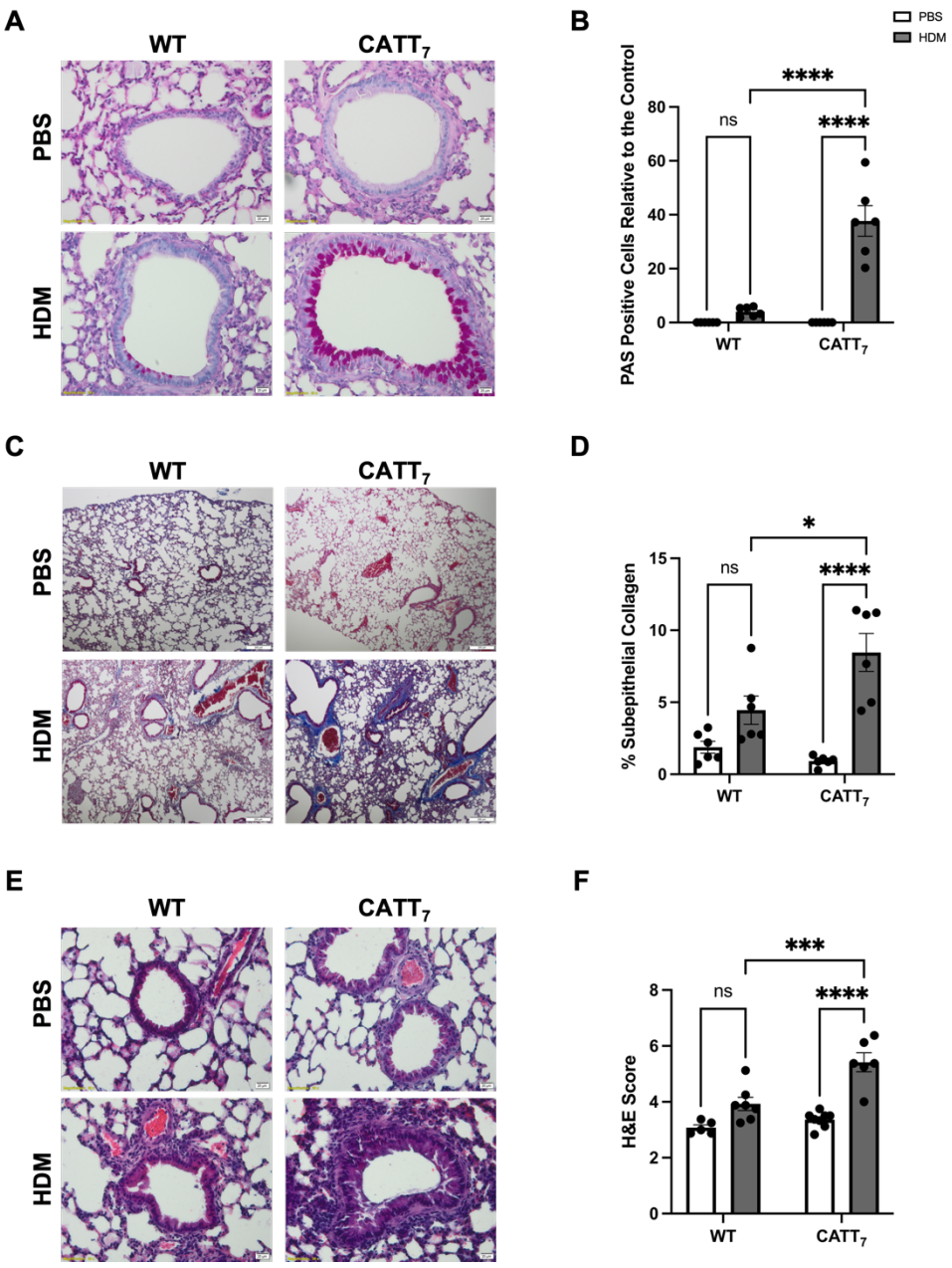


Figure 4. Human CATT$_7$ allele exacerbates airway inflammation in a house dust mite model of allergic asthma. A Representative images of lung tissue stained with Periodic Acid Schiff at 20X magnification, scale bar = 20 µm. **B** Goblet cell hyperplasia was investigated through the quantitation of PAS positive cells relative to the control. **C** Representative images of lung tissue stained with Masson's trichrome at 4X magnification, scale bar = 200 µm. **D** Quantitation of % subepithelial collagen. **E** Representative images of lung tissue stained with H&E from WT and CATT$_7$ mice challenged with HDM or PBS control at 20X magnification, scale bar = 20 µm. **F** Quantitation of airway inflammation in H&E-stained lung tissue. Data are presented as mean ± SEM; N=6 per group. *p<0.05, ***p<0.001, ****p<0.0001. **(*)** *Data in figures B, D and F were generated as part of a shared project and appear in the thesis of Ian Hawthorne.*

2.4.5 Human MIF alters HDM-induced lung mechanics in response to increasing concentrations of methacholine

The airway remodelling associated with allergic airway inflammation has a direct impact on lung function. Using the gold standard FlexiVent® instrument to measure lung function in response to increasing concentrations of the bronchoconstrictor methacholine, $CATT_7$ and WT mice exhibited increased levels of airway resistance (R_N) after repeated HDM challenge compared to PBS controls (Figure 5A). HDM-challenged $CATT_7$ mice that received 25mg/ml of nebulised methacholine had significantly increased airway resistance compared to HDM-challenged $CATT_7$ mice that received a PBS control. Moreover, HDM-challenged $CATT_7$ mice that received 25mg/ml of nebulised methacholine had a significantly elevated percentage airway resistance compared to PBS-challenged mice that also received 25mg/ml of nebulised methacholine (Figure 5A). Although HDM-challenged WT mice had increased airway resistance after methacholine, the percentage of airway resistance was not significantly different to that measured in PBS-challenged mice. The percentage change from baseline measurements of lung mechanics for tissue damping (G) (Figure 5B) and tissue elasticity (H) (Figure 5C) were also increased (not significantly) in HDM-challenged $CATT_7$ and WT mice compared to PBS controls, confirming that HDM challenge induced bronchoconstriction in the airways of these mice.

Our data have demonstrated the correlation between the high expression of human MIF and disease severity. Building on this narrative, we found that increased expression of human MIF exacerbates bronchoconstriction within the airways of these novel transgenic mice. HDM-challenged $CATT_7$ mice exhibited increased levels of airway resistance, tissue damping and tissue elasticity compared to WT mice, however this was no significant. This suggests that human MIF expression and lung bronchoconstriction are directly proportional.

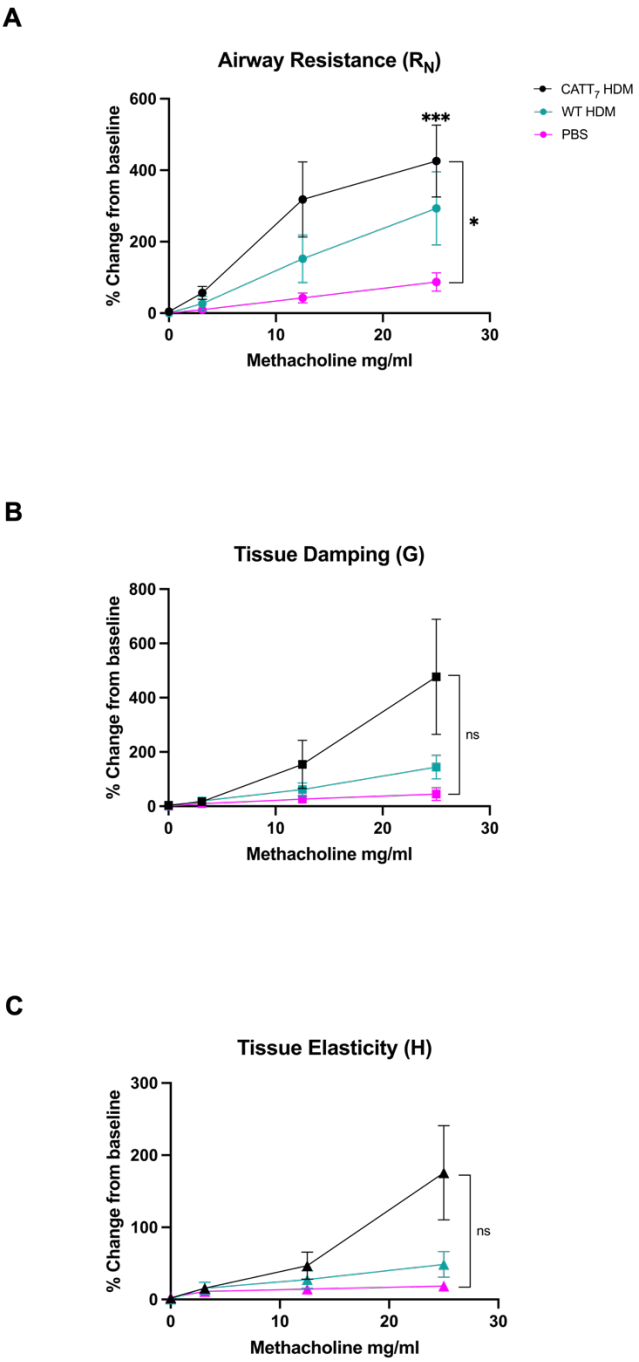


Figure 5. Human MIF increases HDM-induced airway resistance (R_N), tissue damping (G) and tissue elasticity (H) in CATT$_7$ mice. WT and CATT$_7$ mice were challenged with 25µg of HDM or PBS control I.N. three times a week for three weeks. 24hr after last challenge, a tracheostomy was performed, and lung function was measured using a FlexiVent® instrument (SCIREQ) in response to PBS or increasing concentrations of methacholine (3.125, 12.5 and 25mg/ml). **A** Airway Resistance. **B** Tissue Damping. **C** Tissue Elasticity. Data are presented as peak response normalized to the baseline and expressed as % increase over baseline N=6 per group. *p<0.05. **(*)** *These data were generated as part of a shared project and appear in the thesis of Ian Hawthorne.*

2.4.6 MIF Inhibitor SCD-19 decreases HDM-induced total cell counts and Th2 cytokine production in BALF from $CATT_7$ mice

High human MIF expression has been demonstrated to play a role in driving the physiological hallmarks of allergic airway inflammation such as eosinophil infiltration (Figure 3A, B), increased Th2 cytokines (Figure 3D, E, F), goblet cell hyperplasia (Figure 4A, B), subepithelial collagen deposition (Figure 4C, D) and decreased lung function (Figure 5).

MIF is known to initiate its biological effects through its active site (Mawhinney et al., 2014). Therefore, to fully elucidate MIF's role in our model, the MIF antagonist ISO-1 was initially used to block MIF's biological activity (Supplementary Figure 2, 3 and 4), however in light of new studies in the field of MIF inhibitors, questions about the specificity of ISO-1 arose (Ma et al., 2019). Thus, we decided to continue our studies with the highly potent MIF inhibitor SCD-19. SCD-19 acts by altering the conformation of MIF and thus impairs its interaction with other molecules. To investigate the efficacy of this small molecular weight inhibitor to decrease MIF production, MIF production was measured in supernatants generated from bone marrow-derived macrophages of $CATT_7$ mice, where SCD-19 significantly reduced MIF (Supplementary Figure 1).

HDM-challenged $CATT_7$ and WT mice received the MIF inhibitor SCD-19 or vehicle control intraperitoneally twice a week for three weeks and BALF was collected on day 18, 4hrs after last challenge. SCD-19 treated $CATT_7$ mice had a decreased total cell count compared to $CATT_7$ mice that received the vehicle control (Figure 6A). There was no significant difference between WT groups. Using differential cell counts and identifying immune cells by their morphology (Figure 6C), BALF from SCD-19 treated $CATT_7$ mice had decreased numbers of eosinophils compared to $CATT_7$ mice that received the vehicle control (Figure 6B). There was

no significant difference between WT groups. Although not statistically significant, there is a visible trend that when MIF's biological activity is inhibited by SCD-19, there is a decrease in total cellular infiltration in the BALF of SCD-19 treated $CATT_7$ mice compared to vehicle control mice.

Th2 cytokine levels in the BALF from HDM-challenged $CATT_7$ and WT mice that received SCD-19 or vehicle control were measured by ELISA. SCD-19 $CATT_7$ mice had significantly decreased levels of IL-13 compared to vehicle control $CATT_7$ mice (Figure 6F). SCD-19 $CATT_7$ mice had lower levels of IL-5 compared to vehicle control $CATT_7$ mice (Figure 6E). There was no significant difference between SCD-19 and vehicle control WT groups, which was expected as we have previously noted that WT mice already have low levels of MIF production. No SCD-19-specific differences were seen in IL-4 protein levels (Figure 6D).

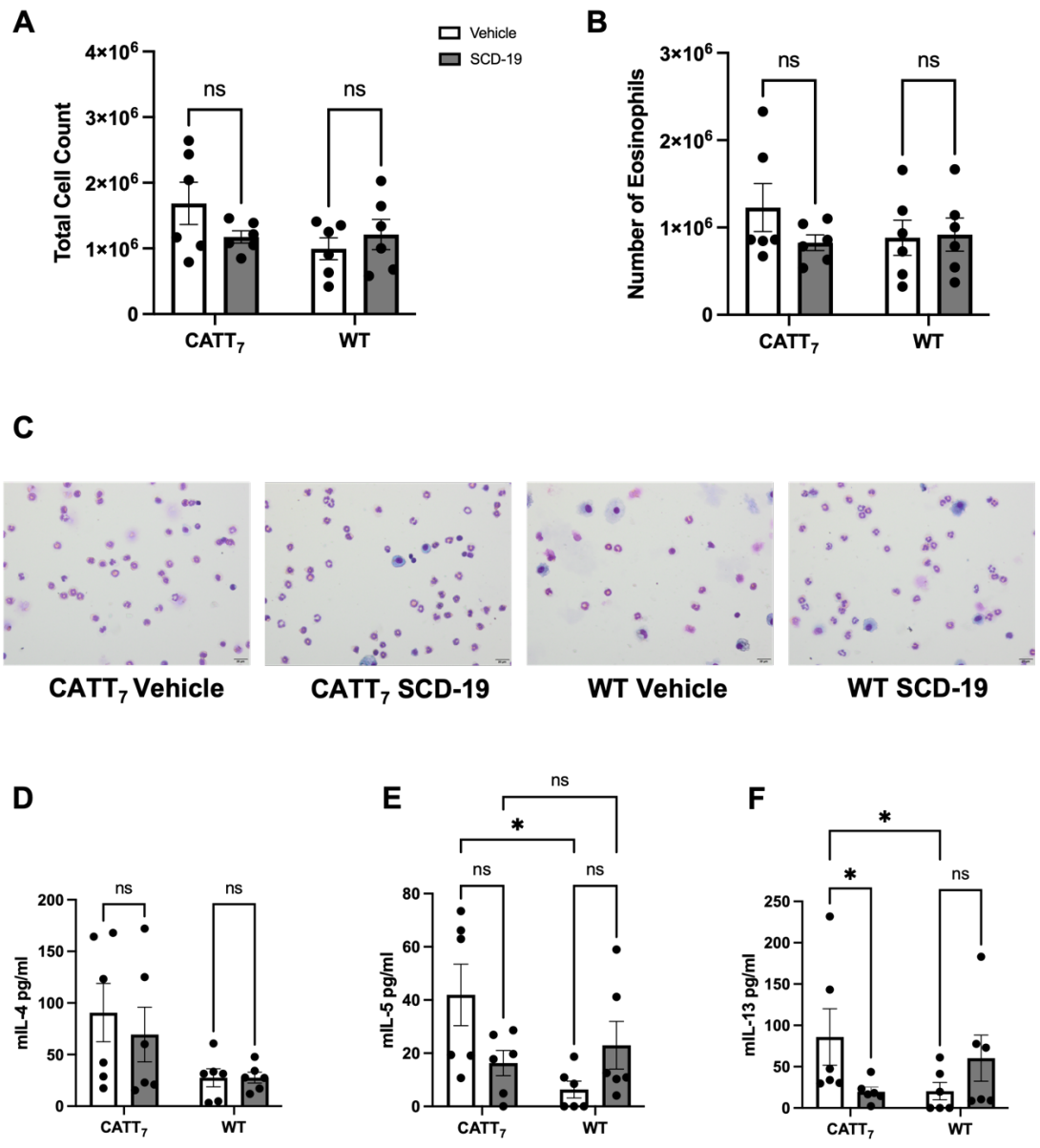


Figure 6. MIF inhibitor SCD-19 decreases HDM-induced total cell counts and Th2 cytokines in $CATT_7$ BALF . Mice were challenged with 25μg of HDM I.N. three times a week for three weeks, in addition to receiving 35mg/kg of SCD-19 or vehicle control I.P. twice weekly for three weeks. **A** Total cell count recovered from BALF. **B** Number of Eosinophils from differential cell counts of BALF from $CATT_7$ and WT mice . **C** 300 cells were counted and identified based on morphology. Cytokine levels of **D** IL-4, **E** IL-5 and **F** IL-13 in the BALF determined by ELISA. Data are presented as mean ± SEM; N=6 per group. *p<0.05.

2.4.7 SCD-19 significantly decreases HDM-induced lung pathology

High levels of human MIF in $CATT_7$ mice drives airway remodelling and inflammation following repeated exposure to the clinically relevant allergen HDM. Here we examined the capacity for a potent MIF inhibitor SCD-19 to prevent this exacerbation-related pathology.

$CATT_7$ and WT mice received SCD-19 at a dose of 35mg/kg or a vehicle control twice a week for three weeks while undergoing our house dust mite model of allergic asthma. PAS staining of goblet cells within the airways of these mice indicated that high levels of MIF was driving this significant increase in goblet cell hyperplasia, as SCD-19 significantly abrogated the number of PAS positive cells present compared to $CATT_7$ mice that received the vehicle control (Figure 7A, B). The vehicle control did not mediate off-target effect as the $CATT_7$ vehicle mice maintained significantly increased numbers of PAS positive cells compared to vehicle WT mice. As expected, there was no significant difference between WT mice that received SCD-19 and the vehicle control (Figure 7A, B). Lung sections from $CATT_7$ mice exhibited significantly increased levels of subepithelial collagen deposition after HDM challenge (Figure 4C, D). To investigate SCD-19's efficacy in halting the process of airway remodelling, lung sections were analysed using the Masson's Trichrome stain. In high human MIF expressing $CATT_7$ mice, SCD-19 significantly reduced the percentage of subepithelial collagen present after three weeks of intervention, compared to the vehicle control (Figure 7C, D). $CATT_7$ vehicle mouse lung sections had statistically significant increased levels of collagen deposition compared to WT vehicle mice. Moreover, SCD-19 had no effect in WT mice (Figure 7C, D). Lastly, lung sections were analysed for cellular infiltration using H&E staining. High expression of human MIF was proven to amplify cellular infiltration, as $CATT_7$ mice that received SCD-19 had a significantly lower H&E score compared to those that received the vehicle control (Figure 7E, F). $CATT_7$ vehicle mice had significantly higher H&E scores

compared to WT vehicle mice. Moreover, similarly to our previous histological findings, SCD-19 had no effect in WT mice (Figure 7E, F). These data support our hypothesis that MIF is a key factor in driving acute airway inflammation in our house dust mite model, as blocking the biological activity of MIF reduced lung inflammation. We next set out to elucidate if SCD-19's protective effects worked in a prophylactic or therapeutic manner. If prophylactic, the blocking of MIF activity would prevent the development or progression of HDM-induced pathology seen in our three week model. Conversely, if MIF inhibition worked in a therapeutic manner, it should have the ability to reverse or halt disease symptoms after the pathology has been established in the lung.

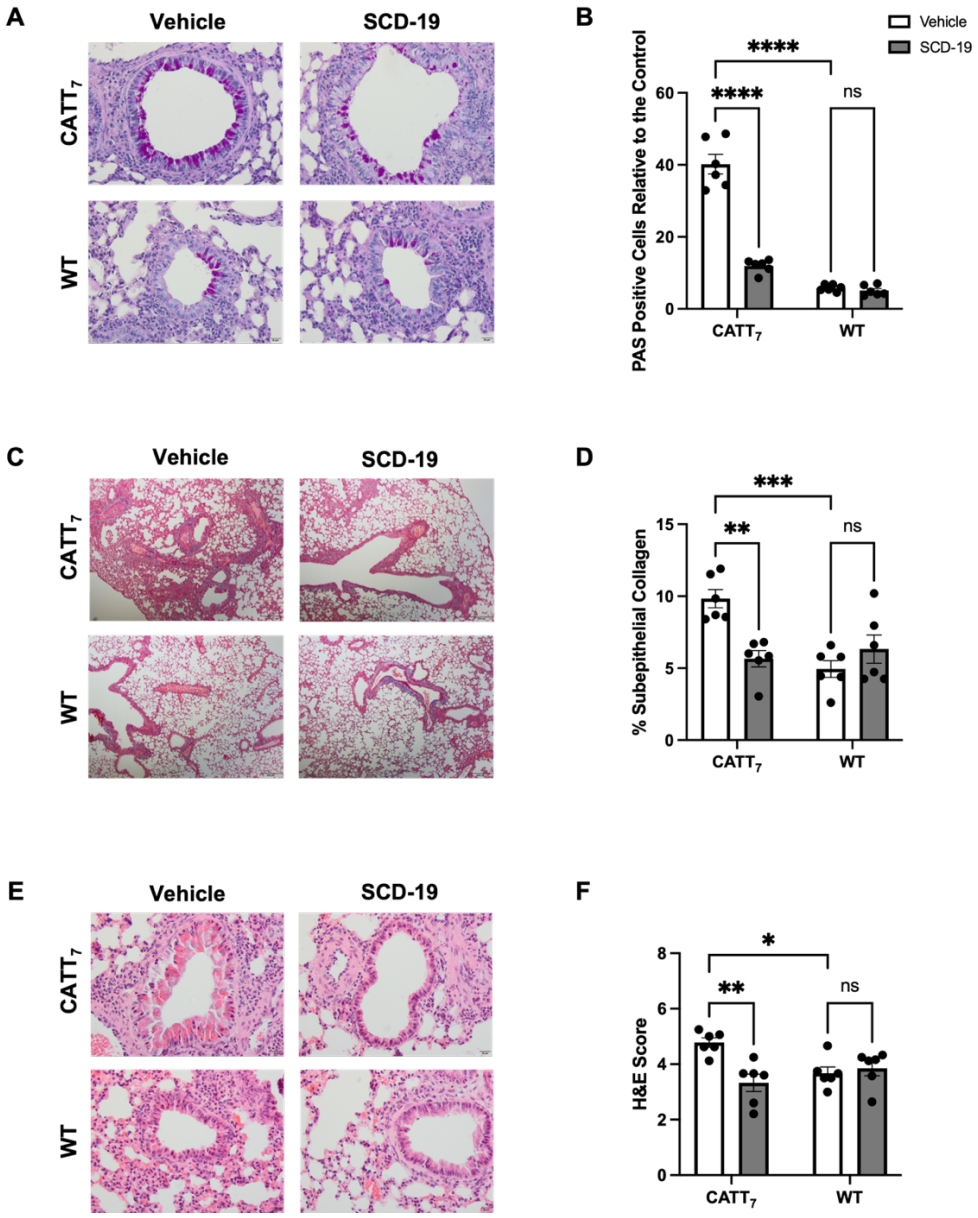


Figure 7. MIF antagonist SCD-19 significantly decreases HDM-induced allergic airway inflammation in CATT$_7$ mice. A Representative images of lung tissue stained with Periodic Acid Schiff at 20X magnification, scale bar = 20 µm. **B** Goblet cell hyperplasia was investigated through the quantitation of PAS positive cells relative to the control. **C** Representative images of lung tissue stained with Masson's trichrome at 4X magnification, scale bar = 200 µm. **D** Quantitation of % subepithelial collagen. **E** Representative images of lung tissue stained with H&E from WT and CATT$_7$ mice challenged with HDM or PBS control and received SCD-19 or vehicle control at 20X magnification, scale bar = 20 µm. **F** Quantitation of airway inflammation in H&E-stained lung tissue. Data are presented as mean ± SEM; N=6 per group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

2.4.8 MIF Inhibitor SCD-19 protects against HDM-induced allergic inflammation in high MIF transgenic mice in a prophylactic manner

In order to elucidate if SCD-19 was preventing the development of an asthmatic phenotype or if it had the ability to reverse established asthma-associated lung pathology, the timing and number of administrations of this MIF antagonist needed to be investigated further. Thus, SCD-19 or vehicle control was administered in week three alone (2 doses) of our 21 day model, before lung tissue was harvested for histopathological analysis. Our findings illustrated that when SCD-19 was only administered in the final week of our model, it no longer had the ability to significantly decrease the number of PAS positive cells in our $CATT_7$ mice (Figure 8A, B). Additionally, 2 doses of SCD-19 in the final week of this model failed to decrease the percentage of subepithelial collagen deposition (Figure 8C, D) or H&E score (Figure 8E, F) in $CATT_7$ mice. Interestingly, the timing and frequency of doses of SCD-19 was extremely important, as $CATT_7$ mice that received 6 doses versus $CATT_7$ mice that received 2 doses exhibited significantly difference results. $CATT_7$ mice that received 6 doses of SCD-19 over the full course of our three week model presented to have significantly lower numbers of PAS positive cells (Figure 8A, B), percentage subepithelial collagen (Figure 8C, D) and H&E score (Figure 8E, F) compared to $CATT_7$ mice that only received 2 doses of SCD-19 in the final week. This concludes that high expression of human MIF may act as a catalyst, augmenting and exacerbating the development of a Th2 response in this house dust mite-induced model of allergic airway inflammation.

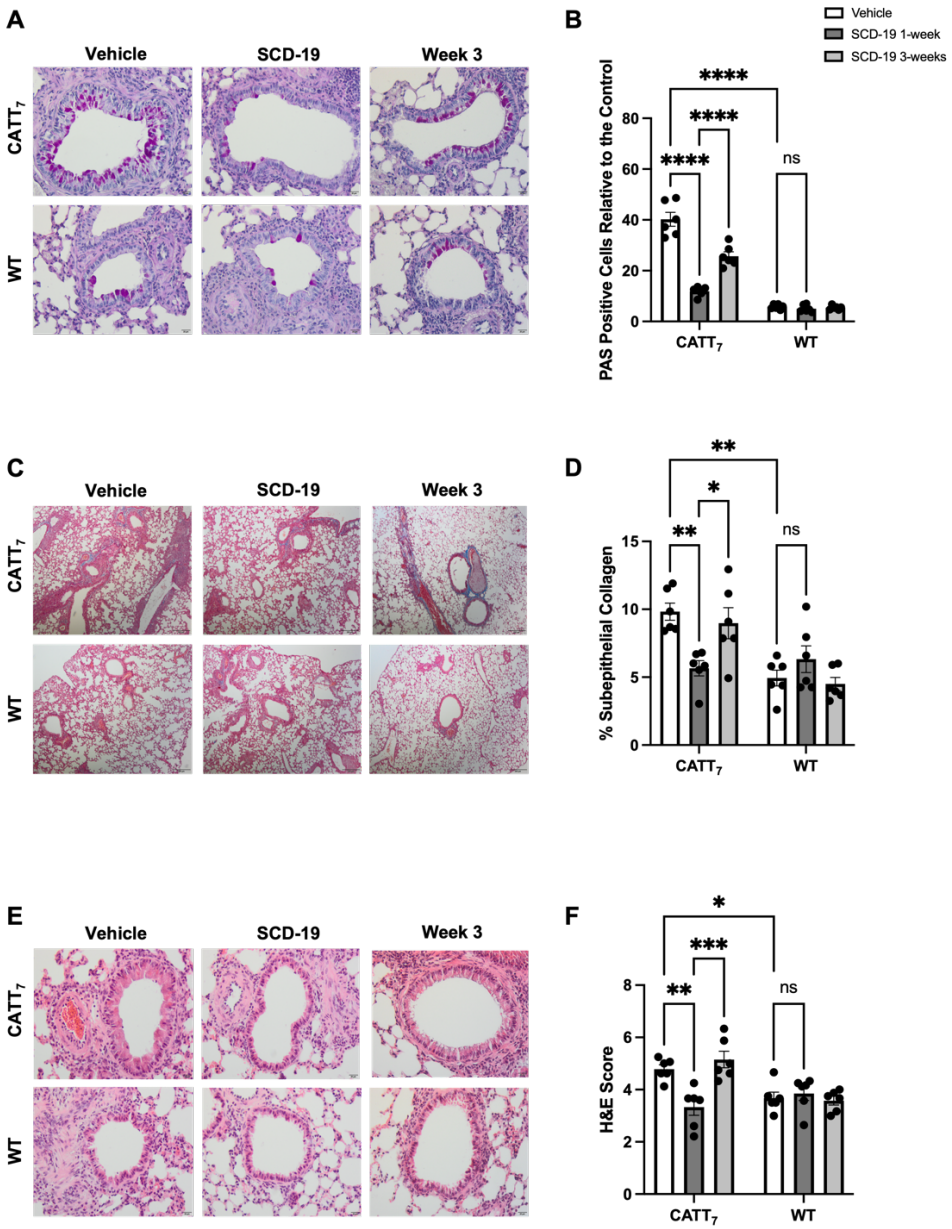


Figure 8. MIF antagonist SCD-19 works in a prophylactic, protective manner. Mice were challenged with 25µg of HDM I.N. three times a week for three weeks, in addition to receiving 35mg/kg of SCD-19 or vehicle control I.P. twice weekly for one or three weeks respectively. **A** Representative images of lung tissue stained with Periodic Acid Schiff at 20X magnification, scale bar = 20 µm. **B** Goblet cell hyperplasia was investigated through the quantitation of PAS positive cells relative to the control. **C** Representative images of lung tissue stained with Masson's trichrome at 4X magnification, scale bar = 200 µm. **D** Quantitation of % subepithelial collagen. **E** Representative images of lung tissue stained with H&E from WT and $CATT_7$ mice challenged with HDM or PBS control and received SCD-19 or vehicle control at 20X magnification, scale bar = 20 µm. **F** Quantitation of airway inflammation in H&E-stained lung tissue. Data are presented as mean ± SEM; N=5-6 per group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

2.4.9 SCD-19 improves lung function by decreasing airway resistance in $CATT_7$ mice in response to increasing concentrations of methacholine

We have previously shown that mice possessing the high human MIF expression allele ($CATT_7$) had increased airway resistance (Figure 5A), tissue damping (Figure 5B) and tissue elasticity (Figure 5C) after inhaling increasing concentrations of methacholine compared to WT mice.

To further investigate our hypothesis that human MIF is driving the development of preclinical signs of asthma in our mice, we measured the respiratory mechanics of mice that received the MIF inhibitor SCD-19 twice a week for three weeks, along with three weeks of HDM challenge. HDM-challenged $CATT_7$ vehicle mice that received methacholine at a dose of 25mg/ml displayed significantly increased airway resistance, damping and elasticity compared to HDM-challenged $CATT_7$ mice that received a PBS control (Figure 9A, B, C). In humanised $CATT_7$ mice, SCD-19 had the ability to decrease, albeit not significantly, HDM-induced inflammation at a mechanical level, by reducing the percentage of airway resistance (Figure 9A), tissue damping (Figure 9B) and tissue elasticity (Figure 9C) from baseline, compared to $CATT_7$ vehicle mice. To accurately determine the effects of SCD-19 on lung function in $CATT_7$ mice, increased numbers of subjects per experimental group are required. To reiterate, the vehicle control was seen to have no non-specific effects, as $CATT_7$ vehicle mice had resistance, damping and elasticity values equivalent to those of $CATT_7$ mice seen in figure 5. As WT mice did not show a severe Th2 asthmatic phenotype in this study up to this point, we decided to only measure the respiratory mechanics of the novel humanised $CATT_7$ mice, +/- SCD-19.

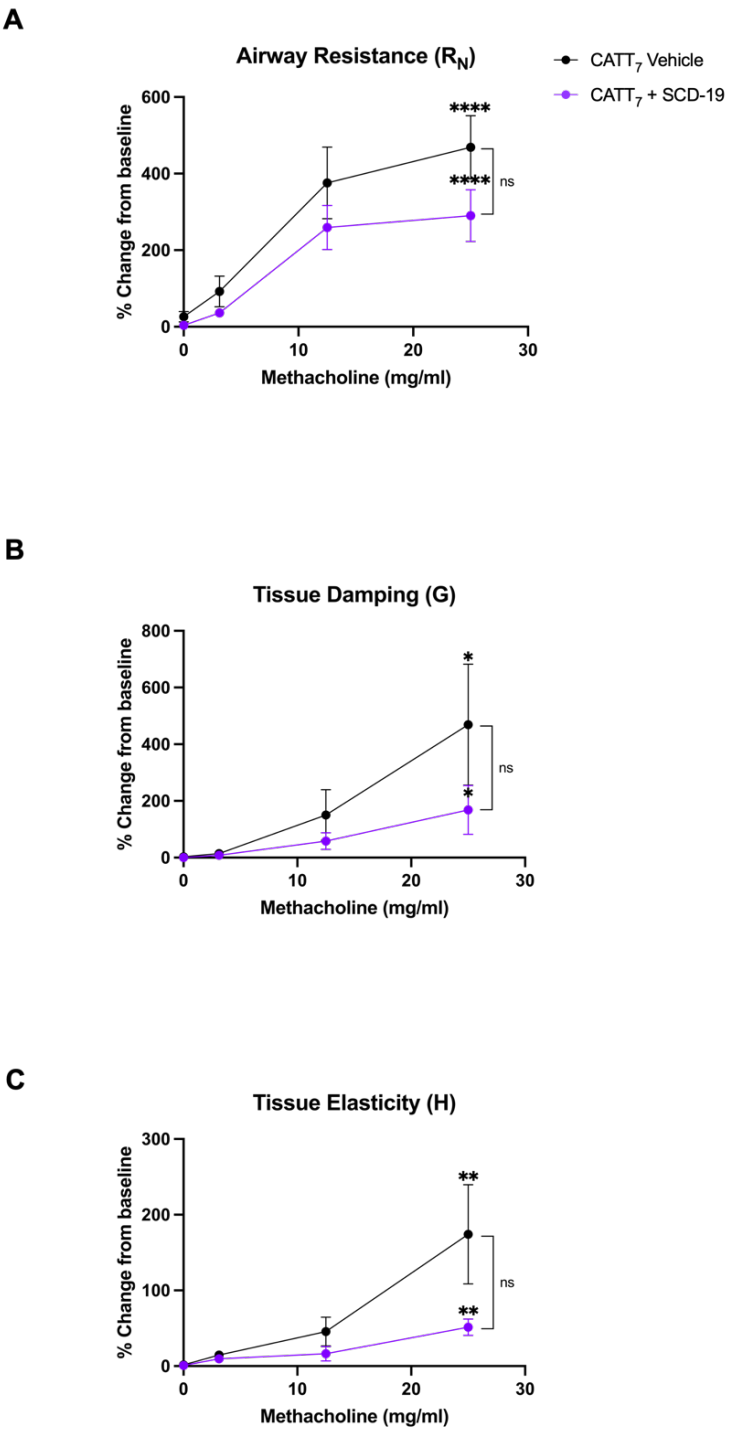


Figure 9. SCD-19 can decrease HDM-induced airway resistance (R_N), tissue damping (G) and tissue elasticity (H) in $CATT_7$ mice compared to vehicle control. $CATT_7$ mice were challenged with 25µg of HDM or PBS control I.N. three times a week for three weeks, in addition to receiving 35mg/kg of SCD-19 or vehicle control I.P. twice weekly for three weeks. 24hr after last challenge, a tracheostomy was performed, and lung function was measured using a FlexiVent® instrument (SCIREQ) in response to PBS or increasing concentrations of methacholine (3.125, 12.5 and 25mg/ml). **A** Airway Resistance. **B** Tissue Damping. **C** Tissue Elasticity. Data are presented as peak response normalized to the baseline and expressed as % increase over baseline. N=4-6 per group.

2.5 Discussion

The biological role of macrophage migration inhibitory factor (MIF) has been previously documented in a plethora of inflammatory lung conditions (Adamali et al., 2012; Florez-Sampedro, Brandsma, et al., 2020; Florez-Sampedro, Soto-Gamez, et al., 2020; Luo et al., 2021; Melotti et al., 2014; Plant et al., 2005, 2006; Shin et al., 2022; Smith et al., 2019), including asthma (Kobayashi, 2006; Li et al., 2021; Magalhães et al., 2007; Mizue et al., 2005; Rossi et al., 1998). The low human MIF expressing $CATT_5$ promotor polymorphism has proved to be linked to a milder manifestation of asthma symptoms (Mizue et al., 2005). We hypothesised that the $CATT_7$ promotor polymorphism which expresses high levels of human MIF could be linked to increased severity of allergic asthma. Using novel humanised MIF mice to create a physiological scale of allergic airway inflammation in response to the clinically relevant aeroallergen house dust mite, this study set out to investigate the biological role of this pro-inflammatory cytokine on key hallmarks associated with this atopic condition.

When comparing MIF production from naïve and HDM-challenged $CATT_7$ and WT mice, it was discovered that intranasal challenge can drive enhanced MIF production in the lung (Figure 2D, E), perhaps illustrating a positive feedback loop resulting in the exacerbations of physiological asthmatic characteristics. In a similar fashion, MIF is known to act in an autocrine and paracrine fashion to promote downstream cytokine production (Calandra et al., 1995; Das et al., 2013; Mitchell et al., 2002; Roger, David, et al., 2001).

Analysis of the bronchoalveolar lavage fluid from mice that received HDM demonstrated that MIF also facilitates the increase in the Th2, eosinophilic profile, as observed in $CATT_7$ mice compared to WT controls (Figure 3). This pleiotropic cytokine is expressed

constitutively by many immune cells, including T cells (Plant et al., 2006). Thus, depicting the role of MIF driving Th2-mediated antibody production.

MIF's crucial contribution in this model of house dust mite-induced allergic airway inflammation was particularly clear at a histological level when PAS and Masson's Trichrome stains showed a significant increase in mucin-producing goblet cell hyperplasia (Figure 4A, B) and subepithelial collagen deposition (Figure 4C, D) in the presence of high levels of human MIF in $CATT_7$ mice. Airway remodelling occurs in uncontrolled cases of asthma, as repeated lung injury by inhaled insults and over-production of fibrotic tissue results in goblet cell hyperplasia and increased subepithelial collagen, as illustrated in figure 4. This alteration in tissue architecture has consequences in the mechanical functioning of the lung, resulting in an increase in airway hyperresponsiveness, resistance, tissue damping and elasticity. After repeated HDM challenge to recapitulate day-to-day exacerbations, the gold standard FlexiVent® system was used to measure various parameters of respiratory mechanics. This system performs the forced oscillation technique, allowing comprehensive and reproducible assessment of lung function. Our findings further clarified the physiological role of MIF in HDM-induced airway inflammation, as humanised high MIF expressing $CATT_7$ mice had significantly increased airway resistance in response to increasing concentrations of the chemical bronchoconstrictor methacholine. Additionally, $CATT_7$ mice displayed increased tissue damping (energy dissipation into alveoli) and elastance (energy conservation in the alveoli) after methacholine challenge, however this was not significant (Figure 5).

To confirm the involvement of the MIF promotor polymorphism and thus high levels of endogenous MIF on the severity and pathogenesis of airway inflammation after HDM challenge, the use of a small molecular weight inhibitor SCD-19 was incorporated into our

model. Although previous studies have utilised anti-MIF antibodies and small molecule inhibitors in mouse models of inflammatory conditions (Amano et al., 2007; Chen et al., 2010; Lan et al., 2020; Luo et al., 2021), this is the first study to do so in humanised MIF mice expressing the relevant human $CATT_7$ MIF polymorphic allele.

In this study, SCD-19 significantly mitigated the MIF-associated increase in inflammatory histopathology, in a time-dependent manner (Figure 8), reinstating the importance of this cytokine in the induction/development and maintenance of asthma, as previously described (Chen et al., 2010). SCD-19 decreased the total cells (Figure 6A) and the number of eosinophils present in bronchoalveolar lavage fluid retrieved from these mice, compared to the mice that received the vehicle control (Figure 6B). However, these results were not statistically significant. SCD-19 had no effect on IL-4 production, but decreased IL-5, and significantly decreased IL-13 production in the BALF from $CATT_7$ mice compared to vehicle control. In line with our data, using an anti-MIF antibody did not affect IL-4 levels in BALF of OVA challenged mice (Magalhães et al., 2007), perhaps suggesting that IL-4 production is independent of MIF expression. Additionally, mice treated with this anti-MIF antibody demonstrated no change in IL-13 production, and IL-5 was not measured (Magalhães et al., 2007). Furthermore, anti-MIF treatment during OVA sensitisation significantly decreased eosinophil infiltration in balb/c mice (Magalhães et al., 2007). Conversely in C57BL/6 mice, MIF inhibition with SCD-19 did not decrease eosinophil infiltration in the BALF of HDM-challenged $CATT_7$ mice. Transgenic mice are routinely generated on a C57BL/6 background, which may be a limitation of this study, as Th2 atopic allergy models, like our HDM model of allergic airway inflammation, would generally be carried out in mice on a balb/c background. As a result, readouts from our study may have a lower baseline than those performed in balb/c mice. Furthermore, SCD-19 may not decrease eosinophil infiltration as efficiently as seen with

an anti-MIF antibody in an OVA model (Magalhães et al., 2007), as our Th2 response was not high enough at baseline levels to be further suppressed by SCD-19. Furthermore, the timing of MIF inhibitor administration is fundamental, as the use of ISO-1 in a model of severe neutrophilic asthma could only abrogate HDM-induced airway inflammation when administered both 30 mins prior and 6 hours after allergen challenge (Allam et al., 2022). Taking into account the SCD-19 dosing regimen, along with the suboptimal genetic background of the mice used, this may help to explain the incomplete effects of SCD-19 in this model. As previously mentioned, collagen deposition and goblet cell hyperplasia were seen to be significantly increased at this acute timepoint, but more HDM challenges might be required to see the full effects of chronic inflammation in our model. To conclude, the blocking of the MIF active site with SCD-19 from first allergen challenge extensively diminishes HDM-induced histopathology in $CATT_7$ mice (Figure 7), however no significant effect was noted when measuring inflammatory mediators in the BALF of these mice (Figure 6).

To investigate if SCD-19 treatment could improve lung function at a mechanical level, respiratory mechanics were measured after mice received 35mg/kg of SCD-19 or vehicle control twice a week for 3 weeks with repeated HDM challenges as described in figure 2C. SCD-19 reduced (although not significantly) the airway resistance (R_N), tissue damping (G) and tissue elasticity (H) in $CATT_7$ mice compared to the vehicle control, indicating an association between lower levels of tissue remodelling at a histological level (Figure 8) and overall lung function (Figure 9), however, increased n numbers would be required to definitively show this.

There are limitations to using small molecular weight MIF inhibitors as a therapeutic approach. This is associated with the lack of reproducibility in their associated IC50 values, as

most are less potent than initially predicted due to their binding to the active site in a time-dependent manner (Cisneros et al., 2016; Kok et al., 2018). In our study, SCD-19 was most efficacious when administered from first HDM challenge, in comparison to only being administered during the third week of HDM challenge. Albeit in a positive light, this category of MIF antagonists boast advantages over anti-MIF antibodies, including their easy uptake by host cells, lower manufacturing costs, potential oral or nasal administration (Doroudian et al., 2020) and most importantly, their ability to avoid triggering a reaction from host immunoglobulins (Chen et al., 2010; Kok et al., 2018; Mawhinney et al., 2014).

As previously mentioned, MIF is ubiquitously expressed throughout the body by a number of cells including epithelial and immune cells in healthy individuals, perhaps explaining its intracellular storage unless exposed to stimulation, protecting bodily homeostasis (Florez-Sampedro, Soto-Gamez, et al., 2020). As MIF is still expressed in healthy individuals, it cannot be utilised as a biomarker for inflammatory disease and may propose problems with specificity when using MIF antagonists as a therapeutic approach. Thiele et al., (2022) recently discussed an alternative approach; targeting the druggable isoform of MIF, known as oxidised MIF or 'oxMIF'. This conformational isoform differs as it is an immunologically distinct disease-related type of MIF that is found in the plasma and tissues of patients with inflammatory diseases, thus being a more specific therapeutic target. A monoclonal anti-oxMIF antibody known as Imalumab or BAX69 has been shown to target oxMIF in a preclinical/phase 1 clinical trial (NCT01765790). Furthermore, inhibitors of MIF's structural homolog D-Dopachrome Tautomerase (D-DT), also known as MIF2, should not be overlooked as inhibitors which also target this molecule, may have increased efficacy for MIF inhibition (Kok et al., 2018; Zhang et al., 2022). Importantly, both MIF inhibitors used in this study, SCD-19 and ISO-1 (supplementary figures) bind competitively to MIF's tautomerase/CD74 receptor active

site (Sinitski et al., 2019), proposing that it would not be useful to combine these antagonists as it is unlikely that they would work synergistically.

2.6 Conclusion

This novel study demonstrates the correlation between the number of tetranucleotide repeats ($CATT_n$) within the human MIF promotor region and the severity of airway inflammation after house dust mite challenge. Novel humanised MIF mice expressing the $CATT_7$ polymorphic allele exhibited significantly increased immune cell infiltration (eosinophils), production of inflammatory mediators (IL-4 and IL-13), goblet cell hyperplasia and percentage of subepithelial collagen deposition compared to WT controls. Together, these cardinal features of allergic asthma collectively result in airway remodelling and changes in lung architecture, leading to an increase in airway resistance, tissue damping and elasticity. Furthermore, administration of the MIF inhibitor SCD-19 effectively alleviated histological features exhibited in high MIF expressing $CATT_7$ mice after HDM challenge, however the administration of this small molecule inhibitor was not effective in decreasing Th2 inflammatory mediator production. Thus, this confirms MIF's role in driving HDM-induced histopathology, associated with the development of airway inflammation *in vivo*.

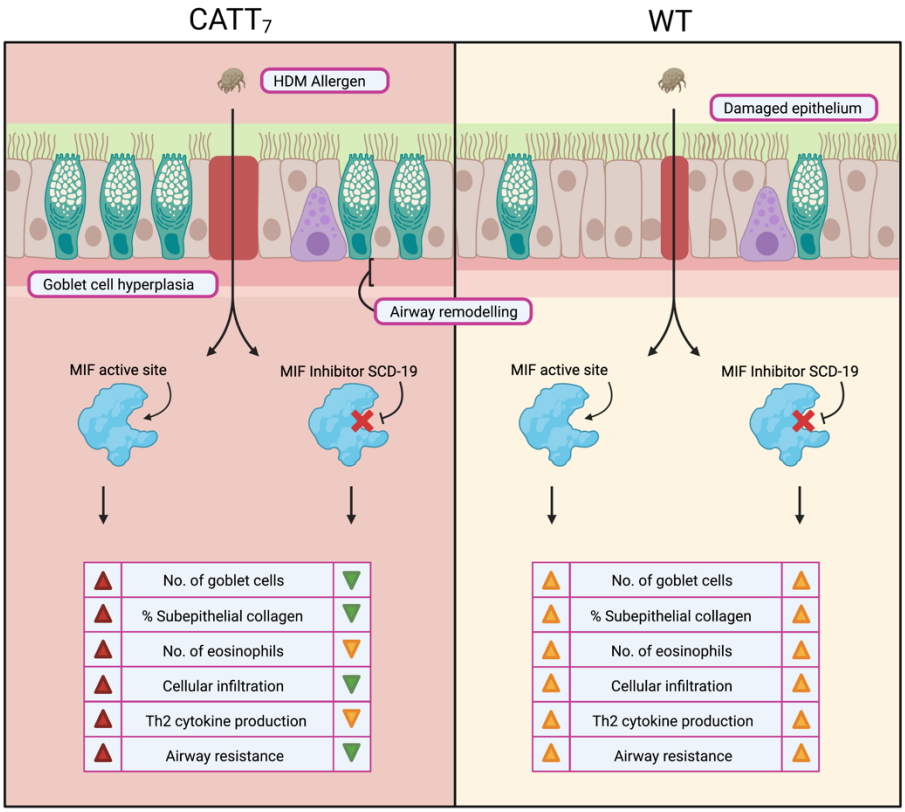
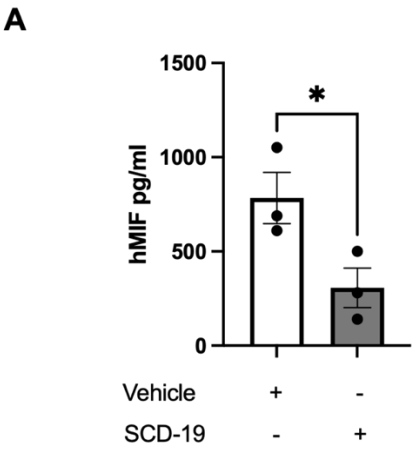
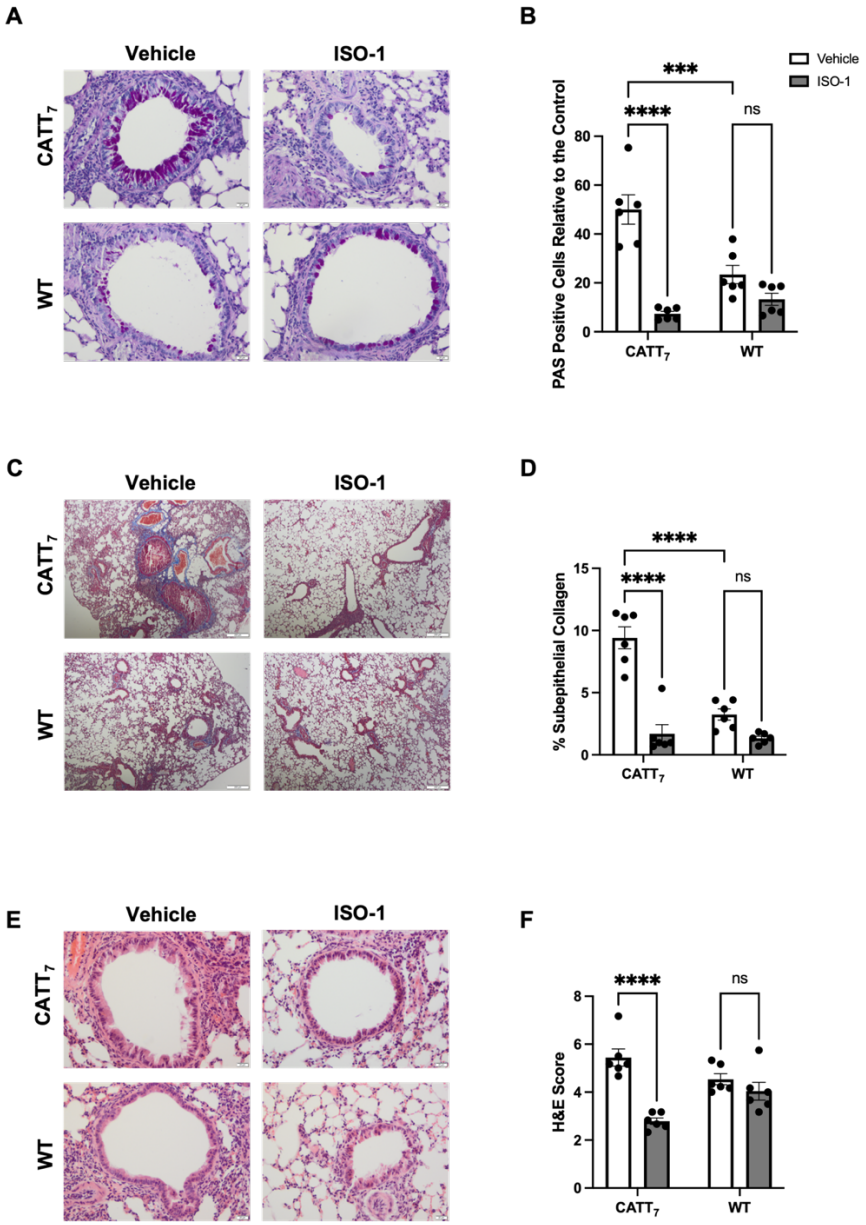


Figure 10. Schematic illustrating $CATT_7$ and WT proximal airway environment after house dust mite challenge. $CATT_7$ and WT mice received HDM challenge, which enters through the damaged epithelium resulting in an increase in goblet cell hyperplasia, airway remodelling, eosinophilia, cellular infiltration, Th2 cytokine production and airway resistance. High human MIF expressing $CATT_7$ mice present a more severe phenotype compared to WT mice. By blocking MIF's active site, MIF inhibitor SCD-19 decreases these hallmark signs in $CATT_7$ mice. No change is seen in WT mice.

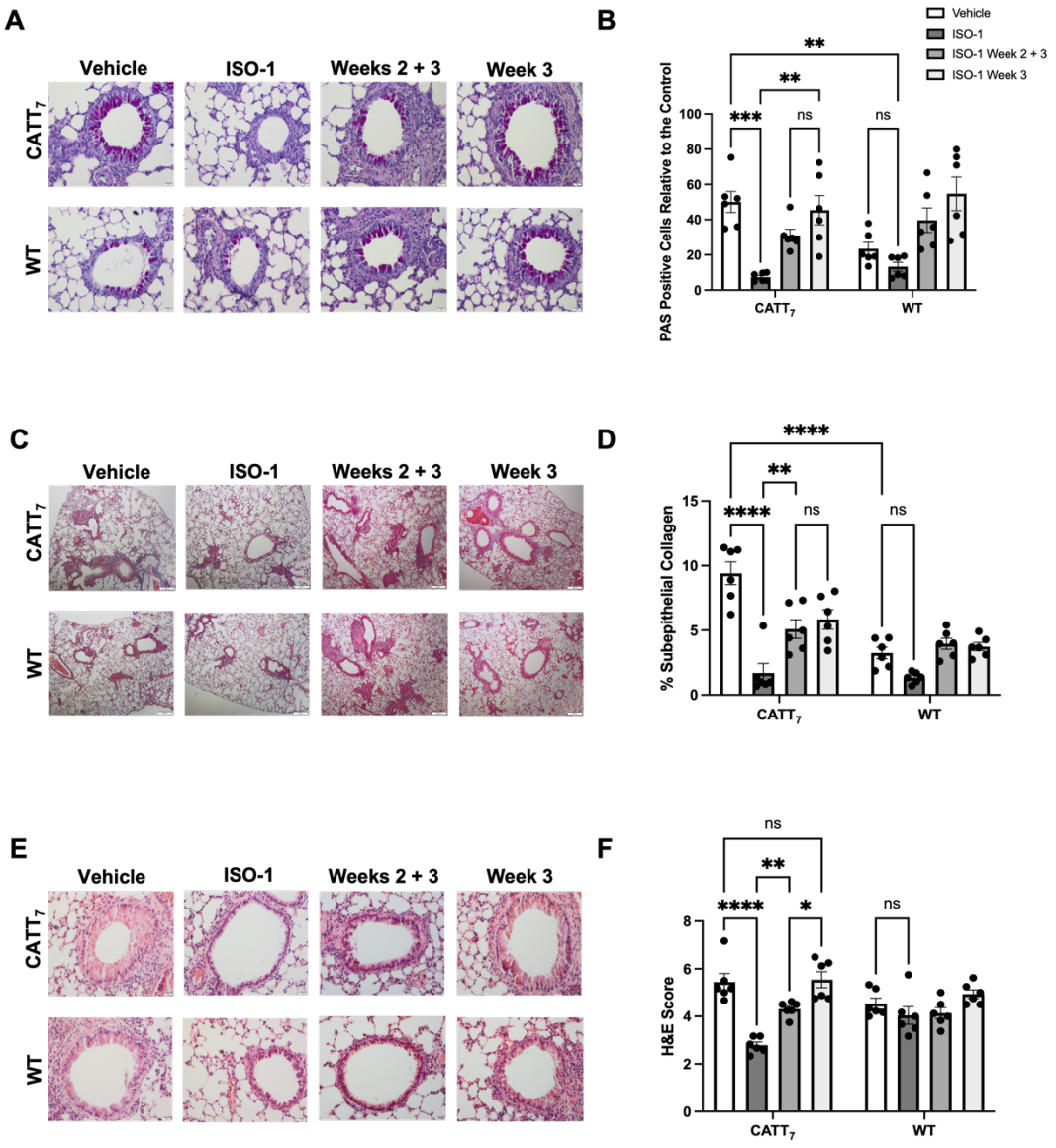
2.7 Supplementary Figures



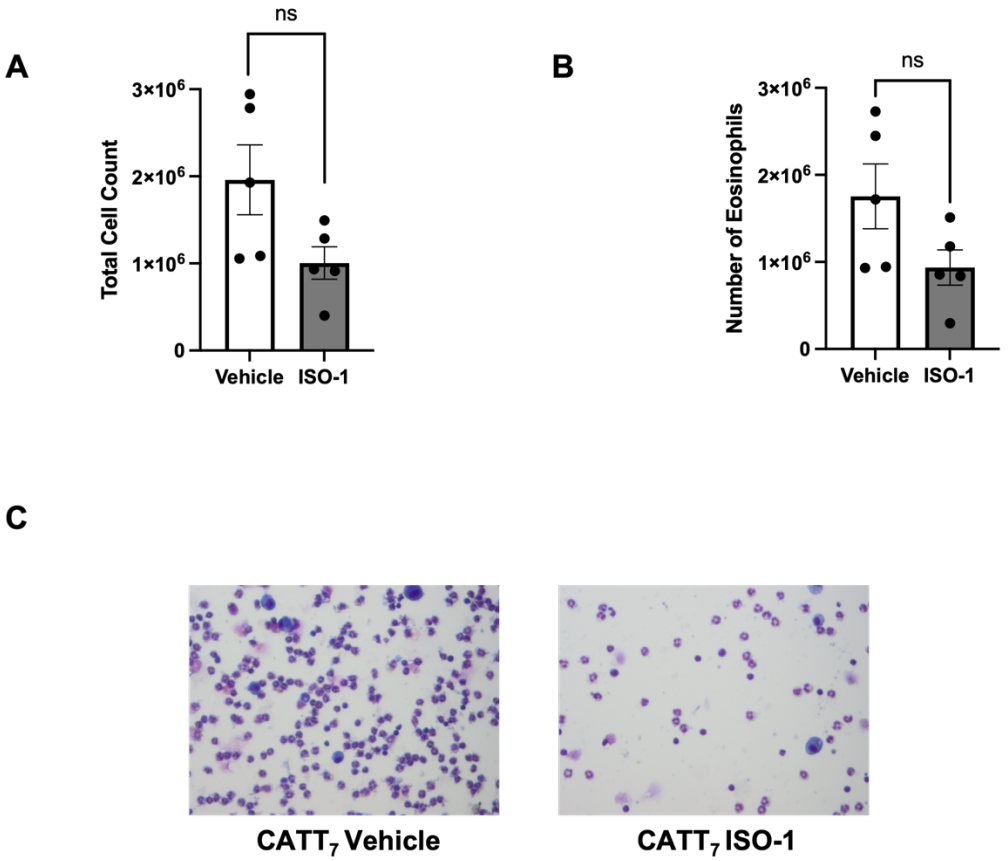
Supplementary Figure 1. MIF antagonist SCD-19 significantly decreases hMIF levels in $CATT_7$ mice. A hMIF production from BMDMs of $CATT_7$ mice that received 35mg/kg of SCD-19 or vehicle control I.P. twice weekly for three weeks. Data are presented as mean ± SEM; N=3 per group. *p<0.05.



Supplementary Figure 2. MIF antagonist ISO-1 ameliorates HDM-induced allergic airway inflammation in CATT$_7$ mice. Mice were challenged with 25µg of HDM I.N. three times a week for three weeks, in addition to receiving 35mg/kg of ISO-1 or vehicle control I.P. twice weekly for three weeks. **A** Representative images of lung tissue stained with Periodic Acid Schiff at 20X magnification, scale bar = 20 µm. **B** Goblet cell hyperplasia was investigated through the quantitation of PAS positive cells relative to the control. **C** Representative images of lung tissue stained with Masson's trichrome at 4X magnification, scale bar = 200 µm. **D** Quantitation of % subepithelial collagen. **E** Representative images of lung tissue stained with H&E from WT and CATT$_7$ mice challenged with HDM or PBS control at 20X magnification, scale bar = 20 µm. **F** Quantitation of airway inflammation in H&E-stained lung tissue. Data are presented as mean ± SEM; N=6 per group. ***p<0.001, ****p<0.0001.



Supplementary Figure 3. MIF antagonist ISO-1 works in a prophylactic, protective manner. Mice were challenged with 25µg of HDM I.N. three times a week for three weeks, in addition to receiving 35mg/kg of SCD-19 or vehicle control I.P. twice weekly for one, two or three weeks respectively. **A** Representative images of lung tissue stained with Periodic Acid Schiff at 20X magnification, scale bar = 20 µm. **B** Goblet cell hyperplasia was investigated through the quantitation of PAS positive cells relative to the control. **C** Representative images of lung tissue stained with Masson's trichrome at 4X magnification, scale bar = 200 µm. **D** Quantitation of % subepithelial collagen. **E** Representative images of lung tissue stained with H&E from WT and $CATT_7$ mice challenged with HDM or PBS control at 20X magnification, scale bar = 20 µm. **F** Quantitation of airway inflammation in H&E-stained lung tissue. Data are presented as mean ± SEM; N=5-6 per group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



Supplementary Figure 4. ISO-1 decreases total cell counts and number of eosinophils in BALF from CATT$_7$ mice. A Total cell count recovered from BAL fluid. **B** Number of Eosinophils from differential cell counts of BALF fluid from CATT$_7$ mice . **C** 300 cells were counted and identified based on morphology. Data are presented as mean ± SEM; N=5 per group.

Chapter 3:

The cytoprotective ability of the MIF-licensed MSC secretome in HDM-induced epithelial damage

3.1 Abstract

Licensed mesenchymal stromal cells (MSCs) and their cytoprotective therapeutic potential are increasing in clinical trials. Moreover, the soluble mediators released into their secretome boast cytoprotective properties equal to those associated with the cell itself, without cellular limitations or adverse effects. In asthma, epithelial barrier damage caused by the inhalation of allergens like house dust mite (HDM) drives type 2 inflammation and airway remodelling. Vascular endothelial growth factor (VEGF) plays a pivotal role in the repair and maintenance of airway epithelial integrity. This study illustrates the novel role of the pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) on MSC licensing, where endogenous MIF from bone marrow-derived macrophages from transgenic high human MIF expressing $CATT_7$ mice increased MSC production of VEGF into their secretome. $CATT_7$-MIF licensed MSC conditioned media containing increased levels of VEGF significantly enhanced bronchial epithelial wound healing and epithelial cell protection from HDM injury *in vitro*. To confirm the role of human MIF in MSC licensing and VEGF-mediated epithelial repair, a MIF antagonist SCD-19 was utilised.

3.2 Introduction

Mesenchymal stromal cells (MSCs) have gained notable interest as a cellular therapy (Mezey, 2022), with their application being tested in clinical trials to treat an array of inflammatory diseases, including lung conditions such as acute respiratory distress syndrome (Wang et al., 2023), pulmonary emphysema (Rodrigues et al., 2021; Weiss et al., 2021), COVID-19 (Shi, Huang, et al., 2021; Weiss et al., 2022), cystic fibrosis (Bonfield et al., 2023) and asthma (Melo et al., 2023; Yu et al., 2020). As of late July 2023, there are currently 55 clinical trials listed for mesenchymal stromal cells in lung disease, with 14 being active. Despite the increasing use of MSCs in clinical trials, their use as a therapeutic intervention in asthma is low, with only 4 clinical trials documented (search conducted on 20th July 2023 with terms: asthma, mesenchymal stromal cells, mesenchymal stem cells). As discrepancies in reported results are still an issue, different therapeutic avenues associated with these stromal cells are increasing in popularity. The soluble immunomodulatory and cytoprotective mediators which MSCs secrete are rapidly becoming an attractive cell-free alternative (Gunawardena et al., 2019; Szabłowska-Gadomska et al., 2023; Xia et al., 2019), harnessing the pro-reparative effects of these cells whilst minimising limitations associated with MSC adverse effects, such as embolism formation (Hackel et al., 2023; Moll et al., 2022).

MSCs are renowned for their cytoprotective abilities, which they can elicit through the production and secretion of soluble mediators in a paracrine fashion. The secretome is defined as products secreted by MSCs to facilitate the cell's communication with their surrounding microenvironment. In addition to soluble mediators, the MSC secretome also contains genetic components (miRNA (Li et al., 2018), mitochondrial DNA (Ahmad et al., 2014; Morrison et al., 2017; Yao et al., 2018)), lipids (Prostaglandin E2), extracellular vesicles (EV) (Cruz et al.,

2015; Park et al., 2019), metabolites (kynurenine) and cytokines (TNF and IL-6) (English, 2013; Szabłowska-Gadomska et al., 2023; Xia et al., 2019). Furthermore, newly emerging data outlines the importance of MSC-derived apoptotic bodies in their therapeutic efficacy, making the secretome their main mechanism of action (Liu et al., 2020; Műzes & Sipos, 2022; Pang et al., 2021; Tang et al., 2022; Weiss et al., 2019). Importantly for this study, MSCs are known to elicit their anti-apoptotic and pro-regenerative effects through the production of vascular endothelial growth factor (VEGF) (Ge et al., 2017; Han et al., 2022; Kozhukharova et al., 2022). Evidence of MSC's increased production of VEGF was illustrated when these cells were in direct contact of B cells, relating back to the effects of how the microenvironment of these cells can boost their cytoprotective efficacy (Healy et al., 2015).

In preclinical models of lung disease, conditioned media from MSCs (MSC CM) are proven to be as potent as their cellular counterpart (Cahill et al., 2015; Cruz et al., 2015; English et al., 2009; Kennelly et al., 2016). After lipopolysaccharide (LPS)-induced injury, conditioned media from human bone marrow-derived MSCs mitigated neutrophil influx and alternatively activated wound healing associated M2 alveolar macrophages, dampening lung injury in an IGF-1 dependent manner (Ionescu et al., 2012). Similarly, conditioned media from murine MSCs recovered lung fibroblasts from cigarette smoked-induced lung injury, illustrating the positive role of MSC secreted factors in facilitating epithelial regeneration (Cahill et al., 2015; Kennelly et al., 2016; Kim et al., 2012). More recent studies also follow this narrative, demonstrating the potent effects of MSC CM in preclinical lung disease (Kruk et al., 2021; Moreira et al., 2020; Su et al., 2019; Zhou et al., 2021).

Extensive literature illustrates the benefits of licensing MSCs prior to administration (Boland et al., 2018; Boyt et al., 2020; Carvalho et al., 2019; Cheng et al., 2021; Dunbar et al.,

2021; English, 2013; Hackel et al., 2023; Noronha et al., 2019), which can be carried out by modifying the environment in which these cells grow through exogenous stimulation, gene manipulation, or even the addition of chemical reagents. The asthmatic lung can act as a suitable environment for the activation of MSCs *in vivo*, as it contains a multitude of pro-inflammatory cytokines including macrophage migration inhibitory factor (MIF). MIF has not only been found at elevated levels in the bronchoalveolar lavage fluid of asthma patients (Mizue et al., 2005), but its level of expression has been linked to disease severity (Plant et al., 2005). Low expression of MIF is associated with a low number of repeats of the tetranucleotide sequence 'CATT', demonstrating the importance of MIF's functional promotor polymorphism (Baugh et al., 2002). Using novel humanised MIF mice expressing the 7-repeat allele termed $CATT_7$, we have shown that high expression of human MIF drives airway inflammation after house dust mite challenge (Dunbar and Hawthorne et al., 2023). Furthermore, MIF production can increase in response to infection or in the case of dysregulated immune systems associated with autoimmune or auto-inflammatory diseases such as asthma or sepsis (Vincent et al., 2018). As a result, this evidence elucidates the important role this pro-inflammatory cytokine can have in MSC licensing, increasing their therapeutic function within the asthmatic lung.

According to Altorki et al., (2019), a healthy lung displays both physical and chemical attributes which together act as a defence mechanism against external pathogens and thus, disease. However in chronic inflammatory diseases such as allergic asthma, these defence mechanisms may be imbalanced or compromised, making the lung susceptible to invasion by environmental particulates. In asthmatics, first described by Huber (1922), the repetitive mechanical exacerbations due to inhaled agents or non-specific stimuli can result in physical or biological injury of the airways and/or abnormal cycles of wound healing (Hsieh et al., 2023; Tatler, 2023; Varricchi et al., 2022), where epithelial cell apoptosis and damage can drive

further airway remodelling (Ke et al., 2019; Yu et al., 2020). HDM is known to increase epithelial cell apoptosis (Hoffman et al., 2013; Liu et al., 2021; Zeng et al., 2022). Furthermore, MSCs have illustrated efficacy in resolving damage inflicted by these repeated exacerbations by repairing endothelial barrier integrity (Pati et al., 2011) and increasing wound healing (Zhou et al., 2013).

This study sets out to investigate the use of the physiologically relevant pro-inflammatory cytokine MIF found in supernatants from novel transgenic high human MIF expressing mice to license human bone marrow-derived MSCs, where the secretome generated from these cells can efficiently promote wound healing and epithelial protection after HDM challenge *in vitro*.

3.3 Materials and Methods

3.3.1 Ethical Approval and HPRA Compliance

All procedures involving the use of animals were carried out by licensed personnel. Ethical approval for all work was granted by the ethics committee of Maynooth University (BRESC-2018-13). Project Authorisation was received from the HPRA (AE19124/P022), whereby the terms of the animal experiments within this project were outlined and adhered to.

3.3.2 Compliance with GMO and Safety Guidelines

All GMO/GMM work was performed according to approved standard operation procedures and recording protocols approved by the Environmental Protection Agency (Ireland). Safe working practices were employed throughout this study as documented in the Biology Department, Maynooth University Safety Manual.

3.3.3 Cell Culture

Human bone marrow-derived mesenchymal stromal cells (RoosterBio Frederick, MD 21703, United States) at passages 2-4 were cultured in complete low glucose DMEM (Sigma) in T175 vented flasks (Sarstedt). Human alveolar epithelial cells (A549) and human normal bronchial epithelial cells (BEAS-2B) were cultured in complete low glucose DMEM (Sigma) in T75 vented flasks. All cells were incubated at 37°C/5% CO_2/20% O_2.

3.3.4 Generation of L929 Conditioned media (M-CSF)

L929 cells were thawed, seeded in complete RPMI and incubated at 37°C/5% CO_2/20% O_2 for 7 days. Supernatant was collected and centrifuged at 300g for 5 minutes to remove cell debris. After being passed through a 0.2µm filter for sterilisation, conditioned media containing M-CSF was aliquoted and stored at -80°C.

3.3.5 Transgenic Humanised MIF Mice

A C57BL/6 mouse strain expressing the human high-expression $CATT_7$ MIF allele (M*IF*^{CATT7} [(C57BL/6NTac-Miftm3884.1(MIF)Tac-Tg(CAG-Flpe)2Arte] mice) was created using vector-based recombinant replacement of murine MIF by Taconic Biosciences (Rensselaer, NY). Validation of human but not murine MIF mRNA expression was verified by qPCR, and -794 CATT-length dependent stimulated MIF production was confirmed *in vivo* (Shin et al., 2022). These humanised MIF transgenic mice ($CATT_7$) were kindly provided by our collaborator Prof. Seamas Donnelly (Trinity College Dublin, Ireland), and $MIF^{--\backslash-}$ (MIF knockout) mice were a kind donation from Prof. Richard Bucala (Yale School of Medicine, Yale University, New Haven, CT, USA). All mice were housed according to the HPRA SAP (Ireland) guidelines and used with ethical approval under the terms of AE19124/P022 project authorisation from HPRA.

3.3.6 *In vivo* House Dust Mite Challenge

$CATT_7$ transgenic humanised MIF mice (C57BL/6NTac-Miftm3884.1(MIF)Tac-Tg(CAG-Flpe)2Arte, wildtype (WT) mice and $MIF^{-/-}$ mice (Trinity College Dublin, Ireland)

were anesthetised using isoflurane and challenged with intranasally (I.N.) with 25μg of *Dermatophagoides pteronyssinus* (endotoxin content of 9937.5 EU/vial) (Greer Laboratories Inc, Lenoir, NC, USA) on days 0, 2, 4, 7, 9, 11, 14, 16 and 18. An animal welfare score sheet was utilised throughout the study, where examining features included: weight loss, activity, general appearance and clinical signs.

3.3.7 Generation of $CATT_7$ MIF Conditioned Media

Mice were humanely sacrificed using the cervical dislocation technique on day 18, 4hr after last HDM challenge. Bone marrow was isolated from femur and tibia and centrifuged at 300g for 5 minutes. Red blood cells were lysed using 1X RBC lysis buffer (eBioscience) for 5 minutes. Cells were centrifuged at 300g for 5 minutes before being split in two T175 flasks per mouse. Cells were grown for 7 days in complete cRPMI supplemented with 20% L929 conditioned media. Supernatants were collected, centrifuged at 300g for 5 minutes and filter sterilised to remove cell debris. Aliquots were stored at -20°C. Aliquots were not freeze thawed. To account for variability of human MIF levels between $CATT_7$ mice and to ensure WT mice did not produce human MIF, supernatants were measured by human MIF ELISA (R&D) (Shown in Chapter 2, Figure 2D).

3.3.8 Generation of MSC Conditioned Media

Human bone marrow-derived mesenchymal stromal (hBM-MSC) cells were cultured as described. Documented concentrations of rhMIF (1, 100 or 400ng/ml) (Mawhinney et al., 2014; Tynan et al., 2017) or conditioned media generated by BMDMs from $CATT_7$, WT or $MIF^{-/-}$ mice was added with fresh cDMEM at a 1:1 ratio for 24hrs. Cells were washed with

warm PBS and serum free media was replaced. After 72hrs, supernatants were collected and centrifuged at 300g for 5 minutes to remove cell debris. Aliquots were stored at -20°C and were not freeze thawed repeatedly.

3.3.9 Generation of MIF Inhibited SCD-19 Conditioned Media

SCD-19 (3-(2-methylphenyl)-1H-isochromen-1-one) (Specs.net, Netherlands) was reconstituted in 70% ethanol and diluted in PBS to a working concentration of 100μM. SCD-19 was added to BMDM-derived $CATT_7$ and WT supernatant for 1hr in a shaking incubator at 37°C, before the supernatants were added at a 1:1 ratio into flasks containing human BM-MSCs.

3.3.10 Wound Healing Assay

The underside of a 6 well plate (Sarstedt) was scratched with 3 horizontal lines using a scalpel and a ruler to allow for accurate analysis. A549 or BEAS-2B cells were seeded out at a density of $1x10^5$/ml. When cells are 60-80% confluent, a single perpendicular vertical scratch was made with a sterile p200 tip. Wells were washed with warm PBS to remove cell debris. cDMEM and MIF-MSC conditioned media was added in a 1:1 ratio. On day 0, baseline measurements (100% open) were taken using Optika imaging software and Nikon imaging microscope. Plates were incubated 37°C/5% CO_2/20% O_2 for 48hrs, or until one scratch has sufficiently closed. Cells were fixed with 10% neutral buffered formalin (NBF) for 8 minutes, air dried and stained with crystal violet (Sigma) for 4 minutes. ImageJ software was used to measure the percentage wound closure of each image, relative to the 100% baseline measurements taken on day 0.

3.3.11 Use of VEGFR2 Inhibitor SU-5416

To investigate if VEGF was facilitating MSCs' ability to enhance wound closure in A549 and BEAS-2B epithelial cells, a VEGFR2 inhibitor SU-5416 (Tocris) was used. 10μM of SU-5416 or a DMSO vehicle control was added to A549 or BEAS-2B cells for 4 hours before the scratch was created and conditioned media was added.

3.3.12 Epithelial Survival Assay

BEAS-2B cells were grown as described. A dose curve of house dust mite (HDM) *Dermatophagoides pteronyssinus* (Greer Laboratories Inc, Lenoir, NC, USA) ranging from 25 to 200μg/ml was carried out, before continuing with a concentration of 100μg/ml. HDM was added to BEAS-2B for 24hrs, before the level of apoptotic cells was measured by flow cytometry. To investigate the effect of $CATT_7$ or WT-MSC conditioned media on BEAS-2B survival, cells were incubated with MIF-MSC CM for 2 hours prior to the addition of HDM.

3.3.13 Annexin V/PI Flow Cytometry

Adherent cells were detached using trypsin-EDTA (Sigma) and centrifuged at 400g for 6 minutes. Spent media from cells was also centrifuged to collect apoptotic cells. After washing cells with Annexin V binding buffer (eBioscience), 1μl of 2% rat serum was added to prevent non-specific staining and cells were incubated for 15 minutes. Cells were stained with 1μl of Annexin V for 45 minutes in the dark at 4°C. Once washed in cold FACS buffer and centrifuged, cells were stained with 1μl of PI immediately before being analysed on an Accuri C6 flow cytometer.

3.3.14 VEGF ELISA

MIF-licensed MSC supernatants were collected and centrifuged at 300g for 5 minutes to remove debris, before being stored at -20°C. ELISAs were carried out according to manufacturer's instructions (R&D Systems).

3.3.15 Statistical Methods

Data are presented as mean ± SEM. Results were analysed using a statistical software package (GraphPad Prism, San Diego, CA). One-way ANOVA Multiple Tukey comparison test was used to test for statistical significance of differences when multiple experimental groups were compared with an independent variable.

3.4 Results

3.4.1 Conditioned media from rhMIF-licensed MSCs increases alveolar epithelial cell wound closure

MSCs need to be activated to boost their cytoprotective effects through the production of soluble mediators (Lynch et al., 2020; Silva et al., 2018). MSCs were licensed with different concentrations of recombinant human MIF (rhMIF) (1, 100 or 400ng/ml) (Figure 1A). As VEGF, an important soluble factor secreted by MSCs (Arutyunyan et al., 2016; Ge et al., 2017; Healy et al., 2015) is a trophic factor known to play a role in wound healing (Johnson & Wilgus, 2014), human VEGF levels in rhMIF-MSC CM supernatants were measured by ELISA. MSCs stimulated with all concentrations of rhMIF exhibited significantly elevated levels of human VEGF (hVEGF) in a dose dependent manner. MSCs licensed with 100 and 400ng/ml rhMIF secreted the highest hVEGF protein levels, with no statistical difference between the two concentrations (Figure 1B). Thus, 100ng/ml of rhMIF was used to license MSCs for the remainder of the study.

To investigate the impact of MSC CM +/- rhMIF on epithelial wound healing, alveolar basal epithelial cells were grown to 80% confluency before a vertical scratch was created. rhMIF-MSC CM, where MSCs were licensed with 0 (MSC CM), 1 or 100ng/ml of rhMIF, was added to wells with equal volume of cDMEM. After 48hrs, wells were fixed and stained with crystal violet prior to imaging (Figure 1D). MSC CM, 1ng/ml rhMIF-MSC CM and 100ng/ml rhMIF-MSC CM all significantly increased the percentage wound closure in A549 cells (Figure 1C). Importantly, MSCs activated with rhMIF significantly elevated the percentage wound closure in a dose dependent manner compared to MSC CM, with 100ng/ml rhMIF-MSC CM displaying the highest level of wound closure (Figure 1C).

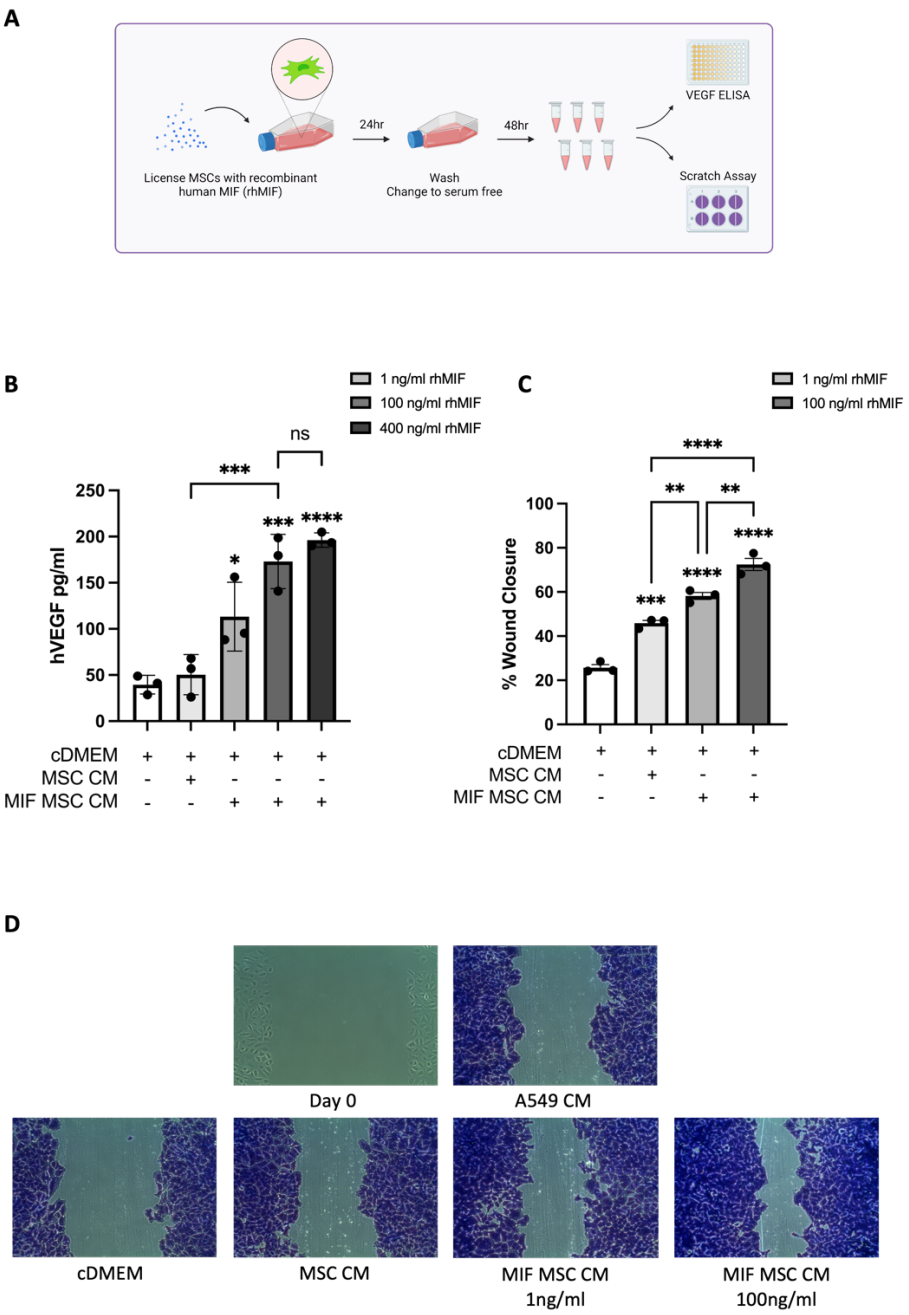


Figure 1. rhMIF licensing increases VEGF in MSC conditioned media, enhancing wound healing in human alveolar basal epithelial cells. A Schematic depicting the generation of rhMIF MSC conditioned media. **B** Human VEGF protein levels in MSC conditioned media supernatants, measured by ELISA, after licensing with different concentrations of recombinant human MIF (1, 100 and 400ng/ml). **C** Percentage wound closure relative to the control in alveolar basal epithelial cells (A549s) after stimulation with rhMIF MSC CM. **D** Images of A549 wound closure after crystal violet stain. Data are presented as mean ± SEM; images are representative of 3 independent experiments, n=3 per group **p<0.01, ***p<0.001, ****p<0.0001.

3.4.2 rhMIF licensing accelerates MSC-mediated wound closure in a VEGF-dependent manner

The role of VEGF in wound healing is established in a variety of different conditions (DiPietro, 2016; Johnson & Wilgus, 2014; Shams et al., 2022), such as type 1 diabetes (White et al., 2021) and pulmonary fibrosis (Murray et al., 2017). Our hypothesis is that the increased levels of VEGF observed in conditioned media from rhMIF-licensed MSCs (Figure 1B) was driving alveolar epithelial cell wound closure. To investigate this further, a potent and specific VEGFR2 inhibitor, SU-5416 was used to block the VEGF receptor on the surface of epithelial cells prior to the addition of rhMIF-MSC CM (Figure 2A). Our findings illustrate that by blocking the VEGFR2 on the A549s, rhMIF-MSC CM containing hVEGF could no longer significantly enhance wound closure (Figure 2B). A549s that received the vehicle control maintained the significant increase in percentage wound closure mediated by rhMIF-MSC CM. Interestingly, the application of SU-5416 had no effect on A549s grown in conditioned media from unlicensed MSCs, again illustrating the important role of rhMIF licensing on MSC's cytoprotective abilities (Figure 2B).

To prove that conditioned media from another cell type could not facilitate enhanced wound closure in alveolar epithelial cells, or that a positive VEGF feedback loop in A549 conditioned media was encouraging self-renewal of these cells, conditioned media from A549 cells was used as a negative control. A549 conditioned media did not significantly increase the percentage of wound closure in A549 epithelial cells (Figure 2B, C). These data illustrate that rhMIF licensing can augment an increase in VEGF production by MSCs, which can have a positive effect on epithelial cell damage by enhancing the percentage wound closure after 48hrs.

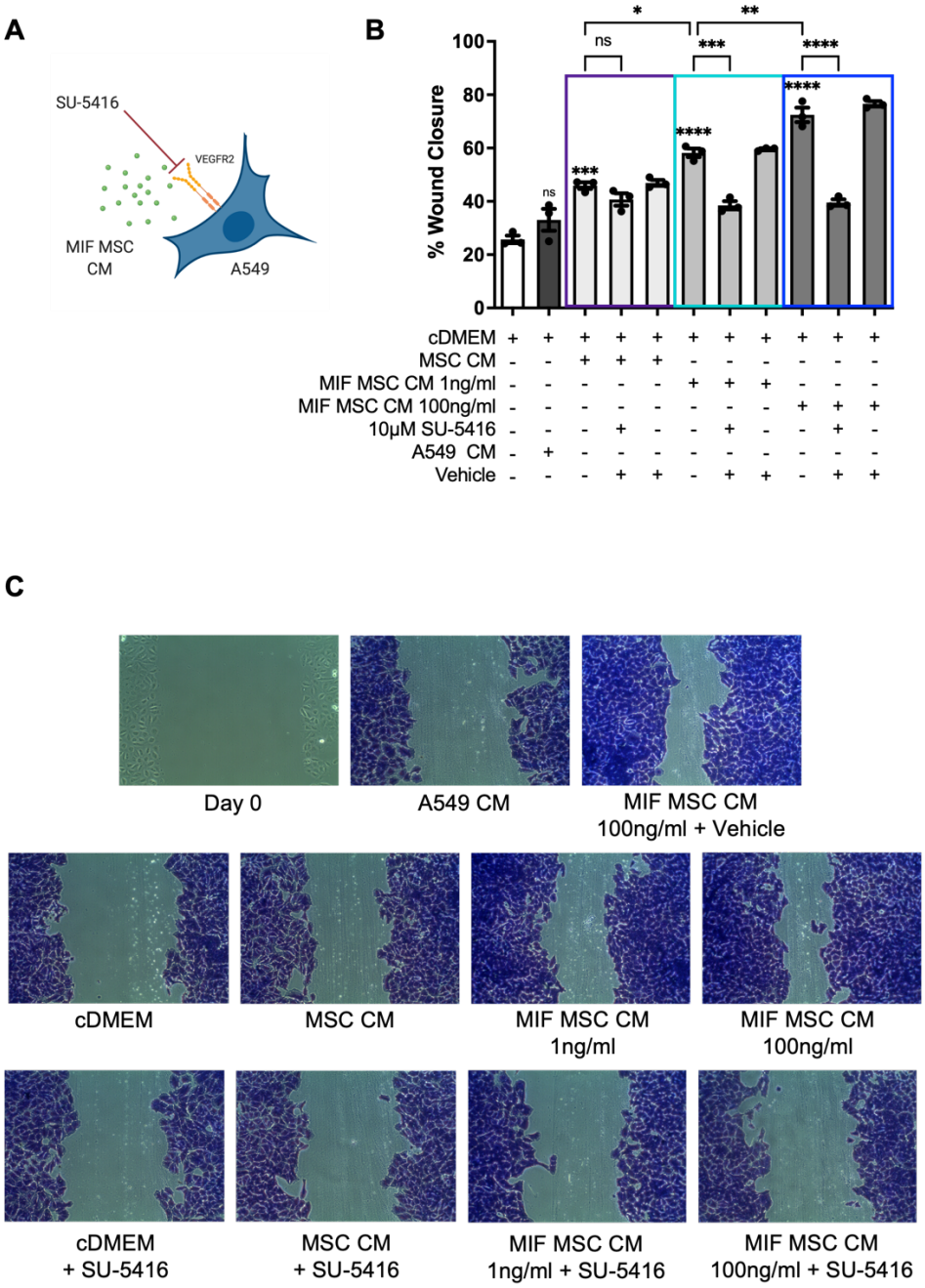


Figure 2. rhMIF licensing accelerates MSC-mediated wound closure in a VEGF-dependent manner. A Human VEGF in recombinant MIF MSC CM binds to A549 epithelial cells through the VEGFR2 receptor. SU-5416, a VEGFR2 inhibitor, blocks this interaction. **B** Percentage wound closure relative to control after use of the VEGFR inhibitor SU-5416 or vehicle control. **C** Images of A549 wound closure after crystal violet stain. Data are presented as mean ± SEM; images are representative of 3 independent experiments, n=3 per group **p<0.01, ***p<0.001, ****p<0.0001.

3.4.3 Endogenous human MIF from $CATT_7$ mice is more efficacious at licensing MSCs than recombinant MIF

Recombinant MIF and its biological effects can vary depending on the manufacturer. Furthermore, a disadvantage to the use of recombinant proteins is their associated short shelf life, as they are predisposed to degrade over a short period of time. Due to a discontinuation of the recombinant MIF used in figures 1 and 2, the remainder of this study utilised endogenous MIF from our humanised $CATT_7$ MIF mice as a preferred method of licensing MSCs. This was highly beneficial as it not only facilitated the incorporation of our novel human MIF expressing mouse strain, but also as the MSC response to MIF produced by these mice was more robust. As MIF is known to be stored in intracellular pools, being secreted only after stimulation (Bacher et al., 1996; Bernhagen, Mitchell, et al., 1994; Calandra et al., 1994; Calandra & Roger, 2003), high MIF expressing $CATT_7$ mice, MIF knockout ($MIF^{-/-}$) mice and wildtype mice were exposed to a model of house dust mite-induced acute allergic airway inflammation. The supernatants generated from bone marrow-derived macrophages isolated from $CATT_7$ mice contained high levels of human MIF (Dunbar and Hawthorne et al., 2023).

After licensing MSCs with $CATT_7$-derived human MIF, WT-derived murine MIF, or no MIF ($MIF^{-/-}$), VEGF protein levels in the endogenous MIF MSC CM were measured by ELISA. $MIF^{-/-}$ and murine MIF-expressing WT mice did not significantly increase VEGF production by MSCs compared to cDMEM control (Figure 3A). Conversely, MSCs licensed with human MIF containing $CATT_7$ supernatants displayed significantly elevated VEGF protein levels in the associated conditioned media, compared to those licensed with $MIF^{-/-}$ or WT supernatants. This proves that human, but not murine MIF, drives enhanced VEGF production by MSCs (Figure 3A). To investigate if endogenous MIF licensed MSC CM could also enhance wound closure in a VEGF-dependent manner as observed in Figure 1 and 2, wound healing

experiments were repeated using $CATT_7$ or WT-derived MIF MSC CM. Previously, alveolar basal epithelial cells, referred to as A549s, were used as a proof of concept and for optimisation of this *in vitro* assay. To improve the physiologically relevance of this assay, a normal bronchial epithelial cell line (BEAS-2B) was used for outstanding experiments. In line with figure 1C and 2B, $CATT_7$-derived MIF MSC CM significantly increased the percentage wound closure in normal bronchial epithelial cells, compared to unlicensed-MSC CM and cDMEM controls (Figure 3B). Moreover, the VEGFR2 inhibitor SU-5416 significantly mitigated this enhanced wound closure in BEAS-2B cells grown in $CATT_7$-MIF MSC CM. SU-5416 had no effect on WT-MIF MSC CM groups, illustrating that murine MIF did not drive wound closure in a VEGF-dependent manner (Figure 3B). The use of a VEGFR2 inhibitor had no off-target, non-specific effects on the general growth of these cells, as cDMEM wells treated with SU-5416 had no significant difference in percentage wound closure compared to cDMEM alone (Figure 3B). On a qualitative level, the evident increase in wound closure associated with conditioned media from $CATT_7$-licensed MSCs is depicted in figure 3C. Furthermore when the VEGFR2 was blocked using SU-5416 but not vehicle control, the inhibition of wound closure is clear (Figure 3C).

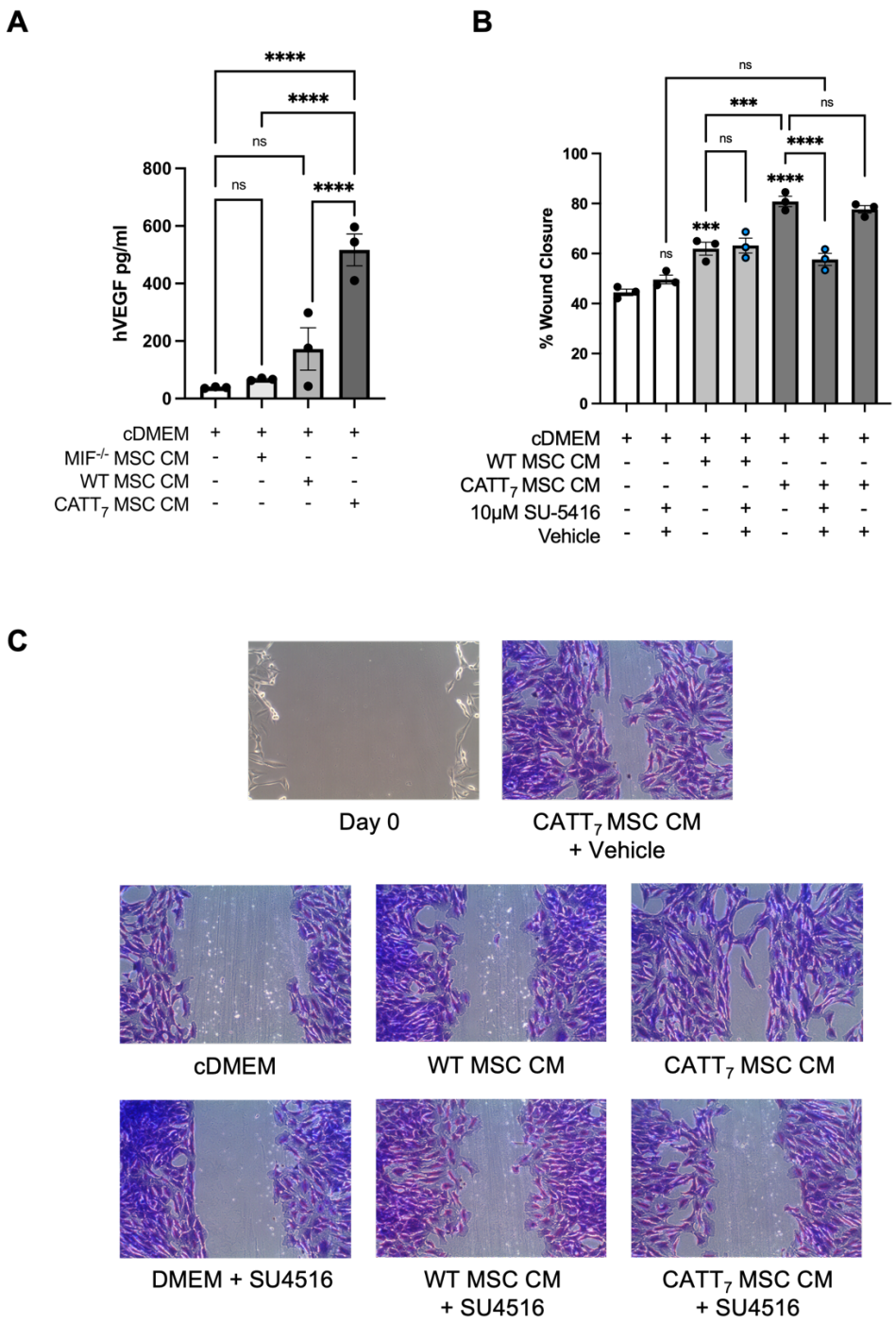


Figure 3. Endogenous CATT$_7$-MIF MSC conditioned media drives bronchial epithelial wound closure in a VEGF-dependent manner. A Human VEGF protein levels in MIF MSC conditioned media supernatants, using bone marrow-derived supernatants from high MIF expressing CATT$_7$ mice, MIF$^{-/-}$ mice or WT mice. **B** Percentage wound closure relative to control after use of the VEGFR2 inhibitor SU-5416 (10μM) or vehicle control. **C** Images of BEAS-2B wound closure after crystal violet stain. Data are presented as mean ± SEM; images are representative of 3 independent experiments, n=3 per group ***p<0.001, ****p<0.0001.

3.3.4 MIF inhibitor SCD-19 blocks VEGF-mediated wound closure in bronchial epithelial cells

We have previously shown that human, but not murine, MIF drives VEGF production from MSCs (Figure 3A) and thus facilitates a significant increase in wound closure (Figure 3B, C). To fully elucidate this MIF-associated increase in wound closure, a MIF inhibitor SCD-19 was used to block MIF's biological activity prior to MSC licensing. When $CATT_7$ endogenous MIF supernatants were incubated with 100μM of SCD-19 for 1 hour prior to MSC licensing, MIF inhibition significantly decreased MSC-mediated VEGF production compared to cDMEM controls (Figure 4A). Importantly, SCD-19 had no effect on WT-derived MIF supernatants, as no significant difference in VEGF production was noted.

SCD-19 effectively decreased the percentage wound closure in $CATT_7$-MSC CM treated bronchial epithelial cells, illustrating the role of human MIF in boosting MSC's cytoprotective, wound healing capabilities (Figure 4B). Interestingly, the use of SCD-19 prior to licensing MSCs with $CATT_7$-containing human MIF supernatants abrogated wound closure levels similar to those treated with WT-MSC conditioned media; illustrating that SCD-19 is more specific at blocking human MIF, than murine MIF. To further prove this, SCD-19 did not significantly decrease VEGF production by WT-licensed MSCs, and thus no significant difference was seen in WT-MSC CM wound closure (Figure 4B). A vehicle control was used to ensure that there were no off-target effects on the wound healing of BEAS-2B cells. No significant difference between $CATT_7$-MSC CM and $CATT_7$-MSC CM + vehicle control was observed (Figure 4B, C). These data conclude that high levels of human MIF from $CATT_7$ BMDM-derived supernatants can license MSCs to produce increased levels of VEGF (Figure 3A), with increased efficacy than those licensed with recombinant MIF (Figure 1B). Following this narrative, conditioned media generated from endogenous human MIF-licensed MSCs can

significantly increase wound closure in BEAS-2B cells in a VEGF-dependent manner (Figure 3B), illustrating MIF's specific role through utilising a potent MIF antagonist SCD-19 (Figure 4B).

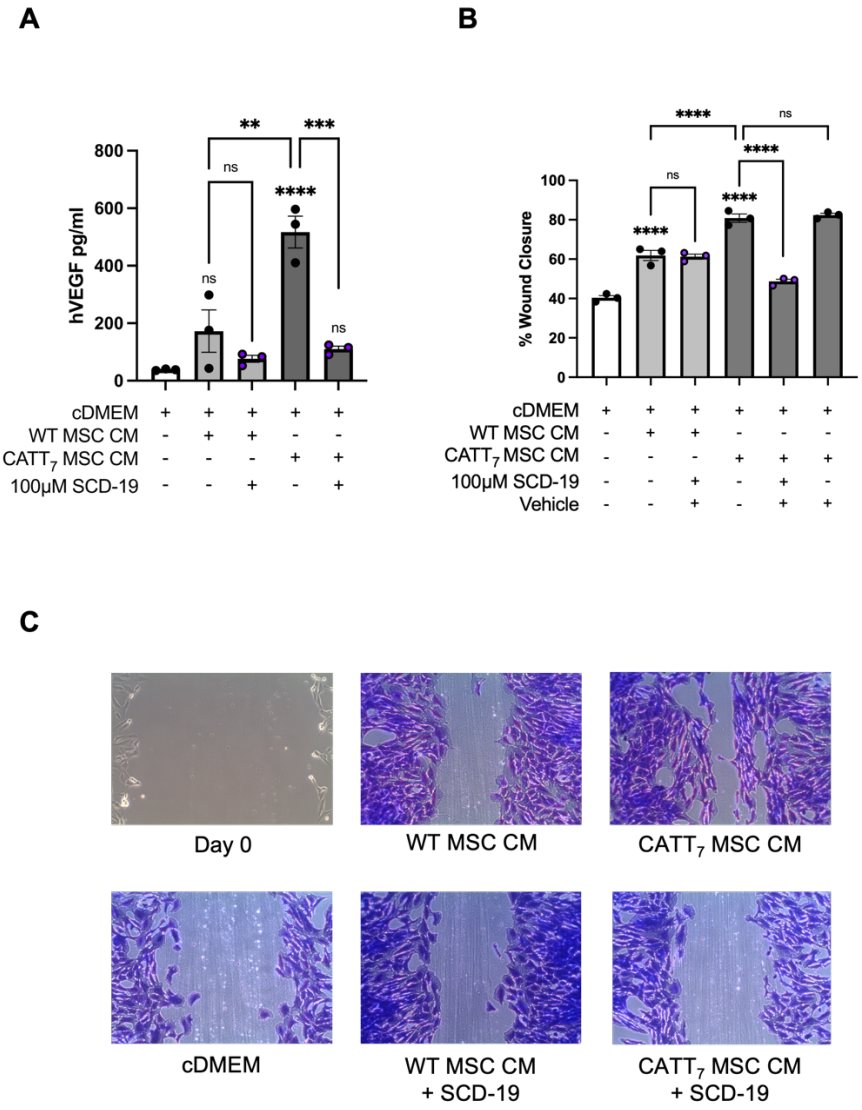


Figure 4. MIF inhibitor SCD-19 prevents increased VEGF-mediated wound closure in bronchial epithelial cells. A Human VEGF protein levels in WT-MSC and CATT$_7$-MSC conditioned media supernatants, with and without the use of SCD-19 (100μM). **B** Percentage wound closure relative to control after use of the MIF inhibitor SCD-19 (100μM) or vehicle control. **C** Images of BEAS-2B wound closure after crystal violet stain. Data are presented as mean ± SEM; representative of 3 independent experiments, n=3 per group **p<0.01, ***p<0.001, ****p<0.0001.

3.4.5 $CATT_7$-MIF licensed MSC conditioned media protects BEAS-2B bronchial epithelial cells from HDM-induced apoptosis

In the asthmatic lung, repeated exacerbations can inflict injury on the membrane epithelium of the lung. To recapitulate this physiological setting, the clinically relevant allergen house dust mite (HDM) was used to induce damage on normal bronchial epithelial BEAS-2B cells. Through measuring epithelial cell apoptosis with Annexin V/PI stain for flow cytometry, HDM was observed to increase apoptosis in a dose dependent manner (Figure 5A). To ensure that effective apoptosis could be induced in these cells, a positive control using the chemotherapeutic drug cisplatin (50ng/ml) was utilised. This study set out to investigate if $CATT_7$-MIF licensed MSC conditioned media could protect against HDM-induced apoptosis in epithelial cells. Interestingly, pre-treatment of BEAS-2B with $CATT_7$-MSC CM for 2 hours prior to HDM stimulation could significantly protect epithelial cells from increased apoptosis compared to cells that did not receive $CATT_7$-MSC CM (Figure 5A). Moreover, this effect was specific to human MIF, as WT-MSC CM could not protect BEAS-2B cells from HDM-induced damage (Figure 5A). To confirm that this protection against HDM injury was specific to human MIF, the MIF inhibitor SCD-19 was used. MSCs licensed with MIF inhibited-$CATT_7$ supernatants no longer decreased BEAS-2B apoptosis, proving our hypothesis that human MIF licensing of MSCs significantly improves their ability to protect against apoptosis caused by allergen insult (Figure 5A) and increases wound healing in bronchial epithelial cells (Figure 4B). Moreover, through licensing MSCS with human MIF, the biological activity of the associated conditioned media is significantly enhanced, illustrating the potential of using MSC's secretome as a therapeutic tool.

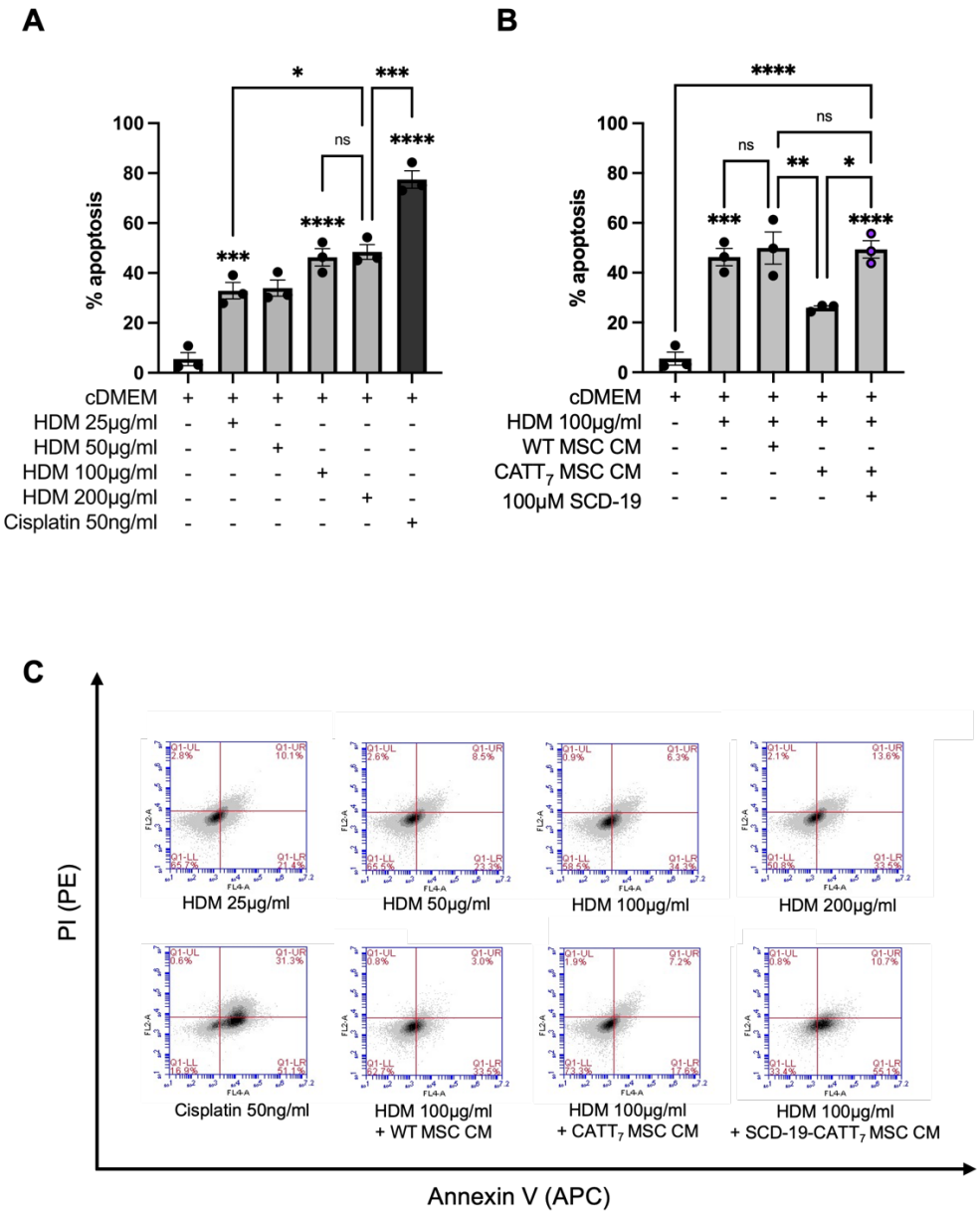


Figure 5. CATT$_7$-MIF MSC CM protects BEAS-2B from HDM-induced apoptosis. A BEAS-2B epithelial cells were exposed to a dose curve of HDM (25, 50, 100, 200µg/ml) for 24hrs. 50ng/ml of cisplatin was used as a positive control. Cells were stained with Annexin V/PI and analysed by flow cytometry. Dead cells were identified as Annexin V+PI- (early apoptosis) and Annexin V+PI+ (late apoptosis). **B** BEAS-2B epithelial cells were grown in WT-MSC and CATT$_7$-MSC conditioned media supernatants, with and without the use of SCD-19 (100µM) for 4hrs before being exposed to 100 µg/ml of HDM. **C** Dot plot images of BEAS-2B stained with Annexin V (APC) and PI (PE). Data are presented as mean ± SEM; representative of 3 independent experiments, n=3 per group *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

3.5 Discussion

Although the use of MSCs in inflammatory conditions has been extensively studied, with over 75,000 publications documenting their capabilities (Mönch et al., 2022, 2023), reproducibility is still an evident problem with these cells in clinical trials. This study set out to investigate the use of macrophage migration inhibitory factor (MIF) as a method of pre-conditioning MSCs, enhancing their performance through utilising their cell-free secretome as a cytoprotective therapy to tackle epithelial injury *in vitro*.

MSCs as a cellular therapy can come with their own limitations, such as the formation of embolisms after intravenous administration of bone marrow or adipose tissue-derived MSCs (Hackel et al., 2023; Moll et al., 2022). To overcome this barrier, their secretome can be used as an alternative cell-free method. Additionally, the use of the secretome rather than the cell itself hosts several benefits; decreased manufacturing costs, more convenient storage, less chance of product delivery failure and lower risk of mounting an unwanted immune response or stimulating tumour progression (Lee & Hong, 2017; Su et al., 2023). A downside however, is that unlike its cellular counterpart, the secretome cannot sense and respond to its surrounding microenvironment. Alas, the concept of optimally pre-licensing MSC prior to administration could resolve this concern, by tailoring the secretome towards the recipient's cellular microenvironment, enhancing the therapeutic outcome (Dunbar et al., 2021; Gorgun et al., 2021). Furthermore, once administered, MSCs have been shown to be viable for 24 hours or less *in vivo*, indicating that the secretome could be an efficacious option (Pang et al., 2021).

As discussed, house dust mite is a clinically relevant aeroallergen which once inhaled, can damage and penetrate the epithelial barrier, activating the immune cascade associated with this type 2 inflammatory condition. Alveolar basal epithelial cells (A549) were used in

preliminary experiments to optimise the wound healing assay. To further recapitulate the asthmatic lung microenvironment and to enhance the physiological relevance, wound healing and epithelial cell survival assays were continued using a normal bronchial epithelial cell line (BEAS-2B). BEAS-2B cells are more suitable when investigating an asthmatic phenotype, as they are isolated from the upper, proximal section of the lung, in comparison to the distally isolated A549 cell line (Hillyer et al., 2018; Schlinkert et al., 2015). Furthermore, as A549 cells are an adenocarcinoma cell line and thus are of tumorigenic origin, they might have an increased interaction with VEGF, as this soluble mediator is known to play a role in the progression of cancer (Ghalehbandi et al., 2023). Taking into account the section of bronchial tree from which the cells are isolated, and if they have known physiological interactions with VEGF and angiogenesis, the normal bronchial cell line was most suitable.

MSC's primed with recombinant human MIF demonstrated enhanced VEGF production, secreting significantly higher levels of this growth factor into their secretome (Figure 1B). Due to questions about the robustness of commercial recombinant MIF and supply issues of purified human MIF from Jurkat T cells (Bernhagen, Mitchell, et al., 1994), our preferred source of human MIF was from the bone marrow-derived macrophages of our high MIF expressing humanised $CATT_7$ mice. A limitation to take into consideration when using endogenous sources of MIF is that other bystander effects and mediators may be at play in the licensing of MSCs. Thus, to confirm the specificity of MIF's role in MSC licensing, the use of a highly potent MIF inhibitor SCD-19 was used.

These data illustrating that human MIF-activated MSCs produced significantly increased levels of VEGF, and thus enhancing the percentage of wound closure in epithelial cells in a VEGF-dependent manner may raise some concerns. As this growth factor is known

for its action in angiogenesis, there is a possibility that increased levels of MSC-derived VEGF alone may have a pathological impact in the lung. We have previously shown the beneficial impact MSCs can have when administered into an acute model of allergic airway inflammation, where MSCs significantly decreased collagen deposition and goblet cell hyperplasia after house dust mite challenge (Hawthorne, Dunbar et al., in review). In an OVA model, MSCs significantly reduced total VEGF levels in lung tissue *in vivo* regardless of source, with bone marrow-derived MSCs being the most efficacious (Abreu et al., 2017). Thus, although MIF-licensed MSCs produce increased levels of VEGF *in vitro*, these cells also produce other regulatory factors such as indoleamine 2,3-dioxygenase (IDO) and cyclo-oxygenase-2 (COX-2) (Hawthorne, 2023., unpublished), illustrating that MSC's immunomodulatory and cytoprotective functions work as a collective and therefore the positive therapeutic effects associated with these cells outweigh the negatives. These data confirm that *in vitro* and *in vivo* work may represent different findings regarding VEGF, as there are more factors at play in the complex lung microenvironment. VEGF has historically played a central role in epithelial repair and the maintenance of epithelial barrier integrity, where VEGF-deficient mice had increased levels of bronchial and alveolar apoptosis (Tang et al., 2004). As illustrated in this study, increased levels of this trophic factor enhanced epithelial wound healing, perhaps through improving the survival and proliferation of airway epithelial cells (Mura et al., 2006; Ohwada, 2003). Furthermore, VEGF overexpression reduced bleomycin-induced cell death in a model of idiopathic pulmonary fibrosis (IPF), again reiterating the protective importance of this growth factor in modulating epithelial repair (Murray et al., 2017). Interestingly, VEGF-A and its receptor VEGFR2 (also known as KDR) have been shown to have a protective role in the defence against mucous cell metaplasia, commonly documented in asthma and cystic fibrosis (Jiang et al., 2021). In asthma, if VEGF-A levels are decreased, the transcription factor Sox9 is upregulated, driving club to goblet cell differentiation (Jiang et al., 2021). These data,

along with the data presented throughout this chapter depict the protective role of VEGF in the repair and regulation of the airway epithelial barrier. Following on from these data shown in this chapter surrounding MSC-derived VEGF, where endogenous human MIF-licensed MSCs secreted increased levels of VEGF, hypothetically, this would also have a positive impact in strengthening the epithelial barrier against house dust mite challenge *in vivo*.

Future experiments investigating the role of MSC's secretome on epithelial permeability would be beneficial, where the impact of MIF licensing on these cells could be further elucidated in the repair of the airway epithelial barrier after house dust mite challenge. Furthermore, MSCs have already illustrated efficacy in a model of bleomycin-induced fibrosis, where they decreased the level of TUNEL stain for DNA strand breaks *in vivo* (Cahill et al., 2016). Similar experiments in an allergic airway inflammation setting would facilitate the translation of the *in vitro* data presented in this study to an *in vivo* setting. Although the role of VEGFR2 has been elucidated using the selective inhibitor SU-5416 (Semaxanib), three subtypes of VEGF (A, C and D) could be involved. A broad-spectrum VEGF ELISA was used to measure protein levels in MIF MSC CM, but the use of subtype-specific inhibitors such as the anti-VEGF-A monoclonal antibody bevacizumab (Avastin®) could be useful to determine what subtype of VEGF is dominant in facilitating epithelial repair (Garcia et al., 2020).

3.6 Conclusion

This study details the importance of epithelial barrier integrity in innate immunity, especially in an age where there is a rapid increase of industrialisation and urbanisation. These evolving environmental conditions could drive an exponential increase in epithelial permeability, giving rise to a plethora of atopic, autoimmune conditions (Akdis, 2021), such as allergic asthma. This study demonstrates the therapeutic efficacy of human MIF-licensed MSC CM, where increased levels of MSC-derived VEGF facilitated epithelial repair and survival *in vitro*.

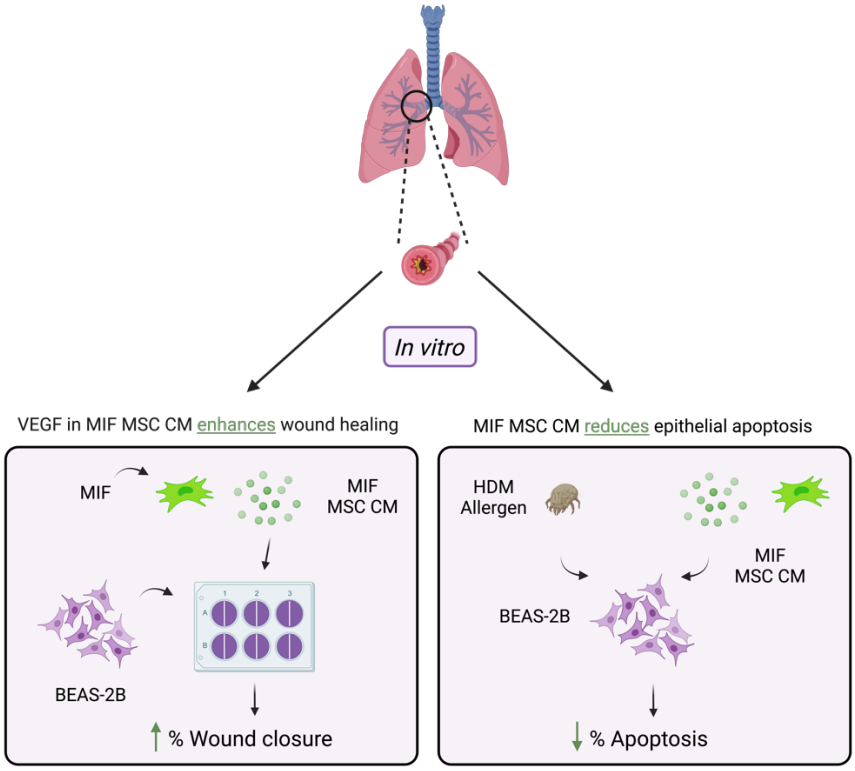


Figure 6. Schematic illustrating effects of rhMIF or endogenous CATT$_7$ MIF licensed MSC conditioned media on airway epithelial wound healing and survival. Conditioned media generated from hBM-MSCs licensed with recombinant human MIF or endogenous human CATT$_7$ MIF has increased levels of VEGF. MIF MSC CM increases percentage wound healing in BEAS-2B cells in a VEGF-dependent manner. MIF MSC CM protects BEAS-2B cells from apoptosis after HDM challenge.

Chapter 4:

MSCs block innate immune priming and training in the bone marrow of humanised MIF mice

4.1 Abstract

Innate immune training is a concept of prolonged immunological memory, where pathogens can educate and re-programme the immune system. The role of MIF and its functional promotor polymorphism in innate immune training is currently unknown. Using novel humanised $CATT_7$ MIF mice, this study is the first to investigate the effect of high human MIF expression on bone marrow-derived macrophage (BMDM) polarisation. Moreover, the effect of human MIF on innate immune training in house dust mite-challenged $CATT_7$ hematopoietic stem and progenitor cells was also examined.

Endogenous expression of human MIF exhibited no definitive impact on the polarisation of naïve $CATT_7$ macrophages. After house dust mite (HDM) challenge *in vivo,* $CATT_7$ BMDMs demonstrated a significant increase in M1 markers, *tnfα*, *il-6*, *il-1β*, *nos2*, and *ifnγ*, and M2 markers, *chi3l3* and *il-10*. The M1 signature was found to be MIF-dependent, as it was diminished with the use of the MIF inhibitor, SCD-19. When co-cultured with $CATT_7$ BMDMs, mesenchymal stromal cells (MSCs) suppressed this HDM-primed effect, with *tnfα* and *rentla* expression being decreased in a COX-1/2 dependent manner. A prominent hallmark of innate immune training is epigenetic re-programming via histone modification. Inhibition of methyltransferase activity in $CATT_7$ hematopoietic stem and progenitor cells (HSPCs) prior to HDM stimulation blocked the enhanced production of pro-inflammatory cytokines following LPS stimulation *in vitro*, confirming the occurrence of this trained immunity phenomenon. Furthermore, MSCs significantly abrogated this HDM-trained effect when co-cultured with HSPCs, displaying their therapeutic efficacy in modulating an over-zealous, human MIF-dependent immune response.

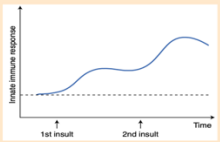
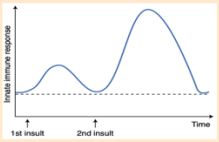
4.2 Introduction

Trained innate immunity has gained significant scientific attention in light of the recent SARS-CoV2 pandemic with vaccine efficacy, where this popular phenomenon illustrates the importance of immune cell memory and how this innate cell training is fundamental to an effective immunological response to pathogens (Divangahi et al., 2021; Netea et al., 2016). A noteworthy example of this non-specific trained immunity is demonstrated in the application of the renowned Bacillus-Calmette Guérin (BCG) vaccine, originally developed to combat tuberculosis in 1928 (Ravenel, 1928). This vaccine is currently acknowledged to provide additional protection against other respiratory tract insults (Chen et al., 2023; Moorlag et al., 2022), including influenza (Kaufmann et al., 2022; Leentjens et al., 2015) and asthma (El-Zein et al., 2010; Marks et al., 2003). Initially, it was speculated that the BCG vaccine could play a role in offering protection against COVID-19 via the concept of innate immune training (Berg et al., 2020; Covián et al., 2020; O'Neill & Netea, 2020), However, recent studies have now contradicted this statement, as healthcare workers that received the BCG vaccination did not have a lower risk of COVID-19 compared to those that received a placebo (Kaufmann et al., 2022; Pittet et al., 2023).

Innate immune cells, specifically phagocytic macrophages, are known to be activated when they interact with a vaccine, adjuvant, or pathogen for the first time (e.g., BCG vaccine, β-glucan or house dust mite). This initial interaction can prime macrophages, preparing them for the recurrence of a secondary infection or stimuli. As a result, the macrophage exhibits an additive or synergistic response between the first and second stimuli. Importantly, when the insult is removed from the macrophage microenvironment, this short-acting priming effect wanes over time, returning active gene transcription of these cells to a non-activated, basal state (Divangahi et al., 2021). In contrast to peripheral innate priming which occurs in differentiated-

macrophages, central innate immune training is an immunologically distinct process, which induces epigenetic and metabolic changes in the bone marrow-resident precursors of these cells. Unlike innate priming, innate immune training results in long-term memory, provoking a faster and greater immune response when the macrophage encounters future, often heterologous stimuli (Table 1). Although innate immune training provides a protective effect from a therapeutic standpoint, where it facilitates enhanced responses to vaccine administration, a highly alert and activated immune system can be detrimental, driving the development or exacerbation of autoimmune conditions, or cardiovascular disease (Funes et al., 2022). Thus, finding a balance between an over- and under-reactive immune system in the context of innate immunity is pivotal for disease resolution.

Table 1. Key disparities between innate priming and training[1]

Priming	Training
• No changes at epigenetic level	• Chromatin remodelling in progenitor cells
• Metabolism is not re-programmed	• Metabolic re-programming
• No rest period b/w stimuli	• Rest period b/w stimuli
• Active gene transcription does not return to basal levels	• Active gene transcription does return to basal levels
• Additive/synergistic response between 1° and 2° stimulus	• Faster and greater response to secondary stimulus
• Short term adaptation (reversible)	• Long term memory (irreversible)
	

[1] **Key disparities between innate priming and training.** Including: epigenetic changes, metabolic re-programming, rest periods, active gene transcription status, speed and strength of innate response and duration of induced memory (Graphs adapted from (Divangahi et al., 2021)).

Macrophages, a population of professional phagocytic cells derived from hematopoietic stem and progenitor cells (HSPCs) within the bone marrow niche, can be categorised as an M1 pro-inflammatory or M2 anti-inflammatory phenotype. Plasticity is a key characteristic of these innate cells, as depending on external factors within their surrounding microenvironment, their activation status (classical – M1, alternative – M2) is a spectrum (Kang & Kumanogoh, 2020; Locati et al., 2020), rather than the two phenotypes being separate, distinct entities. In addition to playing a central role in host defence, macrophages are also a source of the pro-inflammatory cytokine macrophage migration inhibitor factor (MIF) (Calandra et al., 1994). MIF is involved in the pathogenesis of a variety of inflammatory diseases, including asthma, however it remains to be resolved if MIF's role in disease is due to increased susceptibility or severity. Mounting evidence indicating MIF's involvement in asthma severity has been linked to a functional promotor polymorphism, where an increase in the number of repeats of a tetranucleotide sequence $(CATT)_n$, correlates with increased MIF expression (Baugh et al., 2002). Humans that possess a 5-repeat allele of this MIF polymorphism have been noted to have a milder subtype of disease (Plant et al., 2006). Due to its enhanced presence in the bronchoalveolar lavage fluid of asthmatic patients compared to nonatopic volunteers (Mizue et al., 2005), MIF has proved to be an important player in asthma development. We have previously illustrated the role of the high expressing human $CATT_7$ MIF allele in a model of house dust mite (HDM)-induced allergic airway inflammation, where HDM-challenged $CATT_7$ mice exhibited increased airway hyperresponsiveness resulting from increased cellular infiltration, goblet cell hyperplasia, subepithelial collagen and Th2 cytokine mediators with these mice having reduced lung function after methacholine nebulisation. Furthermore, utilising a potent MIF inhibitor SCD-19, MIF's key role in driving disease severity was confirmed (Dunbar and Hawthorne et al., 2023). Although further investigation surrounding innate immunity in type 2 inflammatory disease is required (Hartung & Esser-von Bieren, 2022), evidence shows that HDM, a known

trigger of allergic airway inflammation, plays a role in activating innate immunity (Albers et al., 2022; Branchett et al., 2021; Henkel et al., 2018; Lechner et al., 2022). Our goal in this study was to investigate the role of the high expressing human MIF polymorphism in the innate immunity of macrophages, after exposure to a relevant HDM allergen *in vitro* and *in vivo*.

Markers of innate immunity in macrophages can be defined as enhanced production of pro-inflammatory mediators such as TNFα and IL-6 (Lechner et al., 2022). As previously mentioned, innate immune training occurs in progenitor cells where histone modifications such as DNA methylation (e.g., H3K4me1 and H3K4me3) or acetylation (H3K27ac) occur upon exposure to a primary stimulus, leaving an 'epigenetic scar' resulting in the long-lasting re-programming of daughter cells (i.e., differentiated bone marrow-derived macrophages). Thus, by utilising inhibitors that target areas involved in epigenetic modifications, such as methyltransferase enzymes, one can fully elucidate if innate immunity, rather than priming, is at play. This research utilised a chemical methyltransferase inhibitor, methyltransferase adenosine (MTA), to block DNA methylation and thus investigate the training status of HSPCs and bone marrow-derived macrophages (BMDMs) in transgenic high human MIF expressing $CATT_7$ mice.

Mesenchymal stromal cells (MSCs) have previously demonstrated therapeutic efficacy in inflammatory diseases, such as in a HDM-induced model of allergic airway inflammation, where their effects were mediated by enhanced COX-2 activity (Hawthorne, Dunbar et al, in review). Once administered intravenously, MSCs are known to first migrate to the lung through the pulmonary circulatory system, termed "first passe" (Galipeau & Sensébé, 2018; Sensebé & Fleury-Cappellesso, 2013). Furthermore, due to the large size of these stromal cells (ranging from 16–53 μm) (Furlani et al., 2009), MSCs become trapped within the lung architecture

where they can become licensed by the resident inflammatory microenvironment (Dunbar et al., 2021) and secrete paracrine factors to modulate their surrounding microenvironment (Carty et al., 2021; Ferrini et al., 2021; Leibacher & Henschler, 2016). After 24hrs, MSCs eventually become apoptotic and are cleared by phagocytosing cells such as macrophages (Eggenhofer et al., 2012; Galleu et al., 2017; Giri & Galipeau, 2020; Pang et al., 2021). Our hypothesis states that between encountering and engulfing the MSC, the macrophage may become re-programmed or trained by these stromal cells, influencing its M1/M2 status and thus implicating future protection against re-infection and disease progression (Braza et al., 2016; Liu et al., 2020; Morrison et al., 2017). To elucidate this mechanism, MSCs were co-cultured with WT and $CATT_7$ BMDMs using transwell inserts to investigate their role on innate priming. Additionally, MSCs pre-treated with COX-enzyme inhibitors indomethacin and NS-398 were co-cultured with BMDMs from these mice to demonstrate the mechanistic role of MSCs in innate immunity.

This study is the first to characterise the macrophage phenotype in these novel human $CATT_7$ expressing transgenic mice, both naïve and those exposed to an acute model of HDM-induced asthma. Furthermore, this study is the first to identify the role of this high expressing human MIF polymorphism in the innate immunity of these mice after HDM exposure. This novel research describes the immunosuppressive, partially COX-2 dependent role of human bone marrow-derived mesenchymal stromal cells and the implications of their *in vitro* paracrine communication with $CATT_7$ HSPCs and BMDMs after secondary lipopolysaccharide (LPS) stimulation.

4.3 Materials and Methods

4.3.1 Ethical Approval and HPRA Compliance

All procedures involving the use of animals were carried out by licensed personnel. Ethical approval for all work was granted by the ethics committee of Maynooth University (BRESC-2018-13). Project Authorisation was received from the HPRA (AE19124/P022), whereby the terms of the animal experiments within this project were outlined and adhered to.

4.3.2 Compliance with GMO and Safety Guidelines

All GMO/GMM work was performed according to approved standard operation procedures and recording protocols approved by the Environmental Protection Agency (Ireland). Safe working practices were employed throughout this study as documented in the Biology Department, Maynooth University Safety Manual.

4.3.3 Transgenic Humanised MIF Mice

A C57BL/6 mouse strain expressing the human high expression $CATT_7$ MIF allele (*MIF*^{CATT7} [(C57BL/6NTac-Miftm3884.1(MIF)Tac-Tg(CAG-Flpe)2Arte) was created using vector-based recombinant replacement of murine MIF by Taconic Biosciences (Rensselaer, NY). Validation of human but not murine MIF mRNA expression was verified by qPCR, and -794 CATT-length dependent stimulated MIF production was confirmed *in vivo* (Shin et al., 2022). These humanised MIF transgenic mice were kindly provided by our collaborator Prof. Seamas Donnelly (Trinity College Dublin, Ireland). All mice were housed according to the HPRA SAP (Ireland) guidelines and used with ethical approval under the terms of AE19124/P022 project authorisation from HPRA.

4.3.4 Preclinical model of Acute Allergic Airway Inflammation

$CATT_7$, and WT mice (6-18 weeks old) were challenged with 25μg of house dust mite allergen, *Dermatophagoides pteroyssinus* with an endotoxin content of 9937.5 EU/vial (Greer Labs, Lenoir, USA) or PBS control intranasally (I.N.) under isoflurane anaesthesia, on days 0, 2, 4, 7, 9, 11, 14, 16, 18. After each challenge, mice were returned to their cage and monitored closely. An animal welfare score sheet was utilised throughout the study, where examining features included: weight loss, activity, general appearance and clinical signs.

4.3.5 MIF Inhibitor

SCD-19 (3-(2-methylphenyl)-1H-isochromen-1-one) (Specs.net, Netherlands), a small molecular weight inhibitor of macrophage migration inhibitory factor (MIF) biological activity was used in a house dust mite model of acute allergic asthma. 35mg/kg of SCD-19 or vehicle control (70% ethanol in PBS) was administered intraperitoneally (I.P.) twice weekly for three weeks; day 0, 4, 7, 11, 14, and 18. For dose analysis, SCD-19 was administered twice a week for one week (days 14 and 18).

4.3.6 Isolation of Hematopoietic Stem and Progenitor Cells (HSPCs)

Naïve mice were humanely euthanised using cervical dislocation and the bone marrow was isolated from femur and tibia and centrifuged at 300g for 5 minutes. Red blood cells were lysed using 1X RBC lysis buffer (eBioscience) for 5 minutes. Cells were centrifuged at 300g for 5 minutes before being counted with a haemocytometer and seeded out at $1.5x10^6$ per well of 6 well non-tissue culture grade plate in complete cRPMI supplemented with 20% L929 (M-CSF) conditioned media.

4.3.7 Generation of L929 Conditioned Media (M-CSF)

L929 cells were thawed, seeded in complete RPMI and incubated at 37°C/5% CO_2/20% O_2 for 7 days. Supernatant was collected and centrifuged at 300g for 5 minutes to remove cell debris. After being passed through a 0.2µm filter for sterilisation, conditioned media containing M-CSF was aliquoted and stored at -80°C. L929 conditioned media will be referred to as M-CSF throughout the text.

4.3.8 Generation and Polarisation of BMDM

Cells were isolated from the bone marrow of $CATT_7$ and WT mice as described. $1.5x10^6$ cells were seeded in 2 mls of cRPMI supplemented with 20% M-CSF into non-tissue culture grade 6 well plates (Sarstedt). Media was changed on days 3 and 6 to remove non-adherent cells. On day 7, media was replenished and cells were stimulated for 24hrs with either LPS (from *E. coli* O111:B4) (100ng/ml) (Sigma) or murine IFNγ (10ng/ml) (R&D Systems) to drive M1 polarisation, or murine IL-4 (10ng/ml) (R&D Systems) to drive M2 polarisation.

4.3.9 *In Vitro* Innate Training Assay

For innate training assays, methyltransferase inhibitor, methylthioadenosine (MTA) (1mM) (Sigma) or a DMSO vehicle control was added to HSPCs 30 minutes prior to any stimulus. HSPCs were then stimulated with 10µg of HDM and media was supplemented with 20% M-CSF. After 24hrs, cells were washed in warm PBS and centrifuged to remove HDM stimulus and re-seeded. Cells were grown in 20% M-CSF supplemented cRPMI to facilitate macrophage differentiation, until day 6, when media was changed to non-supplemented cRPMI

to facilitate a rest period. Differentiated macrophages were stimulated with 100ng/ml of LPS on day 10 for 24hrs. Supernatants were collected and analysed for protein production.

4.3.10 Mesenchymal Stromal Cell Co-Culture

Human bone marrow derived mesenchymal stromal cells (MSCs) (RoosterBio Frederick, MD 21703, United States) were cultured in complete DMEM (Sigma) at 37°C/5% CO_2/20% O_2. At passage 2-4, $2x10^5$ of MSCs were seeded into 0.4μM transwell inserts (Greiner) and co-cultured with HSPCs from day 0 (day of isolation) to day 6 for innate training assays or on day 7 for priming assays, for 24hrs.

4.3.11 COX-2 Inhibitor

MSCs were incubated with 10μM Indomethacin (Sigma-Aldrich) or NS-398 (Sigma-Aldrich), or associated vehicle control (1% ethanol in PBS or DMSO/ethanol) for 24hrs. Cells were thoroughly washed with warm PBS prior to co-culture with BMDMs using 0.4μM transwell inserts.

4.3.12 Analysis of Gene Expression

Total RNA was extracted using TRIzol (Ambion Life Sciences) according to manufacturer's instructions. RNA concentrations were measured using a spectrophotometer (Nanodrop 2000, ThermoScientific, Wilmington DE, USA) and were equalised to 100ng/μl before cDNA synthesis. cDNA synthesis was performed using manufacturer's instructions (Quantobio cDNA synthesis kit). Real Time-Polymerase Chain Reaction (RT-PCR) was carried

out using PerfeCta SYBR Green FastMix (Quantbio). Expression was quantified in relation to the housekeeper gene HPRT using the ΔCT method. The fold change in the relative gene expression was determined by calculating the $2^{-\Delta\Delta CT}$ values.

Table 2. Gene name and sequences for RT-PCR

Gene name	Sequence
tnfα	Fwd: AGGGATTTGAATCACGTTTG Rev: TTTACTGGCAACATCAACAG
il-6	Fwd: AAGAAATGATGGATGCTACC Rev: GAGTTTCTGTATCTCTCTGAAG
il-1β	Fwd: CATGGAGAATATCACTTGTTGG Rev: GGATGATGATGATAACCTGC
nos2	Fwd: TTTCCTTTGTTACAGCTTCC Rev: CATCAACCAGTATTATGGCTC
ifnγ	Fwd: CTTATTGGGACAATCTCTTCC Rev: TGAGTATTGCCAAGTTTGAG
arg1	Fwd: CATCTGGGAACTTTCCTTTC Rev: CTGACCTATGTGTCATTTGG
retnla (FIZZ1)	Fwd: GATGAAGACTACAACTTGTTCC Rev: AGGGATAGTTAGCTGGATTG
chi3l3 (Ym1)	Fwd: CTTCTAAGACTGGAATTGGTG Rev: GTACAAACCTCATAGTAAGCC
il-10	Fwd: CAGGACTTTAAGGGTTACTTG Rev: ATTTTCACAGGGGAGAAATC
tgf-β	Fwd: TGTCCAGGCTCCAAATATAG Rev: GGATACCAACTATTGCTTCAG

4.3.13 Protein Quantification

Supernatants were collected and centrifuged at 300g for 5 minutes to remove debris, before being stored at -20°C. All ELISAs were carried out according to manufacturer's instructions for the following proteins: Mouse IL-1β, IL-6 (R&D systems, Abington, UK) and TNFα (Biolegend). The absorbance (optical density (O.D)) of the samples and standards were measured at 450 nm for all ELISAs using a microplate reader (Clariostar Plus, BMG Labtech, Bucks, UK). Arginase Activity Assay (Sigma) and Griess Assay (Abcam) were carried out according to manufacturer's instructions.

4.3.14 Statistical Methods

Data are presented as mean ± SEM. Results were analysed using a statistical software package (GraphPad Prism, San Diego, CA). A one-way ANOVA test was used to test for statistical significance of differences when multiple experimental groups were compared with an independent variable, whilst two-way ANOVA was used for two independent variables. A *post-hoc* Tukey's multiple comparison test was used to compare the means of different treatment groups.

4.4.1 Optimisation of a BMDM polarisation *in vitro* assay

To investigate the inflammatory status of the bone marrow niche, an *in vitro* macrophage polarisation assay was optimised (Figure 1). Bone marrow was isolated from naïve or HDM-challenged WT and $CATT_7$ mice and cells were cultured in M-CSF supplemented (20%) cRPMI for 7 days, washing non-adherent cells off by replacing media on day 3 and 6. On day 7, naïve or HDM-challenged differentiated bone marrow-derived macrophages (BMDMs) were stimulated with polarising conditions for 24hrs as follows; 100ng/ml LPS or 10ng/ml murine IFNγ to drive an M1 pro-inflammatory phenotype, or 10ng/ml murine IL-4 to drive an M2 anti-inflammatory phenotype. Furthermore, to investigate if human bone marrow-derived mesenchymal stromal cells (hBM-MSCs) could influence this polarisation in a paracrine, cell-contact independent manner, $2x10^5$ MSCs were co-cultured with BMDMs using a 0.4µm transwell insert at the same time as stimulation (Figure 1). Gene expression and protein production were investigated after 24hrs.

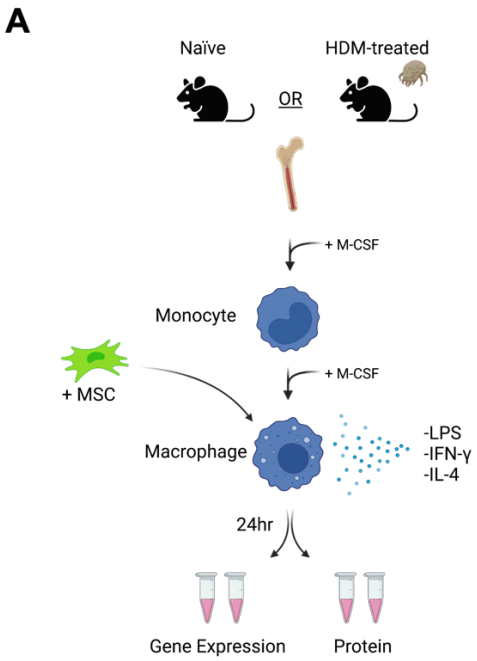


Figure 1. Development of a BMDM polarisation *in vitro* assay. A Schematic depicting workflow of BMDM polarisation from WT and $CATT_7$ mice under basal (Naïve) and diseased (HDM) conditions.

4.4.2 HDM challenge drives type 2 inflammation in the lung of WT and $CATT_7$ mice

Novel transgenic humanised MIF mice were generated to investigate the translational relevance of high levels of human MIF expression, under the functional promotor polymorphism. C57BL/6 mice were humanised by replacing the murine MIF gene with the human counterpart. Within this human MIF gene, 794 downstream of the promotor region, the number of tetranucleotide repeats correlates with MIF allele expression (Baugh et al., 2002), where 7 repeats of this tetranucleotide sequence 'CATT' generated $CATT_7$ mice, containing the high expressing MIF allele (Figure 2A). To characterise the inflammatory status of the lung after WT and $CATT_7$ mice received HDM intranasally in a model of allergic airway inflammation, cDNA from HDM-treated lungs were analysed for target genes of interest: M1 – *tnfα, il-6, il-1β, nos2, ifnγ* or M2 – *arg1, retnla, chi3l3* (Figure 2B). Lower cycle threshold (CT) values (23-30) were observed for the type 2 inflammatory genes *arg1*, *retnla* and *chi3l3*, compared to higher CT values (31-35) for type 1 *tnfα, il-6, il-1β, nos2, ifnγ* genes, indicating that HDM challenge facilitates type 2 inflammation in the lung of WT and $CATT_7$ mice (Supplementary table 1). Average CT values indicate that after HDM challenge, WT mice have higher expression of M2 genes *arg1* and *retnla* (29.38 and 22.94) than those in high human MIF expressing $CATT_7$ mice (30.02 and 23.12). However, $CATT_7$ mice demonstrated higher levels of *chi3l3* (24.66) compared to WT mice (25.06) (Figure 2B).

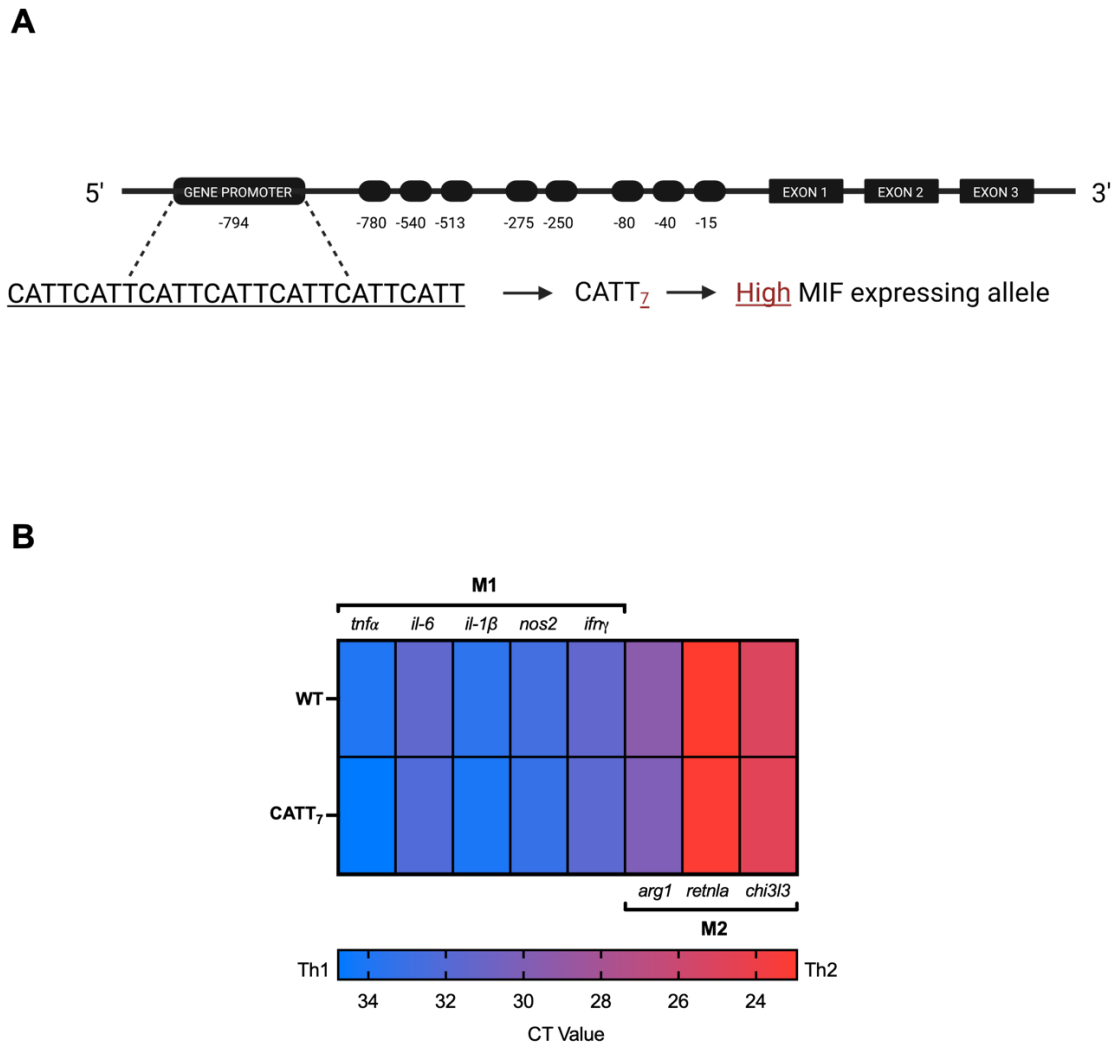


Figure 2. HDM challenge drives type 2 lung inflammation in CATT$_7$ mice expressing high levels of human MIF. A Novel humanised CATT$_7$ MIF mice containing the 7-repeat functional promotor polymorphism at position -794. **B** Average CT values for M1 and M2 macrophage markers from WT and CATT$_7$ lungs exhibit a type 2 phenotype after HDM challenge. Data represented as N=6 mice per group.

4.4.3 High human MIF expression does not significantly alter M1 or M2 polarisation in BMDMs at an mRNA level

Changes in gene expression in BMDMs from naïve WT and high human MIF expressing $CATT_7$ mice after stimulation with LPS, IFNγ and IL-4 were analysed by RT-PCR. $CATT_7$ mice displayed significantly increased levels of *tnfα* expression after LPS stimulation, compared to WT mice (Figure 3A). Conversely, significantly elevated levels of *il-6* and *il-1β* gene expression were observed in WT mice compared to $CATT_7$ mice. No significant changes in relative *nos2* and *ifnγ* expression were noted between WT and $CATT_7$ mice. Additionally, no significant changes were noted in M1 markers after IFNγ stimulation. Upon investigating markers of alternatively activated M2 macrophages, no significant differences in *arg1, retnla, chi3l3, il-10 and tgf-β* expression were seen between mouse groups after LPS or IL-4 polarising conditions (Figure 3B).

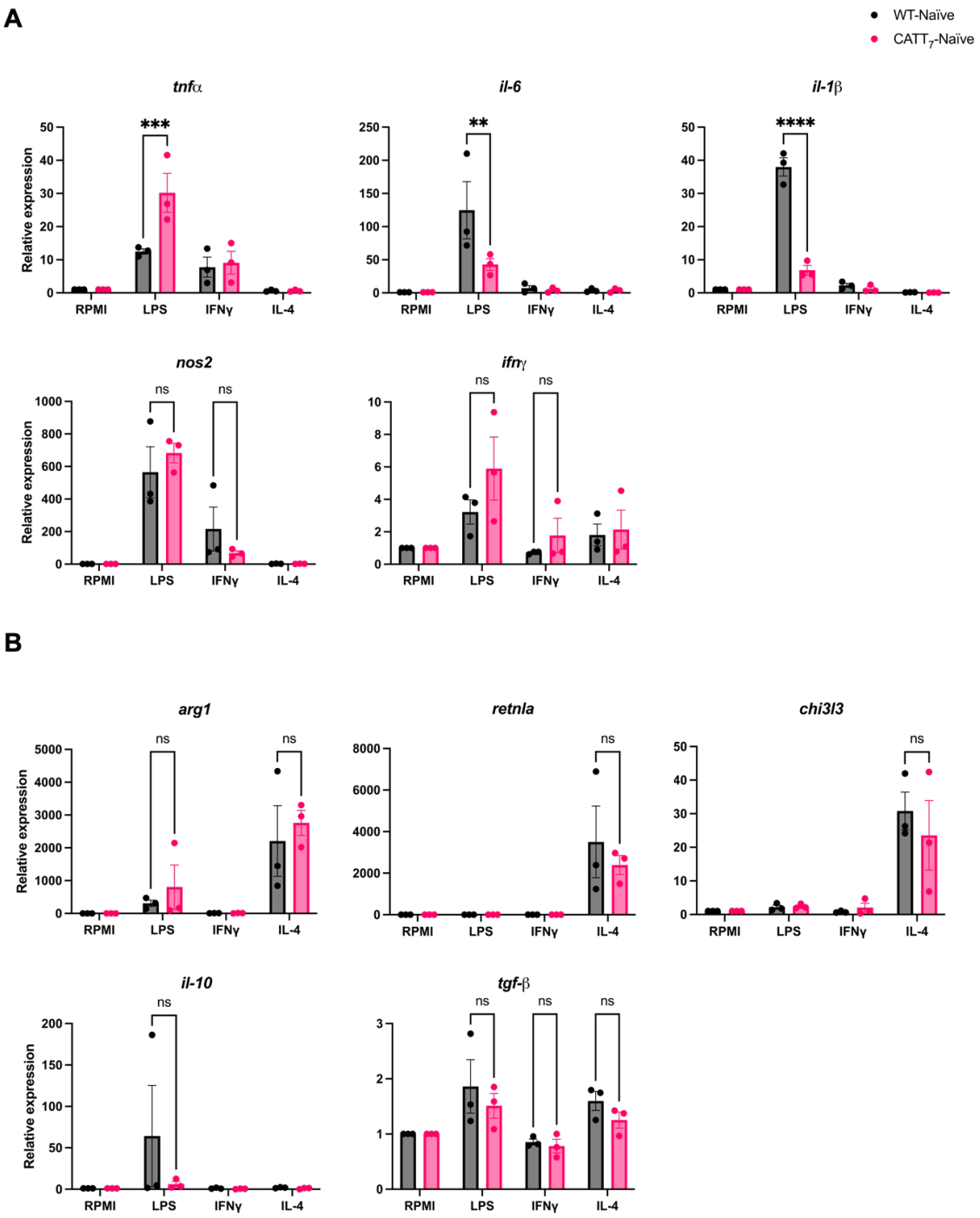


Figure 3. BMDMs from naïve WT and high human MIF CATT$_7$ mice are not predominantly classically nor alternatively activated at the mRNA level. BMDMs from naïve WT and CATT$_7$ mice were stimulated with polarising conditions; LPS, IFNγ or IL-4 for 24hr. Gene expression of macrophage markers **A** M1 (*tnfα*, *il-6*, *il-1β*, *nos2* and *ifnγ*) or **B** M2 (*arg1*, *retnla* and *chi3l3*) were analysed using RT-PCR. Data are presented as mean ± SEM; N=3 mice per group **p<0.01, ***p<0.001, ****p<0.0001.

4.4.4 BMDMs from naïve CATT$_7$ mice have increased M1 protein production compared to WT mice

We previously observed no trend in M1 or M2 gene expression analysis in naïve WT or CATT$_7$ mice (Figure 3). To confirm this at a protein level, TNFα, IL-6, IL-1β, nitrite and arginase activity were measured in the supernatants of naïve WT and CATT$_7$ BMDMs after being treated with polarising conditions. After LPS stimulation for 24hrs, high human MIF expressing CATT$_7$ mice had significantly higher TNFα protein production compared to WT mice. Both WT and CATT$_7$ mice had significantly elevated TNFα after LPS stimulation compared to RPMI control (Figure 4A). Although both naïve WT and CATT$_7$ BMDMs treated with LPS had significantly increased levels of IL-6 compared to RPMI controls, no significant difference was observed between mouse groups (Figure 4A). Similar to TNFα production, CATT$_7$ mice had significantly enhanced IL-1β production compared to WT mice, again with LPS treated BMDMs having significantly elevated levels of this type 1 inflammatory protein compared to the RPMI control (Figure 4A). No statistical difference was noted in nitrate production between naïve WT and CATT$_7$ mice.

As an indicator of M2 type 2 inflammatory protein production, arginase activity was measured in the supernatants of naïve WT and CATT$_7$ mice after polarising conditions (Figure 4B). Specifically, after LPS and IL-4 stimulation, WT mice had significantly increased arginase activity compared to high human MIF expressing CATT$_7$ mice (Figure 4B). Moreover, arginase activity in LPS stimulated BMDMs from WT mice was significantly boosted compared to RPMI controls. Furthermore, no significant difference in arginase activity was noted between any naïve BMDM CATT$_7$ groups (Figure 4B).

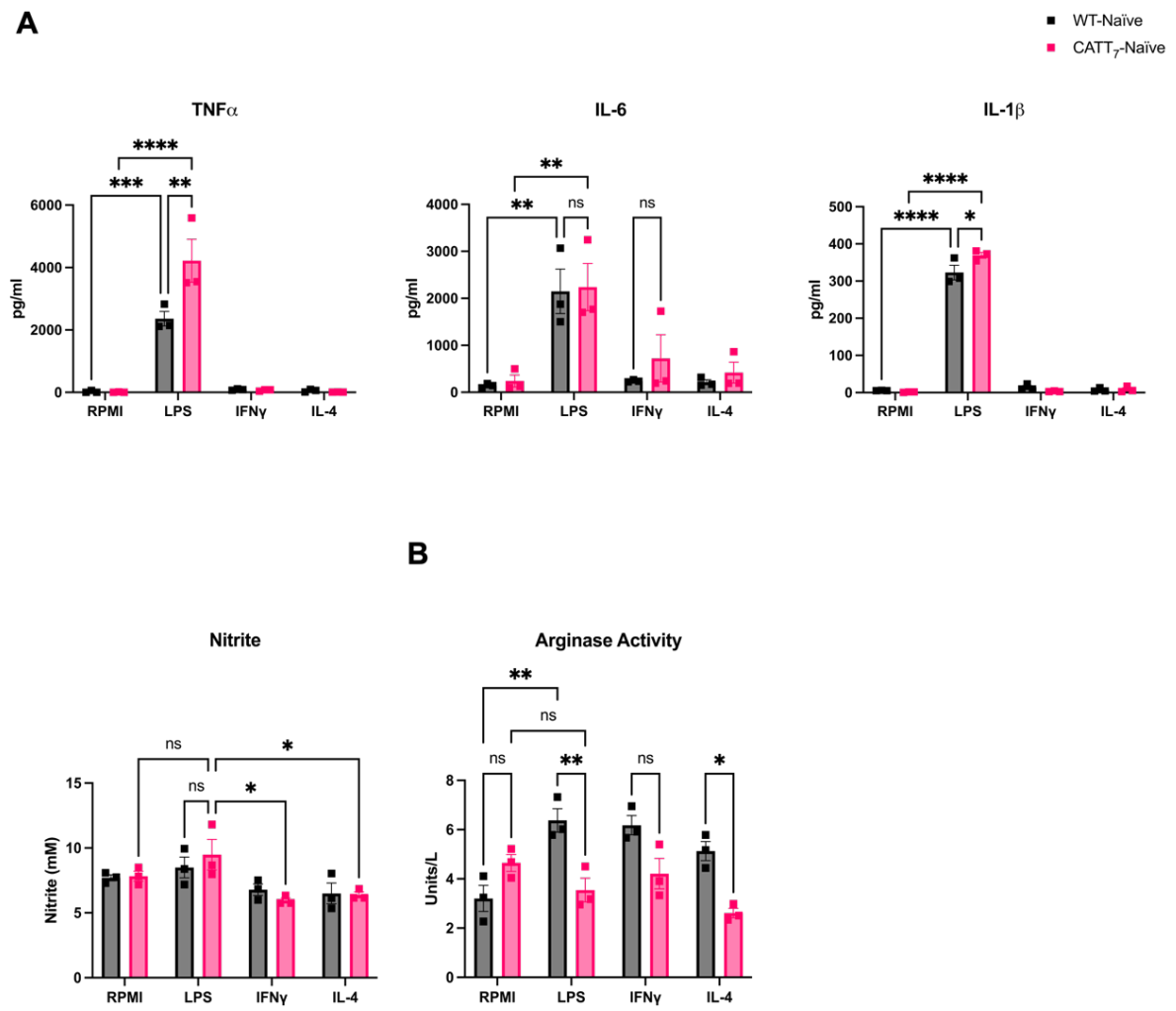


Figure 4. BMDMs from high human MIF CATT$_7$ mice demonstrate elevated M1 protein markers after LPS stimulation. BMDMs from naïve WT and CATT$_7$ mice were stimulated with polarising conditions; LPS, IFNγ or IL-4 for 24hr. Protein production from WT and CATT$_7$ BMDMs were measured. **A** TNFα, IL-6 and IL-1β, nitrite production. **B** Arginase activity. Data are presented as mean ± SEM; N=3 mice per group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

4.4.5 CATT$_7$ mice primed with HDM *in vivo* exhibit an enhanced M1 mRNA signature to a secondary heterologous stimulus

The immune system can be primed by a primary stimulus (e.g., HDM), leading to an activation of gene transcription (Table 1). Upon encountering a secondary stimulus, often heterologous, this results in an additive or synergistic immune response. After investigating the phenotypic status of the BMDM population from WT and high human MIF expressing CATT$_7$ mice, this study set out to directly compare naïve mice and mice exposed to a three week HDM model of allergic airway inflammation (Figure 5A).

Interestingly, CATT$_7$ mice challenged with HDM three times a week for three weeks exhibited a boosted M1 phenotype, with significantly elevated levels of genes associated with classically activated M1 macrophages. BMDMs from HDM-treated CATT$_7$ mice had significantly higher *tnfα* gene expression compared to naïve CATT$_7$ mice, after both LPS and IFNγ stimulation. However, HDM-treated WT mice also had significantly higher levels of *tnfα* gene expression. After LPS stimulation, HDM-treated CATT$_7$ BMDMs demonstrated significantly increased *il-6* expression compared to BMDMs from naïve CATT$_7$ at the mRNA level, whilst no significant difference was seen between HDM-treated and naïve WT mice (Figure 5B). Similarly, a significant increase in *il-1β* expression was noted in HDM-treated CATT$_7$ BMDMs after LPS and IFNγ stimulation, compared to BMDMs from naïve CATT$_7$ mice. No difference was observed between BMDMs from HDM-treated and naïve WT mice. BMDMs from HDM-treated CATT$_7$ mice had significantly increased *nos2* gene expression compared to BMDMs from naïve CATT$_7$ mice (Figure 5B). Although BMDMs from HDM-treated WT mice also had significantly increased *nos2* expression compared to their naïve counterpart, expression levels were still significantly lower compared to BMDMs from HDM-

treated CATT$_7$ mice (Figure 5B). Interestingly, *ifnγ* expression was significantly reduced after HDM challenge, in both WT and CATT$_7$ mice.

Looking at markers of M2 alternatively activated macrophages, the expression of *arg1*, *retnla*, *chi3l3*, *il-10* and *tgf-β* were also investigated in these mice. After HDM challenge, BMDMs from CATT$_7$ were not significantly different to naïve CATT$_7$ BMDMs in their expression of *arg1* and *retnla* (Figure 5C). The opposite was true for BMDMs from HDM-treated WT mice, which had significantly elevated *arg1* and *retnla* expression compared to naïve WT BMDMs, indicating that WT mice may also be primed by the clinically relevant house dust mite allergen prior to IL-4 stimulation. *Il-10* and *chi3l3* expression followed a similar trend to that seen with M1 markers in figure 4B, as BMDMs from HDM-treated CATT$_7$ mice displayed significantly increased relative gene expression compared to BMDMs from naïve CATT$_7$ mice (Figure 5C). HDM-treated WT BMDMs had significantly elevated levels of *il-10* compared to naïve WT BMDMs, albeit still significantly lower than CATT$_7$ under the same conditions. Levels of *chi3l3* expressed by naïve and HDM-treated WT BMDMs were not significantly different. *Tgf-β* gene expression was not statistically different to HDM-treated BMDMs compared to naïve BMDMs, in both mouse groups (Figure 5C).

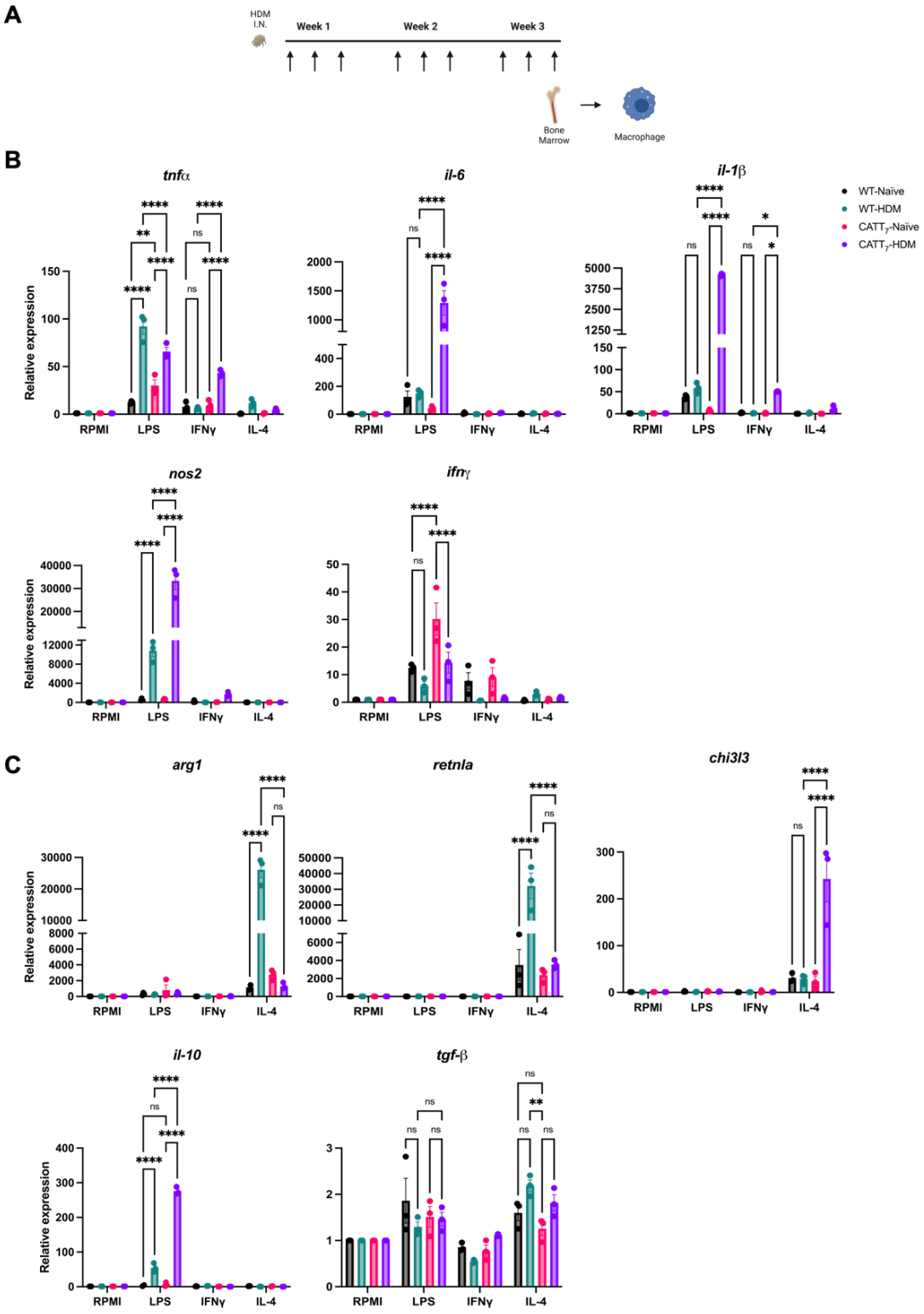


Figure 5. HDM primes BMDMs from CATT$_7$ mice, increasing their relative M1 gene expression. **A** Model of HDM-induced allergic airway inflammation in WT and CATT$_7$ mice, where BMDMs were isolated 4hrs after last challenge on day 18. **B** BMDMs from HDM-treated and naïve CATT$_7$ and WT mice were stimulated with LPS or IFNγ to investigate gene expression of M1 markers **C** BMDMs from

HDM-treated and naïve $CATT_7$ and WT mice were stimulated with IL-4 to investigate gene expression of M2 markers. Data are presented as mean ± SEM; N=3 mice per group. *p<0.05, **p<0.01, ****p<0.0001.

4.4.6 *In vivo* HDM challenge primes $CATT_7$ mouse BMDMs, boosting their M1 response to a heterologous secondary stimuli

Analysis of gene expression of M1 and M2 inflammatory markers previously suggested that BMDMs from these mice, particularly high human MIF expressing $CATT_7$ mice, were primed due to a significant increase in gene expression after a heterologous secondary stimulus (e.g., LPS, IFNγ or IL-4) (Figure 5). A similar trend was noted when protein levels were measured in supernatants of BMDMs from HDM-treated $CATT_7$ mice after LPS stimulation (Figure 6A). HDM-treated $CATT_7$ BMDMs had significantly elevated levels of TNFα and IL-6 compared to naïve $CATT_7$ BMDMs. In contrast to *il-1β* gene expression data shown in figure 4B, HDM-treated $CATT_7$ BMDMs had significantly lower IL-1β protein in their supernatant after LPS stimulation compared to naïve $CATT_7$ BMDMs (Figure 6A). Significantly higher nitrite production was observed in BMDMs from HDM-treated $CATT_7$ mice compared to their naïve counterpart, whilst no significant priming effect was noted between WT BMDM groups (Figure 6A).

Previously, no priming effect was seen in $CATT_7$ mice when investigating anti-inflammatory macrophage-induced *arg1* expression at the mRNA level (Figure 5B). However, although arginase activity in BMDMs from HDM-treated $CATT_7$ mice was significantly increased compared to BMDMs from naïve $CATT_7$ mice, there was no significant difference observed between HDM-treated $CATT_7$ BMDMs that received a secondary LPS stimulation, compared to those that did not (Figure 6B). BMDMs from HDM-treated WT mice exhibited this HDM-priming effect, as they had significantly enhanced arginase activity compared to

BMDMs from naïve WT mice. Moreover, HDM-treated WT BMDMs that were stimulated with LPS had significantly higher arginase activity compared to those that did not receive a secondary stimulus (Figure 6B).

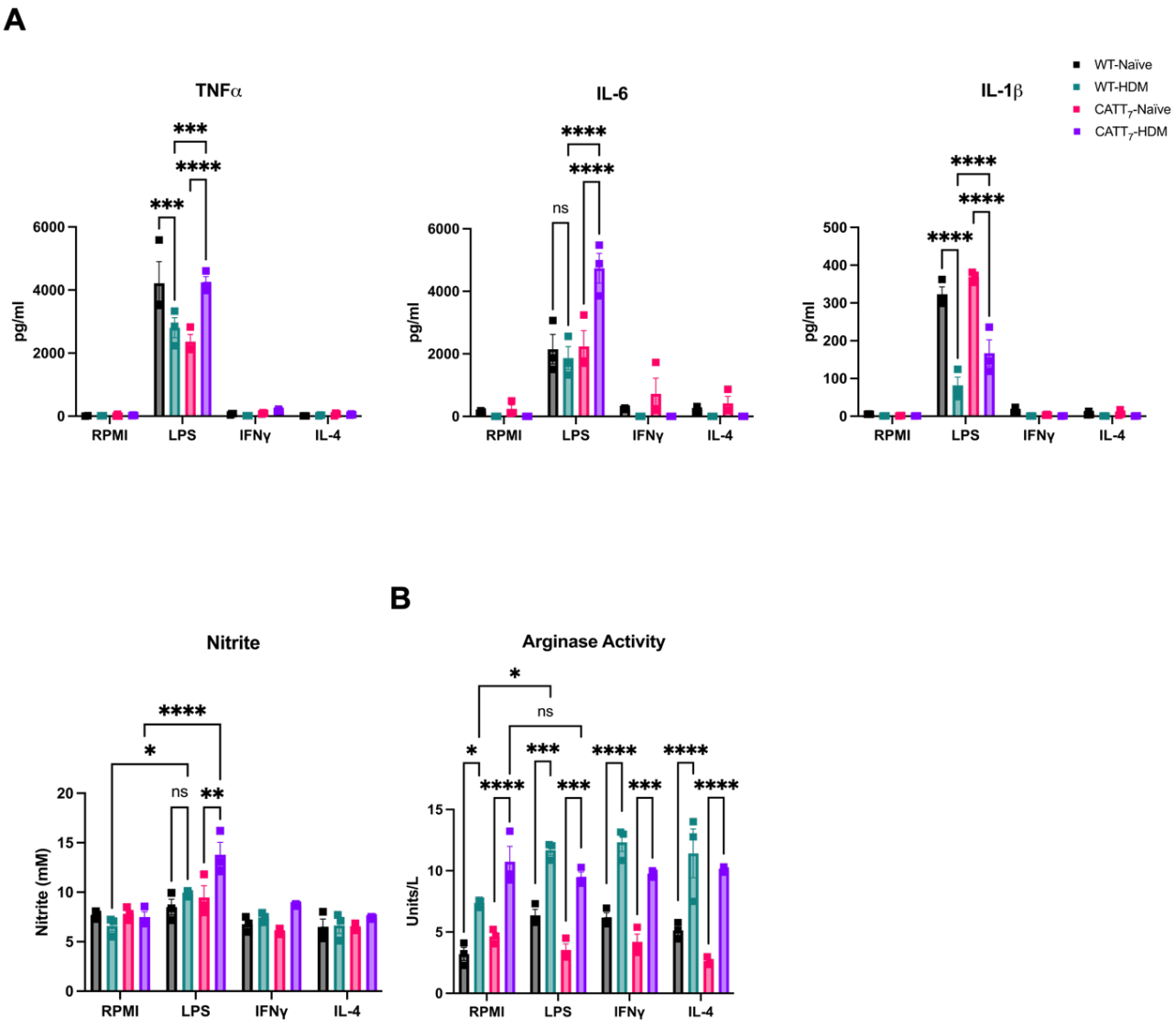


Figure 6. CATT$_7$ mice BMDMs demonstrate a priming effect after HDM challenge, with enhanced protein production. A BMDMs from HDM-treated and naïve CATT$_7$ and WT mice were stimulated with LPS or IFNγ to measure M1 protein markers. **B** BMDMs from HDM-treated and naïve CATT$_7$ and WT mice were stimulated with IL-4 to investigate the production of M2 protein markers. Data are presented as mean ± SEM; N=3 mice per group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

4.4.7 HDM priming of BMDMs is dependent on MIF expression

HDM challenge primes BMDMs from high human MIF expressing $CATT_7$ mice, amplifying their response to LPS by significantly enhancing their gene expression and protein production of M1 markers such as TNFα and IL-6 (Figure 6). This priming effect was also observed in BMDMs from HDM-treated WT mice, but to a lesser extent to that seen in $CATT_7$ BMDMs. Alas, this study set out to investigate if the susceptibility to immune priming by HDM was dependent on the expression of human MIF. WT and human MIF expressing $CATT_7$ mice were challenged intranasally with HDM three times a week for three weeks as described previously. A small molecular weight MIF inhibitor, SCD-19, was administered intraperitoneally twice a week for the full three weeks of this model of allergic airway inflammation (Figure 7A). BMDMs were isolated 4hrs after last challenge and cultured in M-CSF supplemented cRPMI for 7 days, before being stimulated with LPS, IFNγ or IL-4.

SCD-19 significantly blocked the primed immune response observed in BMDMs from $CATT_7$ mice, previously demonstrated in Figure 5 and 6. After receiving this potent MIF inhibitor for the duration of the HDM model, BMDMs from $CATT_7$ mice had significantly lower expression of all M1-associated genes, including *tnfα*, *il-6*, *il-1β*, *nos2* and *ifnγ* compared to $CATT_7$ mice that received the vehicle control. Importantly, BMDMs from WT mice that received SCD-19 had no significant differences in the expression of these genes, with the exception of *tnfα*, which was significantly reduced compared to the vehicle control after LPS stimulation (Figure 7B).

BMDMs from high human MIF expressing $CATT_7$ mice that received SCD-19 exhibited no significant changes in the expression of the M2 markers *arg1*, *retnla* or *chi3l3*

(Figure 7C) compared to those that received the vehicle control. Administration of SCD-19 to WT mice resulted in a significant decrease in their BMDM expression of *retnla* after IL-4 stimulation, with expression levels comparable to those expressed by $CATT_7$ BMDMs. Conversely, BMDMs from SCD-19 treated WT mice displayed increased expression of *arg1* compared to BMDMs from WT mice that received the vehicle control. Similarly, BMDMs from SCD-19 treated WT mice displayed an increase in *chi3l3* expression compared to WT mice that received the vehicle control, however this was not significant (Figure 7C).

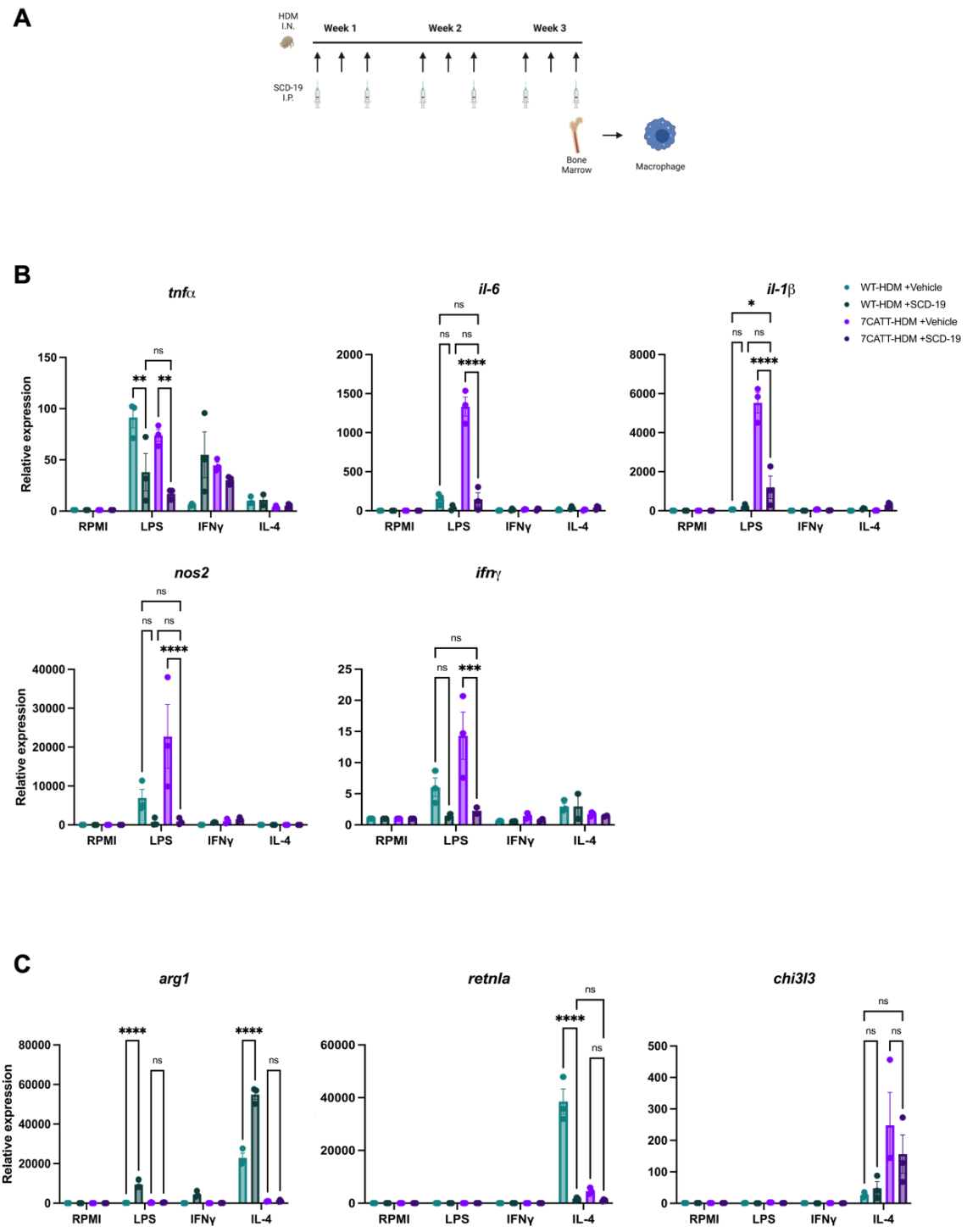


Figure 7. HDM-induced priming in CATT$_7$ mice is human MIF-dependent. A MIF antagonist SCD-19 is administered intraperitoneally twice a week for three weeks in a HDM-induced model of allergic asthma. BMDMs were isolated from WT and CATT$_7$ on day 18, 4hr after last challenge. After being stimulated with polarising conditions; LPS, IFNγ or IL-4 for 24hr, **B** M1 and **C** M2 gene expression was analysed by RT-PCR. Data are presented as mean ± SEM; N=3 mice per group. **p<0.01, ***p<0.001, ****p<0.0001.

4.4.8 SCD-19 attenuates the production of M1 macrophage protein markers in high human MIF $CATT_7$ mice primed with HDM

High human MIF expressing $CATT_7$ mice that received the MIF inhibitor SCD-19 had significantly reduced M1 gene expression compared to $CATT_7$ mice that received vehicle control (Figure 7A). SCD-19 had no effect on WT BMDM M1 gene expression, except where MIF inhibition significantly reduced *tnfα* expression (Figure 7B). Whilst M1 gene expression of SCD-19 $CATT_7$ mice was found to be significantly altered (Figure 7B), protein content was also measured in the supernatants generated from BMDMs of $CATT_7$ mice that received SCD-19 (Figure 8), to validate the gene expression data (Figure 7). SCD-19 significantly reduced TNFα and IL-6 protein production in $CATT_7$ mice compared to vehicle control (Figure 8A). A decrease in IL-1β protein production was also noted in $CATT_7$ mice that received the MIF inhibitor SCD-19, compared to mice that received a vehicle control (Figure 8A). Similar to the data investigating gene expression, SCD-19 had no impact on IL-6 or IL-1β protein production in BMDMs from WT mice, but a significant reduction in TNFα was noted (Figure 8A).

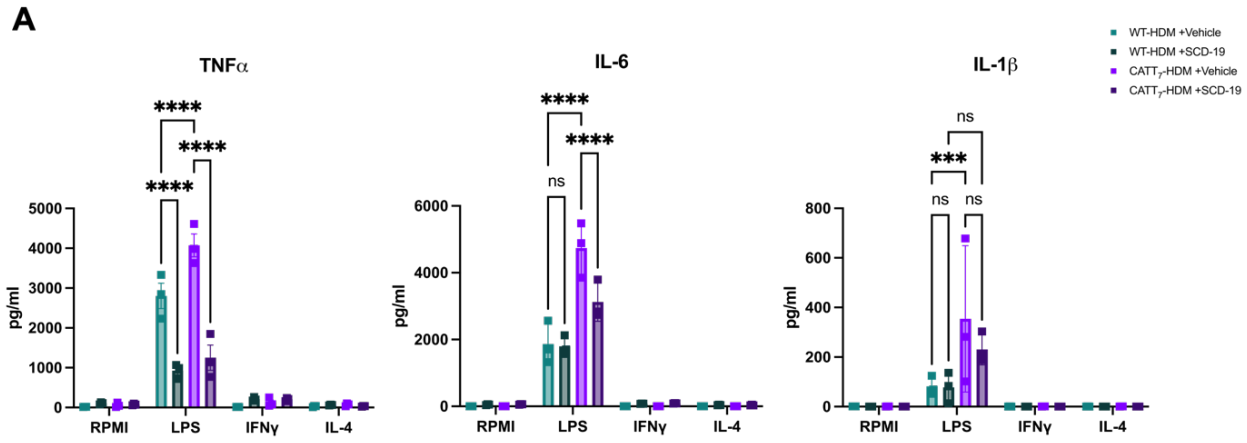


Figure 8. Small molecule MIF inhibitor SCD-19 abrogates protein production associated with priming effect after HDM-challenge in transgenic CATT$_7$ mice. A Protein production from BMDMs isolated from CATT$_7$ and WT mice after HDM challenge and SCD-19 treatment *in vivo*. Data are presented as mean ± SEM; N=3 mice per group. ***p<0.001, ****p<0.0001.

4.4.9 hBM-MSCs block HDM priming effect when co-cultured with BMDMs from CATT$_7$ mice

MSCs are known for their ability to calm an over-zealous immune response through the secretion of paracrine immunomodulatory factors (Cahill et al., 2016; Dunbar et al., 2021; English, 2013; Kennelly et al., 2016; Xia et al., 2019). Novel transgenic mice expressing the human MIF gene containing the CATT$_7$ functional promotor polymorphism exhibited a significant increase in M1 gene and protein expression after HDM challenge, indicating that the immune response of these mice was primed prior to receiving a secondary LPS stimulation (Figure 5 and 6). Moreover, this priming effect was confirmed to be specific to elevated human MIF expression, as this increased M1-priming effect was no longer evident in CATT$_7$ mice that received the potent MIF inhibitor, SCD-19 (Figure 7 and 8).

Using a transwell system, hBM-MSCs co-cultured with differentiated-CATT$_7$ BMDMs on day 7 (Figure 9A) significantly reduced M1 marker gene expression after LPS stimulation (Figure 9B). MSCs significantly decreased not only *tnfα* gene expression in CATT$_7$ mice, but also in WT mice. However after IFNγ stimulation, these stromal cells could only significantly reduce *tnfα* expression in CATT$_7$ mice, as no significant change was seen in BMDMs from WT mice (Figure 9A). Furthermore, when co-cultured with BMDMs from CATT$_7$ mice, MSCs significantly reduced *il-6* and *il-1β* gene expression, again having no effect on WT BMDMs. Following this trend, MSCs also decreased *nos2* and *ifnγ* expression in CATT$_7$ mice, however MSCs significantly increased the expression of *nos2* in BMDMs from WT mice (Figure 9B).

As CATT$_7$ BMDMs did not exhibit increased expression of M2 *arg1* and *retnla* after HDM priming, it is not surprising that no effect was seen with these genes after MSC co-culture (Figure 9C). Although *chi3l3* gene expression was significantly increased in CATT$_7$ mice after HDM challenge (Figure 5C), MSCs decreased, but not significantly, *chi3l3* expression (Figure 9C). However, MSCs effectively abrogated *il-10* gene expression in a significant manner. Striking effects were noted with M2 gene expression when MSCs were co-cultured with BMDMs from WT mice, as MSCs significantly decreased levels of *arg1*, *retnla* and *il-10*. Conversely, MSCs significantly increased expression of *chi3l3* in BMDMs from WT mice compared to BMDMs that were not co-cultured with these stromal cells (Figure 9C).

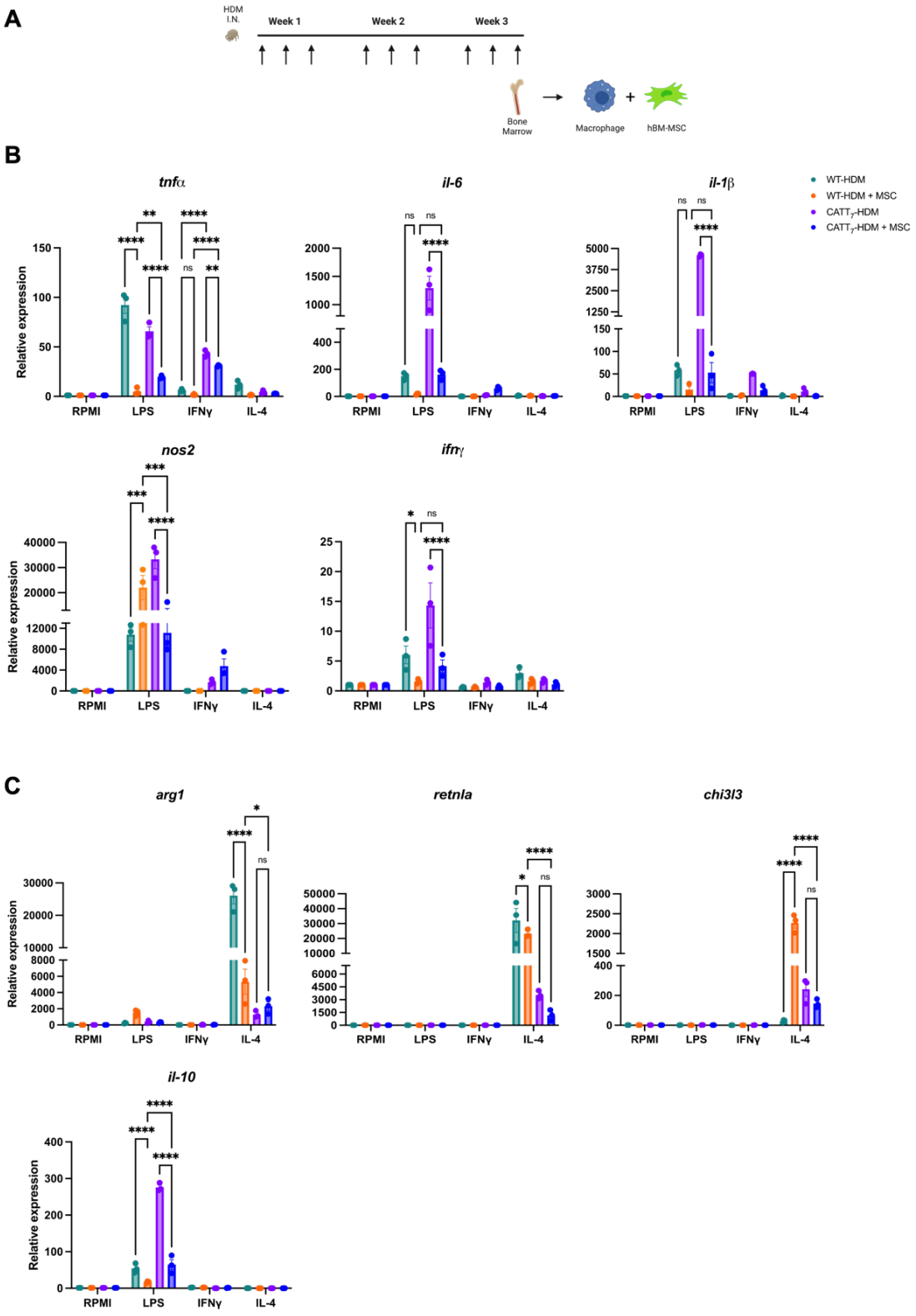


Figure 9. hBM-MSCs block HDM-induced priming effect in CATT$_7$ mice, decreasing their M1 and M2 macrophage gene expression. A BMDMs are isolated from CATT$_7$ and WT mice on day 18 of the HDM model. Once differentiated after culturing in M-CSF supplemented media for 7 days, hBM-MSCs are co-cultured with BMDMs using a 0.4µm transwell insert. **B** hBM-MSCs were co-cultured

with HDM-treated BMDMs from $CATT_7$ and WT mice and after LPS or IFNγ stimulation for 24hrs, the relative gene expression of M1 macrophage markers were analysed. **C** Similarly after IL-4 stimulation, the gene expression of M2 markers *arg1*, *retnla*, *chi3l3* and *il-10* were analysed after MSC co-culture with HDM-treated $CATT_7$ and WT BMDMs. Data are presented as mean ± SEM; N=3 mice per group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

4.4.10 hBM-MSCs decrease M1 protein production in HDM-primed $CATT_7$ mice, but do not alter protein production in WT mice

MSCs effectively calmed BMDMs from HDM-primed $CATT_7$ mice by significantly reducing their pro-inflammatory gene expression after LPS stimulation (Figure 9B). This was confirmed at the protein level, where MSCs significantly reduced the production of TNFα, IL-6, IL-1β and nitrate in the supernatants of $CATT_7$ BMDMs (Figure 10B). Although MSCS significantly altered the expression of these target gene markers of M1 macrophage activation in BMDMs from HDM-treated WT mice (Figure 8A), no significant differences were observed at the protein level (Figure 10A).

Upon investigating changes in M2 protein production, specifically by measuring arginase activity in BMDMs of HDM-treated WT and high human expressing $CATT_7$ mice, MSCs significantly reduced arginase activity in WT mice after LPS and IFNγ stimulation (Figure 10B). Apart from significantly decreasing $CATT_7$ BMDM arginase activity under basal RPMI conditions, MSCs had no significant effects on $CATT_7$ BMDM arginase activity under any polarising conditions (Figure 10B).

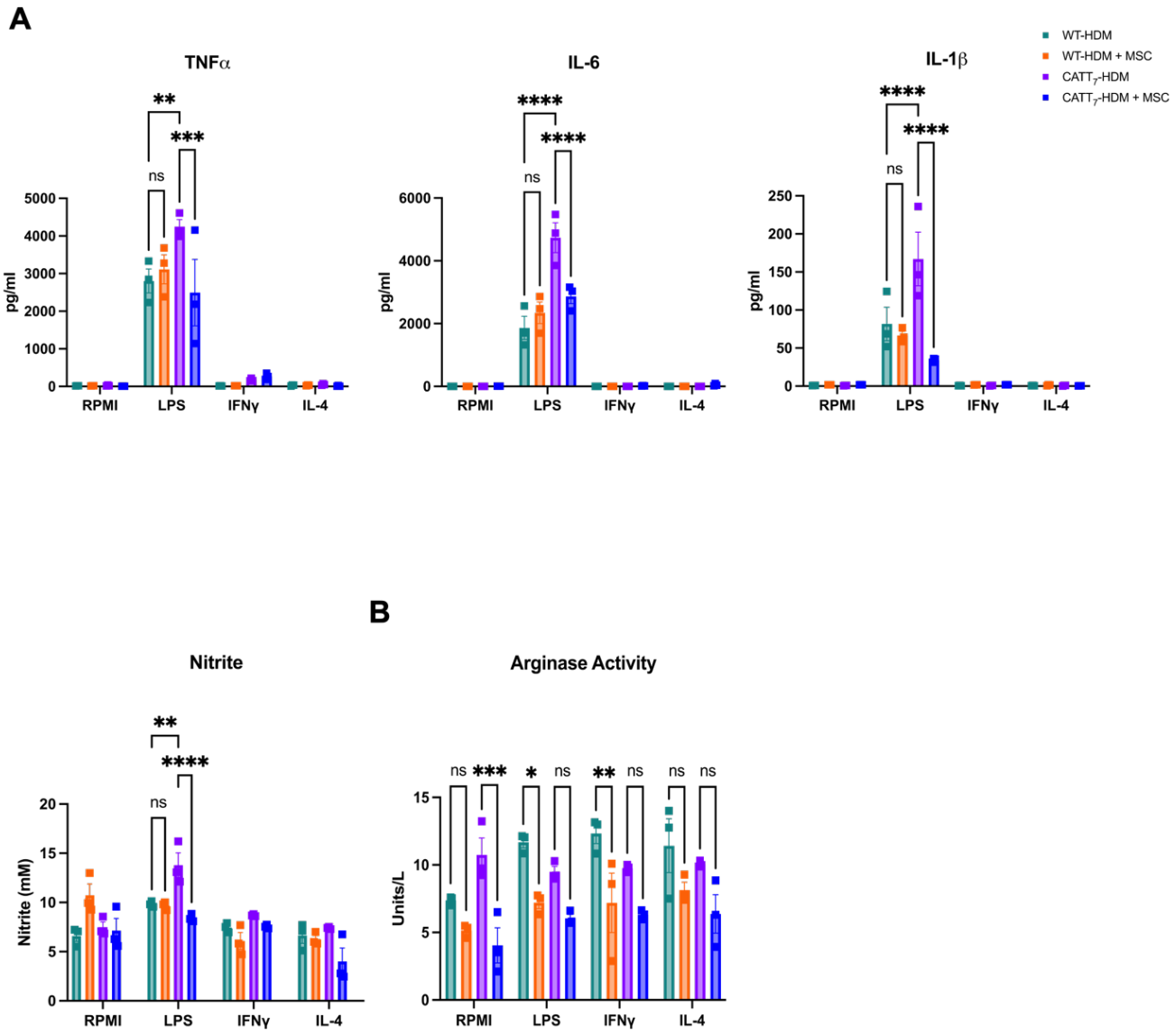


Figure 10. hBM-MSCs block HDM-induced priming effect in CATT$_7$ mice, decreasing their M1 and M2 macrophage activity. **A** M1 protein markers TNFα, IL-6, IL-1β and nitrate were measured in BMDMs from HDM-treated CATT$_7$ and WT mice that were co-cultured with hBM-MSCs when stimulated with LPS and IFNγ. **B** Arginase activity was measured in supernatants of BMDMs from HDM-challenged CATT$_7$ and WT mice after co-cultured with MSCs during polarising conditions. Data are presented as mean ± SEM; N=3 mice per group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

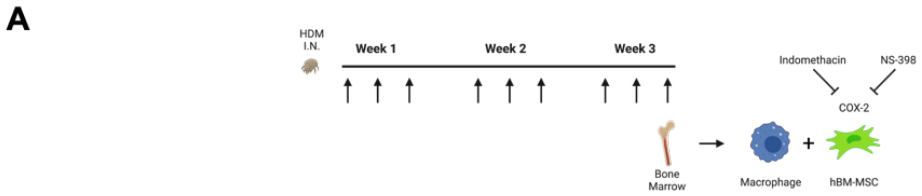
4.4.11 Cyclo-oxygenase enzymes influence MSCs ability to block HDM-priming in $CATT_7$ mice

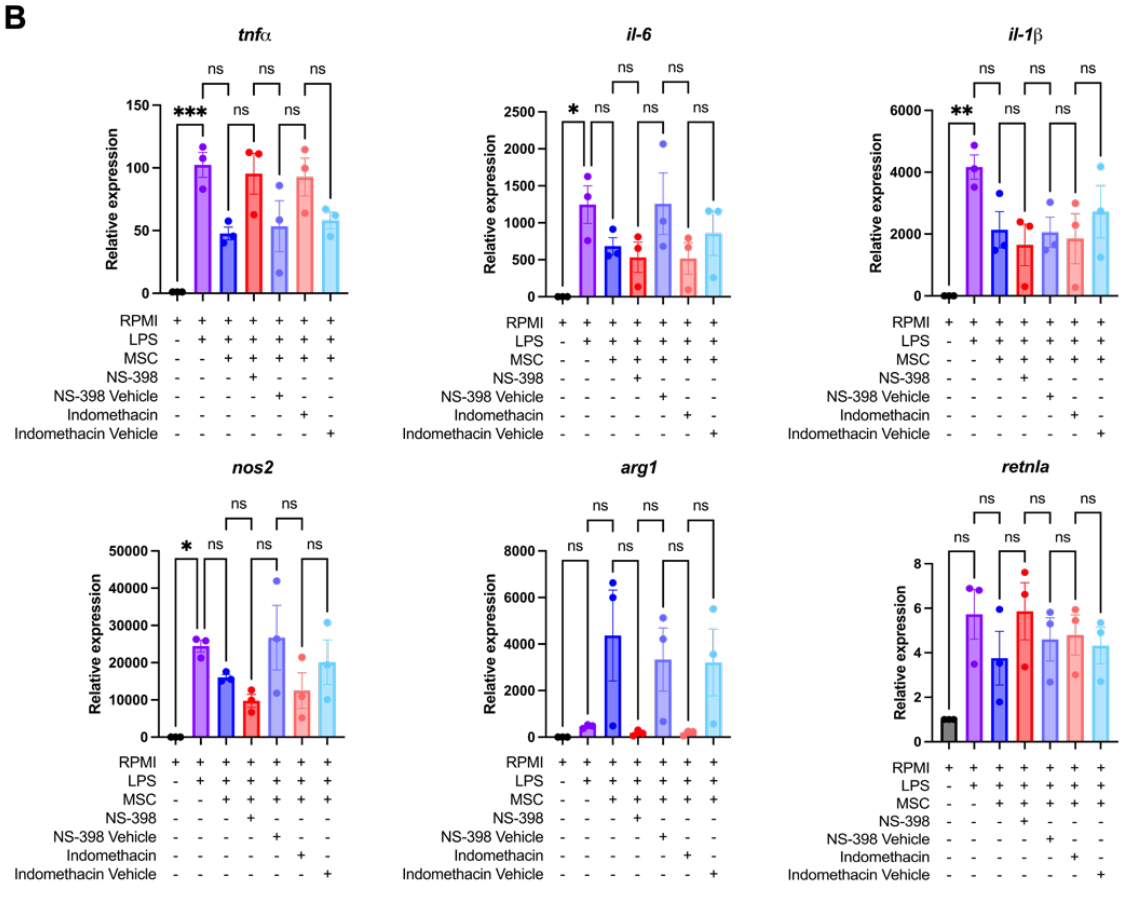
Human bone marrow-derived mesenchymal stromal cells (hBM-MSCs) significantly abrogated the amplified M1-specific immune response in HDM-primed $CATT_7$ BMDMs after a heterologous LPS stimulation (Figure 9 and 10). This study next sought to elucidate the mechanism by which MSCs could significantly calm these phagocytic cells. As cyclo-oxygenase (COX) enzymes are known to facilitate MSC's immunosuppressive capabilities, a non-specific COX-1 and 2 inhibitor indomethacin and a highly-specific COX-2 inhibitor NS-398 were introduced to further investigate the role of COX enzymes in an MSC co-culture with HDM-primed BMDMs from WT or high human MIF expressing $CATT_7$ mice. Notably, after MSCs were incubated with COX inhibitors for 24hrs, cells were sufficiently washed with warm PBS to ensure BMDMs were not cross-contaminated with inhibitor residue when MSCs were transferred to a co-culture using transwell inserts (Figure 11A).

Interestingly, when MSC COX enzymes were inhibited with either indomethacin or NS-398, MSCs could no longer suppress $CATT_7$ BMDM *tnfα* gene expression (Figure 11B) or protein production (Figure 11C). MSCs treated with a vehicle control had no difference in their ability to suppress in *tnfα* expression (Figure 11B) or protein production (Figure 11C) compared to MSCs that received no treatment. When COX enzymes were blocked in MSCs with indomethacin or NS-398 and were co-cultured with BMDMs from $CATT_7$ mice, no effect was seen in IL-6 gene expression (Figure 11B) or protein production (Figure 11C), indicating that MSCs suppressed this cytokine in a COX-independent manner. Similarly, MSCs treated with indomethacin or NS-398 did not significantly differ in their ability to suppress *il-1β* gene expression from non-inhibited MSCs (Figure 11B).

Indomethacin had no effect on MSC's ability to reduce *nos2* expression in HDM-primed $CATT_7$ BMDMs compared to the vehicle control, however NS-398 treated MSCs had improved efficacy by further decreasing *nos2* expression (Figure 11B). Exciting findings were observed in *arg1* gene expression when NS-398 and indomethacin-treated MSCs were co-cultured with BMDMs from HDM-primed $CATT_7$ mice received LPS stimulation, where their immunosuppressive function was boosted, decreasing *arg1* expression (Figure 11B). Lastly, MSCs that had COX-2 inhibited by NS-398 no longer suppressed *retnla* gene expression, but indomethacin treatment had no effect on MSC function relating to *retnla* expression, perhaps indicating a specific role for COX-2 (Figure 11B).

To investigate that the results observed from MSC co-culture in figure 11 and that no off-target effects from indomethacin or NS-398 were indirectly influencing BMDM gene expression, pilot controls involving the direct application of these inhibitors to BMDMs from $CATT_7$ mice were carried out. Importantly, no changes were seen between LPS stimulated HDM-primed $CATT_7$ BMDMs that received indomethacin or NS-398 for 24hrs, and those that did not (Supplementary figure 1).


A
HDM
I.N.
Week 1
Week 2
Week 3
Indomethacin
NS-398
COX-2
Bone
Marrow
Macrophage
hBM-MSC


B
tnfα
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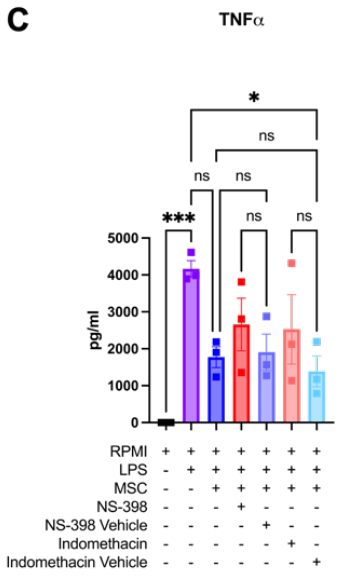
C
TNFα
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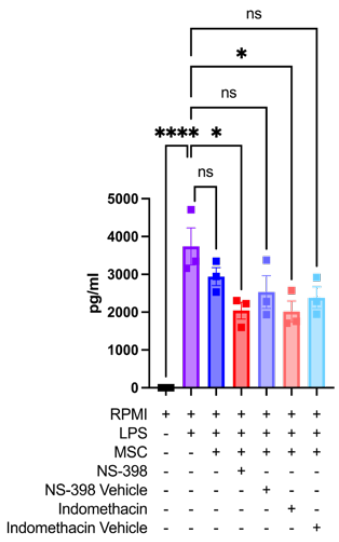
IL-6
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Figure 11. COX1/2 may play a role in MSC suppression of *in vivo* HDM-primed $CATT_7$ mice in response to LPS stimulation. A BMDMs are isolated from $CATT_7$ mice on day 18 of the HDM model. hBM-MSCs are incubated with COX-1 and COX-2 inhibitor indomethacin or COX-2 inhibitor NS-398, or appropriate vehicle controls for 24hrs. MSCs are washed with warm PBS before being co-cultured with BMDMs using a 0.4μm transwell insert, before being subjected to M1 and M2 polarising conditions **B** *tnfα*, *il-6*, *il-1β*, *nos2*, *arg1*, *retnla* and *chi3l3* gene expression from $CATT_7$ BMDMs co-cultured with MSCs were analysed by RT-PCR. **C** TNFα and IL-6 protein production in supernatants from $CATT_7$ BMDMs co-cultured with MSCs were measured by ELISA. Data are presented as mean ± SEM; N=3 mice per group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

4.4.12 HDM not only primes, but increases innate immune training in $CATT_7$ mice, which is blocked using a methyltransferase inhibitor MTA

This study has found that novel transgenic humanised MIF $CATT_7$ mice can be primed by HDM challenge, resulting in a paradigm shift, increasing M1 activation in BMDMs (Figure 5 and 6). This priming effect was also found to be dependent on human MIF expression (Figure 7 and 8).

Priming occurs at the individual cell level (e.g., macrophage), whereas the concept of innate immunity occurs within the bone marrow niche in the hematopoietic stem and progenitor cells (HSPCs). Furthermore, in innate immune training, the chromatin is opened and closed to allow epigenetic re-programming, such as the methylation of DNA controlled by a methyltransferase enzyme. Thus, utilising a methyltransferase inhibitor known as methylthioadenosine (MTA) in HSPCs isolated from the bone marrow of naïve $CATT_7$ mice, prior to the introduction of a stimulus, the concept of HDM-induced innate immunity can be further elucidated (Figure 12A). After the application of MTA, HSPCs were stimulated with HDM *in vitro* for 24hrs, before being washed in PBS and cultured in M-CSF supplemented media to allow for macrophage differentiation (Figure 12B). Importantly, differentiated-

macrophages were then cultured in a stimuli-free environment of cRPMI media alone to facilitate the rest period, that of which is pivotal to classify innate immunity. On day 10, differentiated-macrophages were stimulated with a secondary heterologous stimulus, LPS, before TNFα and IL-6 protein content were measured by ELISA (Figure 12B).

Human MIF expressing $CATT_7$ BMDMs that received the primary stimulus of HDM of this *in vitro* assay had low levels of TNFα and IL-6 production. BMDMs from $CATT_7$ mice stimulated with LPS alone had increased levels of TNFα and IL-6 production. Interestingly, $CATT_7$ HSPCs that received a primary stimulus of HDM on day 0 *in vitro* and their then differentiated BMDMs that received secondary heterologous stimulus of LPS on day 10 had the highest levels of TNFα (Figure 12C) and IL-6 (Figure 12D) production, indicating an innate immune training signature in these novel transgenic mice. Exposing the bone marrow cells at time 0 to a methyltransferase inhibitor (MTA) for 30 mins prior to addition of the primary stimulus (HDM) significantly reduced TNFα production in response to the secondary heterologous stimulus (LPS) (Figure 12C) due to the blocking of methylation of $CATT_7$ DNA and thus preventing any histone modifications occurring at the epigenetic level. A similar result was noted in IL-6 production in BMDMs who's progenitor cells were incubated with MTA on day 0 (Figure 12D), albeit to a lesser extent than observed in TNFα production. This reduction in protein markers associated with innate immunity was not seen when HSPCs were treated with a DMSO vehicle control on day 0. To ensure that the use of a methyltransferase inhibitor had no off-target effects on the bone marrow HSPC-derived macrophages, controls including the use of MTA and a DMSO vehicle control were implicated, concluding that MTA had no undesired effects relating to the general cell wellbeing or protein production (Figure 12C, D).

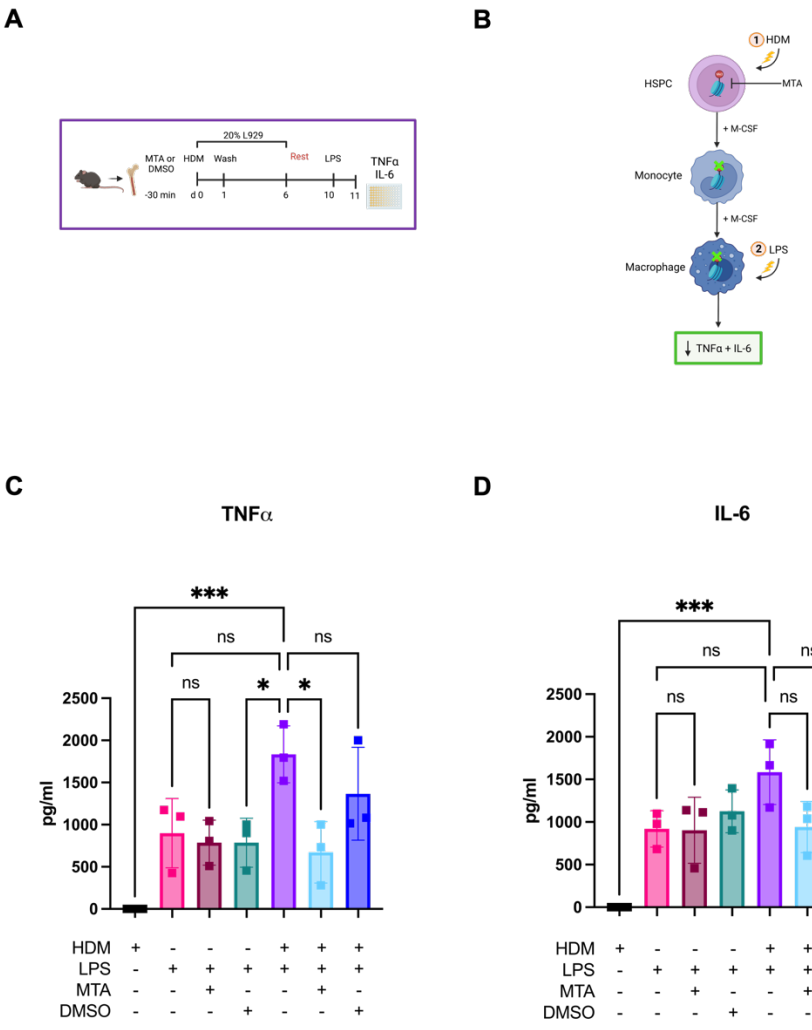

Figure 12. Methyltransferase inhibitor blocks innate immune training in bone marrow hematopoietic stem and progenitor cells from high human CATT$_7$ MIF mice. A To confirm that HDM can induce innate immunity in CATT$_7$ mice, in addition to priming, HSPCs were isolated from CATT$_7$ mice and a methyltransferase inhibitor MTA or vehicle control was added 30 minutes prior to any stimuli. **B** HSPCs were then stimulated with HDM for 24hr and co-cultured with MSCs. Cells were in a rest period from day 6 to 10, before being stimulated with LPS for 24hr, when TNFα and IL-6 protein production in differentiated-BMDM supernatants were analysed. **C** Inhibition of methyltransferase with MTA significantly reduced TNFα production by macrophages and thus trained immunity effect in HSPCs of high human MIF expressing CATT$_7$ mice compared to vehicle control. **D** MTA reduced macrophage IL-6 production, another marker of trained immunity in HSPCs of CATT$_7$ mice. Data are presented as mean ± SEM; N=3 mice per group. *p<0.05, ***p<0.001.

4.4.13 MSCs block trained innate immunity in $CATT_7$ HSPC-derived macrophages

As hBM-MSCs significantly blocked the enhanced M1 phenotype observed in BMDMs from high human MIF expressing $CATT_7$ mice after HDM priming (Figure 9 and 10), with their therapeutic impact on *tnfα* and *retnla* expression being COX-1/2 dependent (Figure 11), we then set out to investigate if MSCs would have a similar effect in suppressing or blocking innate immune training described in these humanised MIF mice (Figure 12C, D).

Hematopoietic stem and progenitor cells (HSPCs) were isolated from naïve $CATT_7$ mice and co-cultured with hBM-MSCs using transwell inserts, while being stimulated with HDM for 24hrs. HSPCs were cultured in M-CSF supplemented cRPMI media to facilitate macrophage differentiation, before entering a rest period from day 6 to day 10, where these BMDMs were then stimulated with LPS for 24hrs (Figure 13A). LPS-stimulated macrophages which originated from HSPCs that previously received a primary HDM stimulation on day 0, exhibited significantly higher levels of TNFα production compared to those that only received LPS alone (Figure 13B). When HSPC-derived macrophages received both a primary (HDM) and secondary (LPS) stimulus, a similar upregulation of IL-6 protein production was observed (Figure 13C). Strikingly, $CATT_7$ BMDMs derived from HSPCs that were co-cultured with MSCs on day 0, illustrated a significant reduction in TNFα production (Figure 13B). MSC transwell co-culture also decreased the level of IL-6 protein production by trained $CATT_7$ BMDMs (Figure 13C). MSCs had no significant effects on TNFα and IL-6 production by $CATT_7$ BMDMs that only received LPS stimulation (Figure 13B, C), proving the importance of having both a primary HDM stimulus on day 0 and a rest period followed by a secondary LPS stimulus in this innate immunity *in vitro* assay.

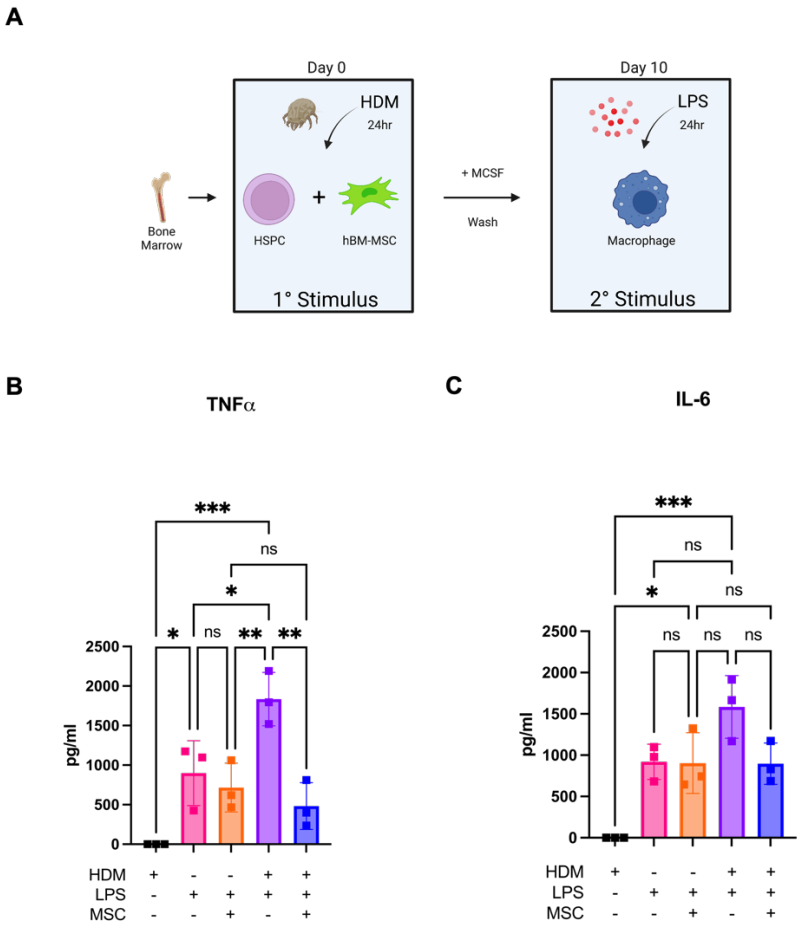

Figure 13. hBM-MSCs calm macrophages by blocking innate immune training effect in HSPCs from CATT$_7$ mice. A After isolation from the bone marrow of CATT$_7$ mice, HSPCs were stimulated with HDM for 24hr and co-cultured with MSCs using a 0.4µm transwell inserts. Cells were in a rest period from day 6 to 10, before being stimulated with LPS for 24hr. **B** TNFα and **C** IL-6 protein production in differentiated-BMDM supernatants were analysed by ELISA. Data are presented as mean ± SEM; N=3 mice per group. *p<0.05, **p<0.01, ***p<0.001.

4.5 Discussion

The concept of immunological memory was first thought to be exclusive to the adaptive immune system, however evidence of adaptation of the immune system has since been defined within the previously thought to be non-specific innate response of certain invertebrates, plants, animals and humans (Conrath et al., 2015; Gourbal et al., 2018; Kurtz, 2005). We have previously illustrated the important role of high levels of human MIF expression in the development and maintenance of asthma severity *in vivo*, using novel transgenic human MIF expressing $CATT_7$ mice (Dunbar and Hawthorne et al., 2023). This study is the first to elucidate the role of this human pro-inflammatory cytokine in facilitating training of the innate immune system, demonstrating that HDM can prime and train human MIF expressing $CATT_7$ macrophages and their precursors, resulting in a heightened inflammatory response to a secondary heterologous insult.

To first characterise their baseline polarisation ability, $CATT_7$ and WT mice were treated with a primary stimulus of LPS, IFNγ or IL-4 and the expression of M1 (*tnfα, il-6, il-1β, nos2, ifnγ*) or M2 (*arg1, retnla, chi3l3)* markers were elucidated through gene expression (Figure 3) and protein analysis (Figure 4). These markers were selected as a representative panel, as they each play pivotal roles in macrophage function. In allergic asthma, TNFα and IL-1β are induced after LPS-stimulation of monocyte-derived macrophages (Haimerl et al., 2021), with macrophages previously shown to acquire an TNF-dependent memory in the type 2 inflammatory asthmatic environment (Lechner et al., 2022). Furthermore when macrophages are classically activated, IL-6 is known to be upregulated (Lechner et al., 2022). Type 2 nitric oxide synthase, referred to as *nos2* or inducible NOS, is highly upregulated in classically activated M1 macrophages, documented to play a defensive role in innate immunity (Bogdan

et al., 2000; Yao et al., 2022). Furthermore, *nos2* is interlinked with IFNγ expression (Gao et al., 2016; Riquelme et al., 2013). Both macrophage phenotypes can act in a pro-inflammatory or anti-inflammatory manner depending on their surrounding microenvironment. However M2 alternatively-activated macrophages tend to sway towards a more anti-inflammatory function, indicated by an increase in *arg1, retnla* and *chi3l3* expression. As nitric oxide (*nos2*) and arginase (*arg1*) both compete within macrophage arginase pathway, perhaps indicating a metabolic switch (Rath et al., 2014), *arg1* expression was a prominent marker of macrophage function throughout this study. As arginase can limit the availability of L-arginine and therefore having a regulatory impact on NO synthesis, despite NOS enzymes being theoretically intracellularly saturated with said L-arginine, this creates somewhat of an "arginine paradox", where macrophage dichotomy relies on the arginine pathway (Lee et al., 2003; Rath et al., 2014). Found in inflammatory zones 1 (FIZZ1), also known as RELM*α* or *retnla* (resistin-like alpha) was first discovered to have an association with pulmonary inflammation in 2000 (Holcomb et al., 2000). Moreover, *retnla* does not have a human homolog and thus is a signature marker of M2 alternatively-activated murine macrophages (Martinez et al., 2013). Lastly, chitinase 3-like 3, *chi3l3* (YM1), also with no known human homolog, is upregulated in allergic asthma due to correlating increased levels of type 2 cytokines IL-4, IL-5 and IL-13 (Abdelaziz et al., 2020). Due to its binding to carbohydrates (Webb et al., 2001; Zhao et al., 2005) and recruitment of eosinophils (Rosenberg et al., 2013; Zhao et al., 2013), this M2 marker is thought to contribute to airway remodelling (Kang et al., 2022). Investigating these markers after polarising conditions, naïve BMDMs from WT and high human MIF expressing $CATT_7$ mice did not show evidence of a significant activation trend. Notably, at both the mRNA and protein level, high human MIF $CATT_7$ BMDMs had significantly increased levels of TNFα. However this was expected, as a profound correlation between LPS-induced macrophage TNFα production and MIF expression is documented, where LPS-induced TNFα

production from peripheral blood mononuclear cells from cystic fibrosis patients was attenuated in the presence of the MIF inhibitor ISO-1 (Adamali et al., 2012). Similarly, in a model of septic shock and also *Salmonella typhiurium* infection, MIF mediated increased TNFα production (Calandra et al., 2000; Koebernick et al., 2002).

We have previously depicted how human MIF drives airway inflammation in a HDM model (Dunbar and Hawthorne et al., 2023). This study sought to elucidate the effects of HDM on $CATT_7$ and WT macrophage polarisation. Mice were challenged with HDM intranasally three times a week for three weeks as depicted in figure 5A and their bone marrow was harvested on day 18, 4hr after the last challenge. On day 7, differentiated BMDMs were stimulated with LPS, IFNγ and IL-4 as described (Figure 1A). Interestingly, BMDMs from high human $CATT_7$ mice that received HDM had a significantly enhanced M1 profile, with increased levels of *tnfα, il-6, il-1β* and *nos2* compared to naïve $CATT_7$ mice (Figure 5, 6). Conversely, whilst WT mice that do not express human MIF had lower levels of M1 markers, BMDMs from these mice had significantly elevated levels of the M2 markers, *arg1* and *retnla*. This was expected as in diseases such as allergic asthma that have a well-documented type 2 inflammatory profile, M2 macrophages are known to have a central impact after HDM challenge, suspected to be orchestrated by the ormyl peptide receptor 2–TNF–2-HG–PGE_2/PGE_2 receptor 2 axis (Lechner et al., 2022). Interestingly, although $CATT_7$ BMDMs illustrated increased arginase activity after HDM challenge (Figure 5), they did not show an increase in relative *arg1* expression, maintaining low levels of this enzyme in both the lung (Figure 2B and supplementary table 1) and bone marrow (Figure 3B). Previously, mice that lack arginase-1 have exhibited increased levels of liver fibrosis (Pesce et al., 2009). As we have illustrated a MIF-dependent significant increased percentage of subepithelial fibrosis in $CATT_7$ mice (Dunbar and Hawthorne et al., 2023), our hypothesis may be that the lack of arginase-1

in both the lung and bone marrow may be contributing to this. Additionally to their striking M1 phenotype, $CATT_7$ BMDMs have demonstrated a significant increase in *chi3l3* and *il-10* expression after HDM challenge (Figure 5). After infection induced by the helminth parasite *Brugia malayi*, homologs of the human cytokine MIF were shown to boost *chi3l3* expression and eosinophil recruitment (Falcone et al., 2001). As MIF is also known to be a eosinophil chemoattractant (Bozza et al., 2020), along with driving significantly increased eosinophilia in a HDM-induced airway inflammation model in high human MIF expressing $CATT_7$ mice (Dunbar and Hawthorne et al., 2023), it is hypothesised that MIF expression is also driving *chi3l3* expression in BMDMs of HDM-treated $CATT_7$ mice. The increased *il-10* expression noted in $CATT_7$ mice after HDM challenge may be indicative of innate immune training, as *il-10* is known to induce T regulatory cells via STAT3, Foxo1 (Hsu et al., 2015) and Jagged-1 (Cahill et al., 2015).

After establishing that this robust pro-inflammatory response after HDM challenge was MIF-dependent by incorporating the potent MIF inhibitor SCD-19 twice a week for three weeks of the allergic asthma model (Figure 7, 8), we next set out to elucidate the influence of hBM-MSCs on BMDM polarisation. Our group and others have continuously demonstrated the powerful immunomodulatory role MSCs exert through paracrine means, primarily via their secretome (Cahill et al., 2016; Kennelly et al., 2016; Szabłowska-Gadomska et al., 2023; Xia et al., 2019) (Dunbar et al, in preparation). Furthermore, MSCs need to be licensed to enhance their therapeutic effects (Carty et al., 2021; Corbett et al., 2021; Dunbar et al., 2021; English, 2013), with high levels of human MIF driving MSC-mediated wound healing (Dunbar et al, in preparation) and the abrogation of HDM-induced pathology (Hawthorne, Dunbar et al, in review). MSCs become apoptotic 24hrs after administration *in vivo*, by which they are phagocytosed by macrophages (Supplementary Figure 2) (Braza et al., 2016; Eggenhofer et al.,

2012; Ferrini et al., 2021; Giri & Galipeau, 2020; Pang et al., 2021). Due to their short-life span, it is hypothesised that MSCs mediate their immunosuppressive effects by re-programming macrophages after being engulfed (Carty et al., 2017), where MSCs are capable of calming macrophages in asthma (Kim et al., 2022; Mo, Kang, et al., 2022; Morrison et al., 2017). Thus, this study set out to further understand if MSCs can calm or re-programme HDM-primed macrophages in an *in vitro* transwell assay (Figure 9A).

hBM-MSCs co-cultured with $CATT_7$ BMDMs significantly suppressed the M1 pro-inflammatory signature previously demonstrated after HDM priming (Figure 9, 10). Furthermore, MSCs significantly reduced M2 markers of *arg1* and *retnla* in BMDMs from WT mice. Investigating *chi3l3*, MSCs significantly increased WT BMDM expression of this eosinophil chemoattractant. As noted, the receiving microenvironment of MSCs need to meet a pro-inflammatory threshold in order to license and activate these cells upon administration. WT mice do not have a sufficient inflammatory status, as our group have seen no significant effects with MSCs in these mice (Hawthorne, Dunbar et al, in review). Thus, in this *in vitro* assay we hypothesise that MSC are not sufficiently activated and thus may actually drive M2 polarisation in the context of *chi3l3* expression in WT mice (Figure 9). When co-cultured with $CATT_7$ BMDMs, MSCs had no significant effect on these M2 markers, but decreased *il-10* expression. Cyclo-oxygenase (COX) enzymes are known to mediate MSC immunomodulation and thus, to elucidate the mechanism by which MSCs can modulate the pro-inflammatory M1 phenotype of $CATT_7$ BMDMs in this study, a non-specific COX-1 and COX-2 inhibitor indomethacin, and a specific COX-2 inhibitor NS-398 were used to pre-treat MSCs prior to co-culture with $CATT_7$ BMDMs. COX inhibition abolished MSCs ability to suppress *tnfα* expression and production, and *retnla* expression (Figure 11), however when this enzyme was inhibited, MSCs further reduced *arg1* expression. Albeit, it is important to note that M2 *arg1*

expression is categorically induced by IL-4 stimulation, yet this enzyme can also be induced through LPS stimulation. To fully elucidate the conclusive role of COX activity in MSC's suppressive function, further investigation with increased numbers of replicates are required. MSCs are known to release intracellular agents that were previously internalised, such as budesonide or cyclosporine (Ankrum, Dastidar, et al., 2014; Girdlestone et al., 2015). To rule out this occurring in our co-cultures, controls of $CATT_7$ BMDMs directly treated with indomethacin or NS-398 for 24hrs were carried out (Supplementary Figure 1). No changes in LPS-induced gene expression were noted between BMDMs that were treated with COX inhibitors and those that were not.

As this *in vitro* assay was carried out utilising differentiated-macrophages that were cultured in M-CSF supplemented media for the duration of the assay, this data can only be classified as innate 'priming', according to table 1. Thus, we set out to investigate if this robust increase in macrophage activation and thus MIF-mediated production of pro-inflammatory cytokines (Figure 7, 8) which were decreased by co-culture with hBM-MSCs (Figure 9, 10) in a possible COX-manner (Figure 11) was evidence of true innate immune training.

We confirmed that HDM primes macrophages from $CATT_7$ mice and so this study next sought to elucidate if HDM also trains macrophages from these novel mice. Innate immune training occurs in the hematopoietic stem and progenitor cells (HSPCs), pre-cursors of our previously analysed BMDMs. Thus, HSPCs were isolated from naïve mice and stimulated with HDM on day 0 in *vitro*. A hallmark indicator of innate immunity is epigenetic re-programming through histone modification, such as DNA methylation. A methyltransferase inhibitor, MTA, was added to HSPCs 30 mins prior to the addition of the primary stimulus (HDM). Importantly, differentiated-macrophages derived from these HSPCs were rested prior to receiving a

secondary heterologous insult of LPS, allowing their active gene transcription to resume to basal levels (Table 1). We found that $CATT_7$ HSPC-derived macrophages that received both a primary and secondary stimulus had increased TNFα and IL-6 production compared to those that only received the first or secondary stimulus alone (Figure 12). This illustrates that *in vitro* HDM exposure on day 0 of this assay trained HSPCs from $CATT_7$ mice. Importantly, we saw no significant signs of innate immunity in BMDMs from WT mice (Supplementary Figure 3), demonstrating that this process was human MIF-dependent. Furthermore, innate immunity was confirmed when DNA methylation in these $CATT_7$ HSPCs was blocked upon receiving a methyltransferase inhibitor MTA, as they had significantly reduced TNFα production, and reduced IL-6 production compared to those that received a vehicle control. The use of MTA not only confirmed that innate immune training was occurring, but that the primary stimulus of HDM was re-programming $CATT_7$ HSPCs at an epigenetic level.

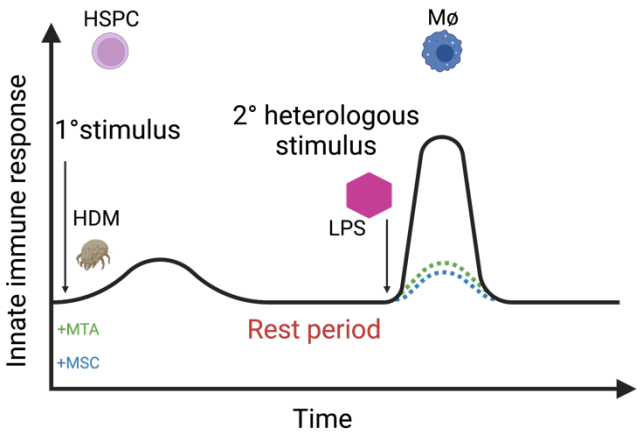

Figure 14. Timeline illustrating innate immune response. HSPCs isolated from the bone marrow of $CATT_7$ mice were stimulated with a primary stimulus of HDM +/- MSCs or methyltransferase inhibitor MTA for 24hr. Cells were washed and left to rest for 7 days. Differentiated-macrophages were treated with a secondary heterologous stimulus of LPS for 24hr.

MSCs can block HDM-priming in $CATT_7$ BMDMs (Figure 9, 10). MSCs can communicate with HSPCs within the bone marrow niche through extracellular soluble mediators and exosomes (Fichtel et al., 2022; Yin et al., 2019), but also by intracellular means through organelles called migrasomes (Deniz et al., 2023). Therefore hBM-MSCs were co-cultured with HSPCs from $CATT_7$ mice using transwell inserts to elucidate if these immunomodulatory cells could block or suppress the occurrence of innate immune training. Strikingly, MSCs were able to significantly reduce TNFα production, and reduce IL-6 production by $CATT_7$ BMDMs when co-cultured with HSPCs from day 0 (Figure 13). MSCs had no significant effect in HSPC-derived BMDMs that only received the secondary LPS stimulus. These data clearly illustrate the ability of MSCs to block HDM-imprinting on HSPCs on day 0, and leave a lasting immunomodulatory memory seen in HSPC-derived macrophages on day 11 (Ng et al., 2015).

These data presented in this novel chapter demonstrate evidence of enhanced innate immune priming and training occurring in the bone marrow niche of transgenic high human MIF expressing $CATT_7$ mice. Furthermore, this short-term (priming) and long-term (training) innate immunity was blocked by co-culturing hBM-MSCs with $CATT_7$ BMDMs and HSPCS respectively. Utilising the aeroallergen HDM, an evident driver of allergic airway inflammation as a primary stimulus to induce innate immune training, it would be beneficial to investigate if alveolar macrophages are also re-programmed in the same manner. However, from an experimental perspective, low cell numbers of alveolar macrophages are obtained from the bronchoalveolar lavage fluid (BALF), especially from naïve mice. Thus, we chose to work with BMDMs due to their ease of isolation, high cell return and also in the interest of reducing the number of animals used. Importantly, we did see the same polarisation trends in the lung and bone marrow, with WT mice having an increased M2 phenotype compared to $CATT_7$ mice

(Figure 2, 3, Supplementary Table 1). Another limitation important to note is the presence of endotoxin within house dust mite batches, perhaps acknowledging the argument that the primary and secondary stimuli used in our assay were not heterogeneous. However the literature has indicated the importance of endotoxin in facilitating a HDM-induced inflammatory response (Patil et al., 2013), especially when driving a type 2 response in C57BL/6 mice, as illustrated in this chapter. To ensure reproducible and robust results utilising this allergen, transparency surrounding endotoxin levels in batches of HDM is encouraged (Supplementary Table 2) (Pascoe et al., 2020).

To further elucidate the longevity of this trained immunity, it would be beneficial to translate this work to an *in vivo* model in future experiments. As in the context of helminth infection, trained HSPCs can generate immunosuppressive monocytes for up to 8 months (Cunningham et al., 2021). Additionally, analysing other aspects of innate re-programming such as the metabolism of these cells before and after HDM-induced training would be invaluable, using SCENITH or seahorse assays to probe the impact of a glycolytic to oxidative phosphorylation metabolic switch (Lundahl et al., 2022). Furthermore, the incorporation of different histone modification inhibitors such as those that target histone deacetylases (HDAC, Givinostat) (Ripamonti et al., 2022; Roger et al., 2011) or bromodomains (I-BET151) (Domínguez-Andrés et al., 2019; Xu et al., 2021), would assist in describing the full extent of epigenetic re-programming happening in these $CATT_7$ mice (Domínguez-Andrés et al., 2023; Mulder et al., 2019).

Overall, this unique study elucidates how macrophages and innate immunity can act as a bridge between the two-armed innate and adaptive immune response, in addition to the ability

of MSCS to calm macrophages and their HSPC precursors in novel humanised high human MIF expressing $CATT_7$ mice in a model of HDM-induced allergic airway inflammation.

4.6 Conclusion

The concept of immunological memory and how exposure to consecutive stimuli can train the immune response to have a heightened response to future pathogens has become increasingly popular in recent years. Moreover, the induction of innate immune training can occur in a subtle manner, where evidence of repeated day-to-day habits such as eating a western diet can trigger this trained immunity (Christ & Latz, 2019). This research utilises novel humanised $CATT_7$ MIF mice expressing the 7-repeat tetranucleotide MIF promotor polymorphism known to exist in the human population (Plant et al., 2006), demonstrating that high human MIF expression enhances trained immunity and its associated pro-inflammatory response to stimuli such as HDM and LPS. When progenitor-derived macrophages from $CATT_7$ mice were co-cultured with MSCs using transwell inserts, this trained innate immunological response was suppressed, illustrating the ability of MSCs to modulate $CATT_7$ pro-inflammatory M1 macrophages by paracrine means. Collectively, these data demonstrate the role of the highly ubiquitous HDM allergen to prime and train the immune response in mice expressing the human MIF polymorphism, perhaps suggesting a pathogenic role of this pro-inflammatory cytokine in the development of human disease. Furthermore, this research illustrates that MSCs may be a suitable therapeutic intervention, where they could act to calm an over-zealous, trained immune response in individuals expressing the 7-7 MIF polymorphism.

4.7 Supplementary Figures

Supplementary Table 1. Mean CT values generated by investigating gene expression by RT-PCR[2]

Gene	*tnfα*	*il-6*	*il-1β*	*nos2*	*ifnγ*	*arg1*	*retnla*	*chi3l3*
WT	33.90	31.49	33.24	32.70	31.35	29.38	22.94	25.06
$CATT_7$	34.77	32.12	34.07	33.15	31.62	30.02	23.12	24.66

Supplementary Table 2. House dust mite species used, with details on source material, lot number, endotoxin levels, protein content, dry weight and der p1 content.

House Dust Mite	Content	Testing Method
Species	*Dermatophagoides pteronyssinus*	-
Source Material	Whole bodies crushed by mortar and pestle	-
Lot Number	394844	-
Endotoxin	9937.5 EU/vial	Limulus Amoebocyte Lysate (LAL)
Protein	50.75 mg/vial	Bradford
Dry Weight	247.1 mg/vial	Gravimetric
Der p 1	3003.75 mcg/vial	Double Blind ELISA

[2] **HDM drives type 2 lung inflammation in WT and $CATT_7$ mice.** WT and high human $CATT_7$ MIF mice were challenged with HDM three times a week for three weeks. Lungs were harvested and snap frozen in liquid nitrogen. Once homogenised, RNA was isolated and cDNA was synthesised. RT-PCR was carried out to measure the expression of genes indicating macrophage polarisation: M1; *tnfα, il-6, il-1β, nos2* and *ifnγ*. M2; *arg1, retnla* and *chi3l3*. Data represented as mean of N=6 mice per group.

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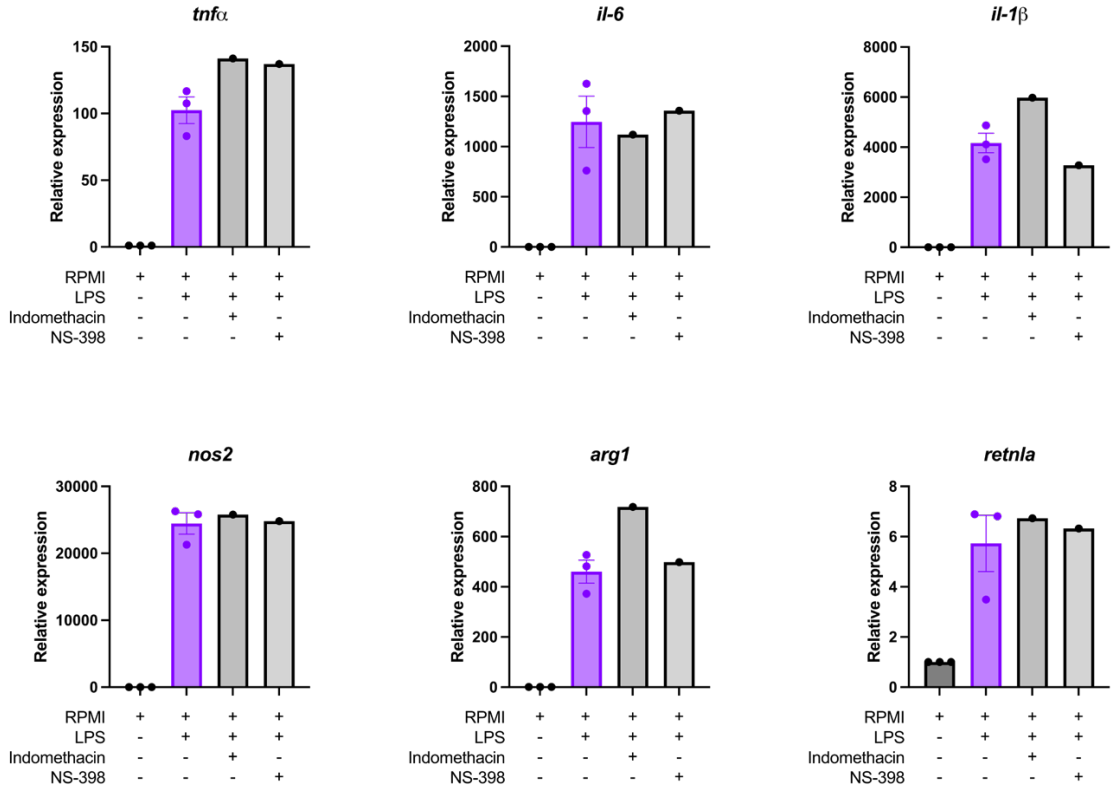
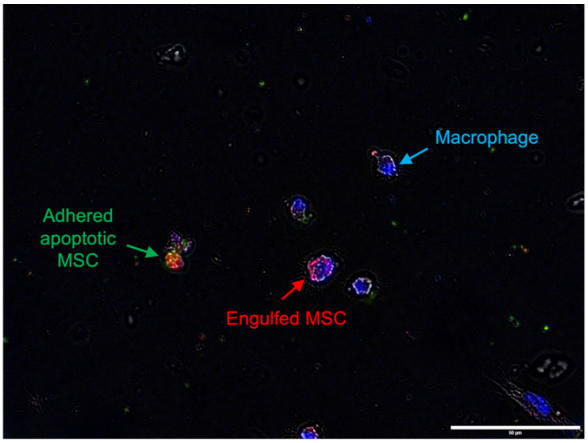
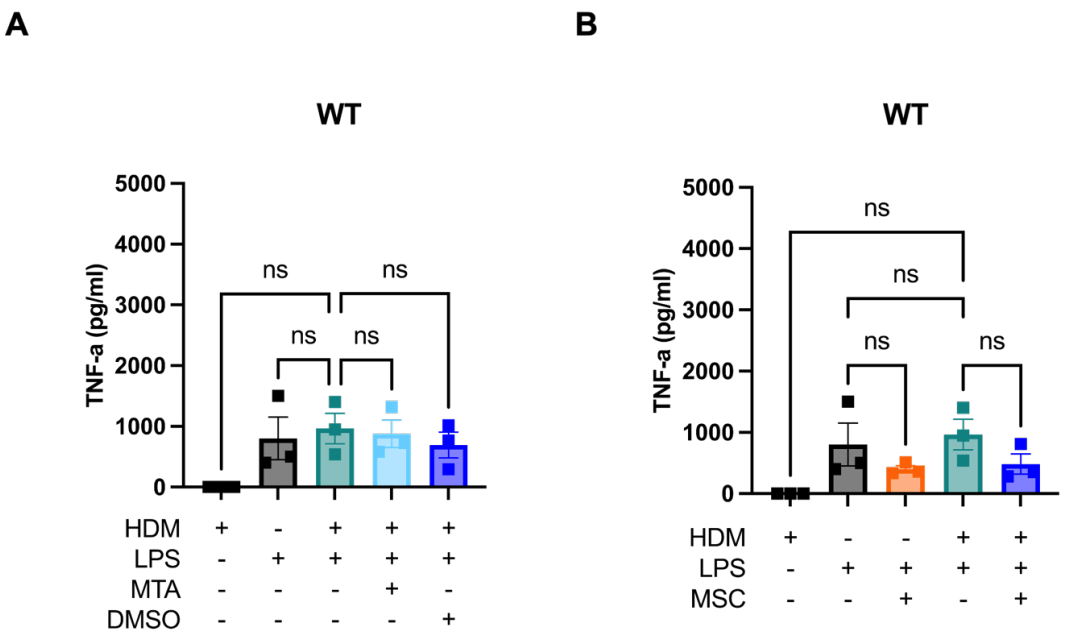


Supplementary Figure 1. COX inhibitors Indomethacin and NS-398 have no indirect effect on BMDMs. To investigate if the COX-1/2 inhibitors indomethacin and NS-398 used on MSCs prior to MSC co-culture with $CATT_7$ BMDMs were having indirect effects on the macrophage population, BMDMs from $CATT_7$ mice were incubated with indomethacin or NS-398 for 24hrs and gene expression was analysed. **A** Indomethacin or NS-398 had no non-specific effects on $CATT_7$ BMDMs. Data are presented as mean ± SEM; N=1-3 mice per group.



Supplementary Figure 2. Alveolar macrophages engulf apoptotic hBM-MSCs. Alveolar macrophages (AMs) were isolated from bronchoalveolar lavage fluid from naïve WT mice and seeded onto a coverslip. Apoptosis was induced in MSCs using with 0.5 μM of staurosporine for 18hr. Biotinylated deep red dye stained apoptotic MSCs were added to the DAPI stained AMs at a ratio of 1:4 (AM:MSC). Wells were centrifuged to ensure cell contact and cells were incubated for 1hr at 37°C. Cells were washed with warm PBS to remove any non-engulfed MSCs, before staining remaining cells with FITC dye. Cells were washed, fixed with 4% paraformaldehyde before being mounted onto a slide with aqueous mounting medium. Slides were imaged at 20X using an Olympus fluorescent microscope. Image representative of N=3 mice.



Supplementary Figure 3. HDM does not induce trained immunity in WT BM-derived HSPCs. A HSPCS were isolated from bone marrow of WT mice and incubated with a methyltransferase inhibitor MTA 30 minutes prior to HDM stimulation. Differentiated BMDMs were stimulated with LPS on day 10 for 24hr. No changes in TNFα production and thus no evidence of trained immunity was observed in WT mice. MTA had no significant effects. **B** MSCs co-cultured with HSPCs from WT mice on day 0 using transwell inserts had no effect on TNFα production. Data are presented as mean ± SEM; N=3 mice per group.

Chapter 5:

General Discussion

Discussion

Allergic asthma is a chronic atopic respiratory disease affecting millions of people worldwide, carrying a substantial economic burden. 50-85% of asthmatics are allergic to the house dust mite (HDM) aeroallergen, which can trigger symptoms such as a recurrent cough, chest tightness and wheezing (Gregory & Lloyd, 2011). After inhalation, HDM can damage the airway epithelial barrier, compromising the integrity of this protective layer of mucosa. This facilitates the infiltration of this allergen to the lung submucosa, where it can prime and train macrophages, a population of phagocytic cells that are pivotal in asthma pathogenesis (Abdelaziz et al., 2020; Jacquet, 2013; Lechner et al., 2022). Innate immune training results in a modification of the epigenetics within the bone marrow-resident progenitor cells of macrophages, causing a rapid, intense inflammatory response to subsequent infection or inflammatory insults (Domínguez-Andrés et al., 2023; Netea et al., 2016). Current prescription medications and biologics struggle to efficiently control asthma at a physiological level. Furthermore, medical non-compliance is a problem associated with asthma treatment, as therapeutics are often high in cost, forcing those in lower socio-economic backgrounds to go without. As a result, approximately half of the asthmatic population have uncontrolled symptoms ("The Global Asthma Report 2022," 2022).

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that contains a functional promotor polymorphism at position -794 in the MIF gene. Found to be secreted by eosinophils and thus having a role in asthma (Rossi et al., 1998), MIF levels are elevated in the bronchoalveolar lavage fluid (BALF) and sputum of asthmatic patients (Mizue et al., 2005). Furthermore, the 5-repeat variant of this MIF polymorphism, termed $CATT_5$, has been associated with milder forms of disease (Baugh et al., 2002; Plant et al., 2006). This

research set out to evaluate the role of the high 7-repeat allele, $CATT_7$, and its implications on disease severity in a mouse model of house dust mite-induced airway inflammation. To confirm MIF's involvement in this airway inflammation a potent MIF antagonist, SCD-19, was utilised. To increase the translational relevance of this study, novel transgenic mice expressing the human MIF gene were utilised throughout.

Mesenchymal stromal cells (MSCs) are a population of heterogenous cells which can be isolated from the bone marrow, adipose tissue and even the umbilical cord. Boosting their immunomodulatory and cytoprotective properties, the activation or licensing of these cells augments their therapeutic efficacy (Boland et al., 2018; Carty et al., 2021; Corbett et al., 2021; Dunbar et al., 2021). With MIF levels being increased in response to infection and inflammation, this innate cytokine can act to license MSCs in the inflammatory lung after they are administered intravenously (Hawthorne, Dunbar et al., in review). This Ph.D. thesis evaluates the impact of MIF-licensing on MSC efficacy *in vitro*, investigating the impact of their secretome to enhance bronchial epithelial repair after injury. Moreover, this research elucidated the ability of MSCs to modulate macrophage polarisation from $CATT_7$ and WT mice, in addition to their ability to reduce or abrogate innate immune training after HDM challenge.

At the outset of this work, there was a lack of robust acute house dust mite models which depicted both cellular inflammation and airway remodelling. Furthermore, the interplay between high human MIF expression and asthma severity was unproven. Chapter 2 of this thesis illustrates for the first time the impact of the human $CATT_7$ MIF polymorphism in a preclinical asthma mouse model. We investigated clinically relevant readouts which capture the pathophysiology of asthma, at a cellular, histological and functional level. BALF retrieved

from $CATT_7$ mice that received HDM intranasally three times a week for three weeks demonstrated a significant increase in eosinophil infiltration and Th2 cytokine production compared to WT mice. At a histological level, high human MIF expressing $CATT_7$ mice exhibited significantly increased numbers of periodic acid schiff (PAS) positive cells relative to the control, along with a significantly increased percentage of subepithelial collagen deposition. This increase of PAS-stained goblet cells, indicative of goblet cell hyperplasia, and masson's trichrome staining of collagen deposition, an indirect marker of fibrosis, indicate that high levels of human MIF exacerbated changes in the lung architecture after HDM challenge. Thus, we investigated the functional impact of high MIF expression using the gold standard FlexiVent® system. Mice were challenged with increasing doses of the bronchoconstrictor methacholine and their lung mechanics were measured. Using this state of the art instrument, $CATT_7$ mice exhibited an increased percentage of airway resistance, damping and elasticity compared to WT mice. To confirm the role of MIF in this model, 35mg/kg of the isocoumarin small molecular weight MIF inhibitor SCD-19 was administered intraperitoneally twice a week for the duration of this model. $CATT_7$ mice that received SCD-19 had significantly decreased levels of HDM-induced inflammation and airway remodelling. After SCD-19 administration, $CATT_7$ lung function measurements also returned to levels comparable with those measured in WT mice, illustrating that a decline in lung function after HDM challenge was MIF-dependent. By generating a robust physiological scale of airway inflammation implicating the human $CATT_7$ MIF polymorphism, our findings demonstrate that high levels of human MIF increased the severity of allergic asthma after HDM challenge (Dunbar and Hawthorne et al., 2023). This novel research is of translational significance, as it depicts the important role of human MIF in the development and maintenance of a highly prevalent atopic respiratory disease, induced by an environmentally-relevant dust allergen. Moreover, as this MIF-dependent exacerbation of allergic asthma was abrogated using a small molecular weight MIF inhibitor, the data presented

in this chapter highlights the potential of therapeutically targeting this pro-inflammatory cytokine to suppress rampant inflammation and tissue remodelling in patients possessing the high MIF expressing $CATT_7$ allele. Going forward, this humanised MIF mouse model can be utilised to facilitate the pharmacological development of therapeutics aimed at blocking MIF biological activity, having a positive impact in not only asthma, but also other MIF-dependent diseases in the realm of respiratory conditions, oncology, infection, and autoimmunity.

In environments of excessive inflammation and airway remodelling, such as in the lung of HDM-challenge $CATT_7$ mice, the airway epithelial barrier may become compromised. The mucosal epithelium acts as a line of defence against invading pathogens, specifically those that are airborne and known to trigger asthmatic exacerbations. Thus, if this epithelial barrier is compromised or dysregulated, allergens can infiltrate into the airway submucosa and mount an immune response after being phagocytosed by professional antigen presenting cells such as dendritic cells. Importantly, MIF is produced by bronchial and alveolar epithelial cells (Donnelly et al., 1997; Kevill et al., 2008). The MIF-CD74 axis plays a role in the proliferation of alveolar epithelial cells by activating Akt survival pathways (Marsh et al., 2009). However in an allergic asthma microenvironment, HDM challenge can promote MIF expression (Figure 1), where increased MIF can accelerate aerobic glycolysis in bronchial epithelial cells and facilitate the secretion of Th2 cytokines, thus contributing to airway inflammation (Lan et al., 2020). There is evidence that MIF can also enhance the survival and protection of MSCs, specifically against antibiotic and hypoxic injury (Palumbo et al., 2014; Xia et al., 2018; Xia & Hou, 2017), however little is known about the role of MIF-licensing on MSCs' cytoprotective function. The MSC secretome is gaining traction as an alternative cell-free therapy (Műzes & Sipos, 2022; Szabłowska-Gadomska et al., 2023; Xia et al., 2019), as it contains soluble mediators which can have distal cytoprotective and reparative effects (Cahill

et al., 2016; Carty et al., 2021; Corbett et al., 2021; Kennelly et al., 2016). Thus, we sought to investigate if these high levels of human MIF, indicative of those found during epithelial-MSC interactions *in vivo*, could license and enhance MSC's pro-reparative function *in vitro*, utilising an airway epithelial wound healing assay. Chapter 3 illustrated the effects of human endogenous MIF on MSC cytoprotective function, where $CATT_7$-licensed MSCs, but not WT or $MIF^{-/-}$-licensed MSCs, significantly promoted bronchial epithelial wound healing in a VEGF-dependent manner *in vitro*. Similarly, MIF has previously been shown to upregulate VEGF production in the conditioned media of synovial fluid mononuclear cells, stimulating endothelial tube formation in the context of rheumatoid arthritis (Kim et al., 2007). Thus, this correlates with our data illustrating that MIF-licensed MSCs exhibit a significant increase in VEGF production. However, although increased levels of VEGF have been shown to possibly contribute to airway remodelling in the context of asthma, VEGF-A and its receptor VEGFR2 have demonstrated a protective role by preventing the metaplasia of goblet cells in this atopic condition (Jiang et al., 2021). Moreover, this growth factor also has a positive impact in diseases such as idiopathic pulmonary fibrosis (Murray et al., 2017; Stockmann et al., 2010), emphysema (Byers, 2019) or even coronary heart disease (Zhou et al., 2021). The question regarding if VEGF is a friend or foe, further elucidates the importance of VEGF homeostasis, where this growth factor can support epithelial repair, without promoting excessive tissue remodelling or fibrosis (Tuder & Yun, 2008). We believe that the MSC secretome, known to contain a plethora of both cytoprotective and immunomodulatory factors can facilitate this homeostasis, as we have shown that MIF-licensing also increases the activity of MSC immunomodulatory enzymes such as COX-2 (Hawthorne, unpublished), leading to increased production of anti-inflammatory mediators such as PGE_2 which could balance the possible negative effects of VEGF in an asthmatic *in vivo* setting. Thus, although we identified human

MIF to be the key driver of asthma pathogenesis *in vivo* (chapter 2), we have also discovered a novel positive effect this pro-inflammatory cytokine can have on MSC therapeutic efficacy.

In addition to the mechanical damage to the airway epithelium caused by frequent exacerbations, HDM is known to directly induce airway epithelial apoptosis after inhalation (Hoffman et al., 2013). Using an *in vitro* bronchial epithelial survival assay, MSCs licensed with endogenous human $CATT_7$ MIF, but not murine MIF from WT mice, offered protection from HDM-induced apoptosis. We speculate that this protection against HDM-induced apoptosis is VEGF-dependent, as VEGF is known to upregulate the Akt survival pathway (Fujio & Walsh, 1999; Gerber, McMurtrey, et al., 1998; Mondru et al., 2023), along with increasing the expression of survival genes such as Bcl-2, A1, survivin and XIAP in cells (Gerber, Dixit, et al., 1998; Nör et al., 1999; Pidgeon et al., 2001; Tran et al., 1999; Wang et al., 2020), including MSCs (Ni et al., 2017). Furthermore, these cytoprotective effects were noted to be MIF-dependent, as addition of the MIF inhibitor SCD-19 to $CATT_7$ supernatants prior to MSC licensing, blocked the enhanced wound healing and epithelial protective effects. This provides a novel mechanistic insight into how MIF, possibly produced by damaged epithelial cells, can license MSCs and enhance their cytoprotective function, specifically their ability to provide protection against a dysregulated airway epithelial barrier and thus, perhaps even prevent the development of airway inflammation after allergen challenge *in vivo* (Figure 1). As asthma patients are likely to be sensitised by new allergens over the course of their life time, these data illustrate the potential benefit of the prophylactic administration of human MIF-licensed MSC or their secretome, to fortify the integrity of the airway epithelial barrier and thus, prevent disease development and future exacerbations. Furthermore, epithelial barrier disruption not only has implications for asthma, but also in other membrane-dependent diseases such as atopic dermatitis, allergic rhinitis, eosinophilic oesophagitis or inflammatory bowel

disease, some of which are associated with elevated levels of MIF (de Souza et al., 2015; Oliver et al., 2007; Shimizu et al., 1999). Thus, this research demonstrates the beneficial consequences of licensing these stromal cells with this pro-inflammatory cytokine, bringing awareness to the possibility of utilising MIF-licensed MSCs, or their secretome as a therapeutic intervention in a broad range of inflammatory diseases.

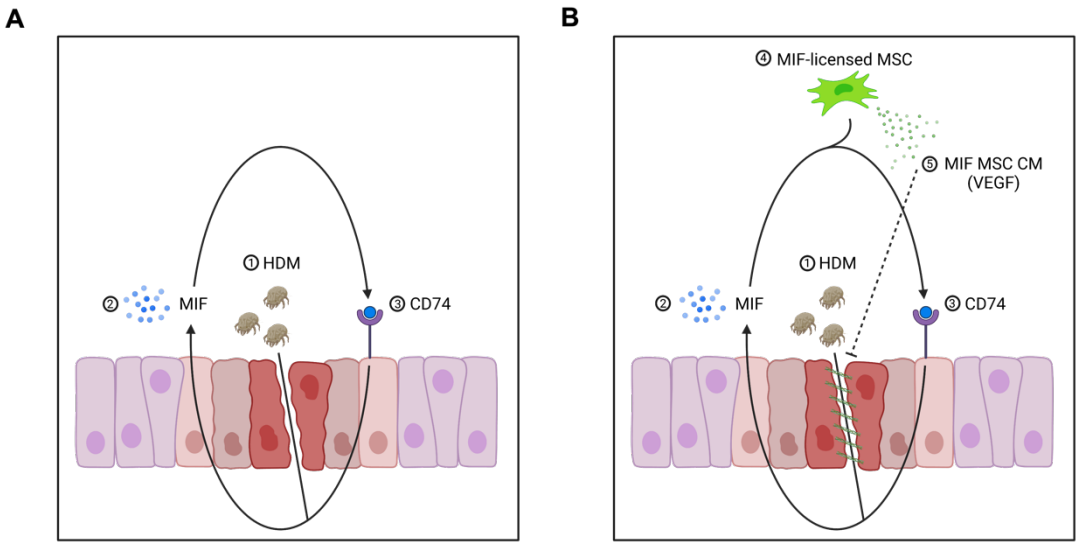


Figure 1. CATT$_7$ MIF-licensed MSC CM promotes wound healing. A. (1) HDM can damage the airway epithelium, (2) increasing MIF expression. (3) MIF can bind to a CD74 receptor on the surface of epithelial cells, creating a positive feedback loop. **B.** (4) MIF can license MSCs and increase their production of VEGF into their secretome.(5) MIF MSC CM promotes airway epithelial wound healing in a VEGF-dependent manner, preventing the future infiltration of airborne pathogens.

Macrophages play a dominant role in the innate immune system and the development of airway inflammation. Responsible for the detection, phagocytosis and disposal of foreign pathogens in the airways, macrophages act as a link to the adaptive immune system, by alerting and recruiting effector cells to sites of inflammation. Depending on the inflammatory profile of their microenvironment, macrophages can be polarised to a pro-inflammatory M1 or anti-

inflammatory M2 phenotype. Specifically in the context of allergic asthma, type 2 helper T cells pump out IL-4, a cytokine involved in immunoglobulin class switching (Pelaia et al., 2022). These increased levels of IL-4 alternatively activate macrophages to a reparative M2 subtype. However as airway remodelling is a problematic feature of this respiratory disease, macrophages can accelerate and worsen this process, as they are trying to 'heal' this damage (Abdelaziz et al., 2020). Furthermore, MIF is also known to have a detrimental effect on macrophages, as this pro-inflammatory cytokine is known to inhibit p53, which is pivotal for the apoptosis and removal of an excessive accumulation of these cells (Mitchell et al., 2002). As a result, MIF can drive macrophage-mediated inflammation.

Continuing with this narrative, chapter 4 of this thesis first set out to elucidate the role of the human $CATT_7$ MIF allele on macrophage polarisation. Besides the expected significant increase in TNFα production, as MIF is known to drive the production of TNFα after lipopolysaccharide (LPS) stimulation (Calandra et al., 2000), no clear M1 or M2 activation trend was identified in naïve $CATT_7$ or WT mice. After generating our novel model of HDM-induced allergic airway inflammation in humanised MIF mice, we next sought to investigate if HDM exposure would drive a specific macrophage phenotype in these mice. Bone marrow-derived macrophages (BMDMs) from HDM-challenged $CATT_7$ mice displayed a significant M1 signature, compared to WT controls. This pro-inflammatory macrophage activation was MIF-dependent, as M1 markers were suppressed when $CATT_7$ mice received the MIF inhibitor SCD-19. However it is important to note, that MIF has also been documented to favour monocyte differentiation, and thus perhaps high human MIF expressing $CATT_7$ mice may have increased levels of monocyte differentiation in comparison to WT mice (Pronier et al., 2022; Sorg et al., 1984). More research through the use of flow cytometry could clarify if this is the case in our *in vitro* assay. The data depicted in this chapter demonstrates that high expression

of human MIF can favour, and amplify a pro-inflammatory response driven by macrophages in a disease setting. Although alternatively-activated M2 macrophages are known to have a pathogenic role in the exacerbation of airway inflammation in asthma (Abdelaziz et al., 2020), these novel data describing the impact of the human MIF polymorphism, specifically the high $CATT_7$ allele, provides a novel insight into the M1 activation status of macrophages in asthmatics expressing this high MIF allele. Furthermore, these data also contribute to the field of knowledge surrounding the role of pro-inflammatory macrophages in other MIF-dependent diseases, such as rheumatoid arthritis (Bilsborrow et al., 2019; Xu et al., 2022).

As mounting evidence has found that MSC therapeutic efficacy is dependent on the presence of macrophages (Carty et al., 2017; Galipeau, 2021; Mathias et al., 2013), we next set out to investigate if MSCs could suppress this human MIF-mediated M1 macrophage signature after HDM challenge. When co-cultured with BMDMs from $CATT_7$ mice using transwell inserts, MSCs significantly reduced this pro-inflammatory macrophage polarisation, demonstrating their ability to modulate these cells and curate a more-balanced M1/M2 macrophage population in $CATT_7$ mice. Our hypothesis is that MSCs are more efficacious at calming macrophages from $CATT_7$ mice than WT mice, as these $CATT_7$ macrophages are also pumping out human MIF and thus, licensing MSCs when in a co-culture system. MIF has been shown to upregulate COX-2 expression and thus prostaglandin E_2 synthesis (Sampey et al., 2001; Zhang, Zhou, et al., 2019), a lipid mediator which is known to be vital in MSCs' immunomodulatory function (Burand et al., 2020; Carty et al., 2021; English, 2013; English et al., 2009; Kulesza et al., 2023). Thus to further understand MSCs' mechanism of action when modulating macrophage polarisation, MSCs' were pre-incubated with a COX-1/2 inhibitor indomethacin or a COX-2 specific inhibitor NS-398. After gene expression analysis, COX-enzymes were seen to be involved in MSC's ability to suppress *tnfα* and *retnla* expression in

$CATT_7$ macrophages, as when COX was inhibited MSCs could no longer suppress these macrophage markers. However, not all macrophage markers were impacted when MSC COX enzymes were inhibited, implicating that other mechanisms of action are at play. MSCs may also be suppressing this HDM-induced primed effect by altering macrophage metabolism (Luque-Campos et al., 2021; Vasandan et al., 2016), or perhaps through the secretion of other mediators such as kynurenine or IL-10 (Carty et al., 2017; English, 2013). This chapter not only elucidates the role of the $CATT_7$ MIF allele in differential macrophage polarisation under basal (naïve) and disease (HDM exposure) conditions, but also the ability of MSCs to modulate this process, suggesting the use of MSCs as a potential therapeutic approach for controlling macrophage-mediated inflammation in a wide range of chronic inflammatory diseases.

Upon directly comparing macrophages from naïve and HDM-challenged $CATT_7$ mice, we noted a striking increase in pro-inflammatory markers at the mRNA and protein level in mice that encountered HDM prior to being stimulated with polarising conditions such as LPS. This indicated that after being exposed to a primary stimulus of HDM, BMDMs from $CATT_7$ and WT mice were primed prior to receiving a secondary heterologous stimulus, resulting in an enhanced inflammatory response. Importantly, this priming effect was significantly amplified in BMDMs from $CATT_7$ mice that express high levels of human MIF, compared to WT mice, illustrating that this innate priming was specific to human MIF. This was confirmed as this heightened inflammatory response was abrogated with the incorporation of the MIF inhibitor SCD-19. As previously stated, evidence has indicated that HDM can prime the immune system after challenge. A distinct difference between *in vivo* innate priming and innate training is that innate training occurs centrally in the hematopoietic stem and progenitor cells (HSPCs) within the bone marrow niche (Ochando et al., 2023). Another key difference between the priming and training of the innate immune system, is that training occurs at an epigenetic

level (Netea et al., 2020). Thus, bone marrow cells containing HSPCs were isolated from naïve $CATT_7$ mice and treated with a methyltransferase inhibitor (MTA) to inhibit DNA methylation. This demonstrated that HDM-stimulation was training the bone marrow-derived progenitor cells from $CATT_7$ mice at an epigenetic level, further solidifying our data that high levels of human MIF expression augments the ability of HDM to train the innate immune system. In the first paper illustrating the ability of HDM to train macrophages in a model of allergic asthma, macrophages from WT mice exhibited an M1 profile mediated through the formyl peptide receptor 2–TNF–2-HG–PGE2/PGE2 receptor 2 axis (Lechner et al., 2022). Similarly, we also saw this M1 profile in macrophages from our HDM-challenged WT mice. However our humanised $CATT_7$ BMDMs demonstrated an amplified M1 pro-inflammatory signature, compared to WT mice (Chapter 4). We are the first to present evidence of HDM training in humanised $CATT_7$ MIF mice, whilst also documenting the immunological response to a secondary LPS stimulation *in vitro*. Firstly, this is important as trained immunity characteristically facilitates an immune response to heterogenous stimuli (i.e., HDM and LPS). Secondly, these data are not only relevant in the context of allergic asthma, but also for bacterial infections such as sepsis, as it indicates that asthmatics that possess the $CATT_7$ allele may have an amplified immune response to microbial infection. This research is also the first to demonstrate that the inhibition of methyltransferase enzymes using methylthioadenosine (MTA) blocks this HDM-training imprint in HSPCs from $CATT_7$ mice, strengthening this evidence of HDM-induced epigenetic re-programming in HSPCs from the bone marrow of $CATT_7$ mice. Overall, the data presented throughout chapter 4 unravels a novel story elucidating the impact of human MIF on macrophage polarisation in naïve and HDM-challenge mice. Furthermore, this research reveals a novel finding regarding the MIF-specific macrophage activation profile in the context of allergic asthma, where this study demonstrated a distinct, amplified M1 pro-inflammatory signature in macrophages from humanised $CATT_7$

mice. Importantly, this suggests that the presence of high levels of human MIF alters the macrophage response to HDM exposure, leading to an activation profile that can have implications for asthma pathogenesis in patients expressing this $CATT_7$ human MIF allele. Lastly, these data not only demonstrate that human MIF expression enhances the ability of HDM to induce an epigenetic imprint in bone marrow progenitor cells, but it also captures the pro-inflammatory consequences after a secondary LPS stimulation. Overall, these data illustrate a possible mechanism explaining why $CATT_7$ mice expressing high levels of human MIF exhibited exacerbated HDM-induced airway inflammation, discussed in chapter 2 of this thesis. Moreover, this novel information can be applied translationally to humans, perhaps uncovering why specific subsets of people may exhibit an exacerbated immune response to environmental triggers.

Our final experiments sought to elucidate MSCs' capacity to inhibit this epigenetic re-programming in $CATT_7$ HSPCs. Therefore, when HSPCs were isolated from the bone marrow of these mice and stimulated with HDM, they were co-cultured with MSCs in transwell inserts. Importantly, MSCs significantly reduced TNFα and reduced IL-6 production, demonstrating that these stromal cells could inhibit the process of epigenetic modification in response to HDM training. This ability of MSCs to inhibit epigenetic modifications in response to HDM training within $CATT_7$ HSPCs has novel clinical implications. By disrupting the epigenetic programming of immune cells, MSCs can exert immunomodulatory effects and reduce amplified inflammatory responses associated with a trained immune system. These findings strengthen the argument that MSC-based therapies could be beneficial for individuals with conditions characterised by these aberrant immune responses, such as asthma. In addition to the data depicted in chapter 3, these data further demonstrate the universal therapeutic efficacy of MSCs at different time points of disease progression, where they can not only calm an

established immune response (Hawthorne, Dunbar et al., in review), but also prophylactically prevent HDM-induced epigenetic re-wiring, thus modulating the immune response against future infection or immunological insults.

An Important question arising from"this'research is does this pro-inflammatory cytokine, MIF, play a crucial role in the susceptibility or the severity of disease? In a Ugandan cohort, low MIF expressers were shown to have a 2.4 increased risk of *Mycobacterium tuberculosis* infection (Das et al., 2013). Furthermore, mice deficient in MIF had a higher level of infection, but a lower innate immune response measured by TNFα. Macrophages from these MIF deficient mice were less effective at killing *mycobacterium*, likely to be associated with their lower expression of the pattern recognition receptor Dectin-1 (Das et al., 2013). Moreover, MIF deficient mice were also more susceptible to a cutaneous *Leishmania* infection as $MIF^{-/-}$ macrophages also demonstrated impaired killing activity (Satoskar et al., 2001). Lastly, $MIF^{-/-}$ mice failed to control a *Salmonella typhimurium* infection, with this increased susceptibility being due to a reduced Th1 response (Koebernick et al., 2002). These data, along with our own, demonstrates the important role of MIF in driving an innate immune response against invading pathogens and subsequent infection or inflammation (Figure 2). Thus, lower levels of MIF may correlate with increased susceptibility to infection, with low MIF expressers succumbing to infection quicker than those expressing high levels of this cytokine (Flores et al., 2008; Satoskar et al., 2001). Conversely, high levels of MIF may increase protection against infection (Roger, David, et al., 2001), although an over-active MIF-mediated immune response may be maladaptive, exacerbating the chronicity or severity of disease (Mitchell et al., 2002), as demonstrated in our $CATT_7$ mice after HDM challenge (Dunbar and Hawthorne et al., 2023). This argument further demonstrates the importance of MIF-mediated macrophage activity within the innate response and the clinical relevance of our work.

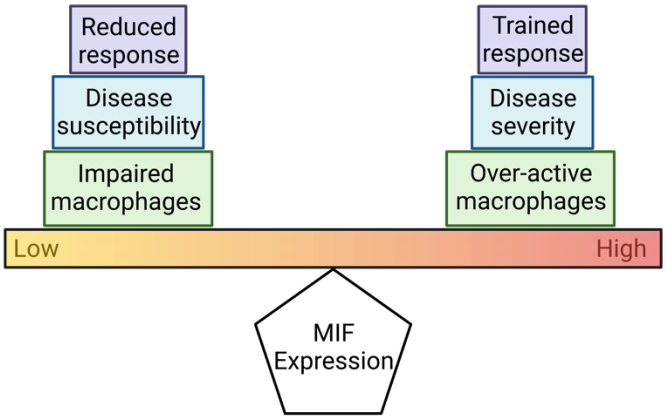


Figure 2. MIF expression controls the intensity of the innate immune response. The expression of MIF is controlled by its gene promotor. A functional promotor polymorphism in the MIF gene can cause MIF expression to be up- or down-regulated, depending on the number of repeats of a tetranucleotide 'CATT' sequence. MIF is important for innate immunity, where lower levels of this cytokine are associated with an increased risk of susceptibility to infection or disease due to a decreased immune response and impaired macrophage function. Conversely, high MIF expression results in an increased immune response and thus can protect against infection. However, if high MIF expressers develop disease, high levels of MIF can contribute to the severity and chronicity of the condition due its ability to promote an over-zealous, maladaptive inflammatory response, with this excessive inflammation being mediated by pro-inflammatory, over-active macrophages.

As there is currently no cure for asthma, effective treatments that can reverse or modulate the associated immune response are still required. Using genotyping techniques to stratify patients on the basis of a high expressing MIF promotor polymorphism or perhaps utilising the new state of the art digital tool 'INCA' developed in Ireland to differentiate patients who have severe asthma versus those with difficult-to-treat asthma (Hale et al., 2023), mesenchymal stromal cell therapeutic efficacy can be predicted prior to administration, as threshold levels of inflammation are required to license these cells *in vivo* (Hawthorne, Dunbar et al, in review) (Dunbar et al., 2021). This study has shown that high human MIF expression exacerbates HDM-induced allergic airway inflammation at both a cellular and histological

level, with MIF-associated pathology being abrogated after the administration of a small molecular weight MIF inhibitor SCD-19. When human bone marrow-derived MSCs were licensed with endogenous MIF from humanised $CATT_7$ mice, MSCs demonstrated a significant increased production of VEGF, increasing their ability to promote wound closure in bronchial epithelial cells, and protect against HDM-induced apoptosis *in vitro*. As MSCs are known to interact with macrophages *in vivo*, this work sought to investigate if MSCs could calm pro-inflammatory macrophages from these $CATT_7$ mice after HDM challenge. Excitingly, MSCs could not only modulate macrophage polarisation, but also reduce MIF-dependent, HDM-induced innate immune training in $CATT_7$ macrophage-precursors. This research is of great clinical importance, as we hypothesise that asthmatics who possess this $CATT_7$ genetic allele may have a heightened immune response to infection resulting in chronic inflammation, as their immune system is trained to exert a strong inflammatory response. Moreover, this thesis demonstrates that the administration of MSCs into a high human MIF microenvironment may be a promising therapeutic intervention to calm an over-zealous, trained immune system, halting disease progression.

To conclude, this thesis thoroughly investigates the complex relationship between the human MIF polymorphism and the innate immune response, investigating its impact on asthma pathogenesis using novel humanised MIF mice in a robust model of HDM-induced airway inflammation. Moreover, these data elucidated the ability of human MIF to license MSCs, boosting the protective effects of their secretome to heal mechanical damage in airway epithelial cells and protect them against subsequent HDM-induced damage *in vitro*. For the first time, this thesis shows that MSCs can efficiently modulate an over-active, MIF-dependent trained immune response, exhibited by macrophages from $CATT_7$ mice after HDM and LPS exposure. Along with elucidating the causative role of the human MIF polymorphism in the

development and severity of allergic asthma, this thesis also proposes a novel cell-based approach, utilising the elevated levels of this MIF cytokine to license MSCs and administer them or their secretome to address epithelial damage, modulate macrophage polarisation, suppress innate immune training and reduce subsequent airway inflammation. The findings presented throughout this thesis hold significant clinical importance and make a substantial contribution to the fields of MIF and MSC research, by offering promising prospects for the development of a new therapeutic approach to target uncontrolled allergic airway asthma.

Future work

To continue this work, the significance of the cytoprotective role of MSCs and their increased production of VEGF after human MIF exposure on the airway epithelial barrier should be elucidated in an *in vivo* model of HDM-induced allergic asthma. To further explore the phenomenon of innate immune training, it would be beneficial to investigate if intranasal HDM challenge can reach the bone marrow niche and train the resident hematopoietic stem and progenitor cells of human MIF expressing $CATT_7$ mice *in vivo*. As innate immunity is associated with long term memory, it is necessary to understand how long $CATT_7$ macrophages maintain this pro-inflammatory, boosted response, both *in vitro* and *in vivo*. Furthermore, to elucidate the role of HDM-trained macrophages in asthma pathogenesis, and also the impact of these macrophages on MSC efficacy *in vivo*, the depletion of resident-macrophages would facilitate a mechanistic insight to this research. To clarify if MSCs reduce HDM-primed macrophage production of *tnfα* and *retnla* in a COX-2 dependent manner, the measurement of PGE_2 production, along with utilising inhibitors which target prostaglandin E_2 receptors EP2 and EP4 would be invaluable to definitively determine MSCs' mechanism of action. Furthermore, elucidating the metabolic status of naïve and HDM-trained macrophages would add value to this research, as the metabolism is also modified in cells under-going trained innate immunity. Lastly, new evidence has demonstrated MSCs can communicate with HSPCs through migrasomes, thus this could be an alternative avenue to investigate their mechanism of action in blocking innate immune training in progenitor cells (Deniz et al., 2023).

Chapter 6:

Bibliography

Abdelaziz, M. H., Abdelwahab, S. F., Wan, J., Cai, W., Huixuan, W., Jianjun, C., Kumar, K. D., Vasudevan, A., Sadek, A., Su, Z., Wang, S., & Xu, H. (2020). Alternatively Activated Macrophages; A Double-Edged Sword In Allergic Asthma. *Journal of Translational Medicine*, *18*(1), 58. https://doi.org/10.1186/s12967-020-02251-w

Abdul-Aziz, A. M., Shafat, M. S., Mehta, T. K., Di Palma, F., Lawes, M. J., Rushworth, S. A., & Bowles, K. M. (2017). MIF-Induced Stromal PKCβ/IL8 Is Essential in Human Acute Myeloid Leukemia. *Cancer Research*, *77*(2), 303–311. https://doi.org/10.1158/0008-5472.CAN-16-1095

Abreu, S. C., Antunes, M. A., Xisto, D. G., Cruz, F. F., Branco, V. C., Bandeira, E., Zola Kitoko, J., de Araújo, A. F., Dellatorre-Texeira, L., Olsen, P. C., Weiss, D. J., Diaz, B. L., Morales, M. M., & Rocco, P. R. M. (2017). Bone Marrow, Adipose, and Lung Tissue-Derived Murine Mesenchymal Stromal Cells Release Different Mediators and Differentially Affect Airway and Lung Parenchyma in Experimental Asthma. *Stem Cells Translational Medicine*, *6*(6), 1557–1567. https://doi.org/10.1002/sctm.16-0398

Abreu, S. C., Lopes-Pacheco, M., da Silva, A. L., Xisto, D. G., de Oliveira, T. B., Kitoko, J. Z., de Castro, L. L., Amorim, N. R., Martins, V., Silva, L. H. A., Gonçalves-de-Albuquerque, C. F., Castro Faria-Neto, H. C. de, Olsen, P. C., Weiss, D. J., Morales, M. M., Diaz, B. L., & Rocco, P. R. M. (2018). Eicosapentaenoic Acid Enhances the Effects of Mesenchymal Stromal Cell Therapy in Experimental Allergic Asthma. *Frontiers in Immunology*, *9*. https://doi.org/10.3389/fimmu.2018.01147

Abreu, S. C., Rolandsson Enes, S., Dearborn, J., Goodwin, M., Coffey, A., Borg, Z. D., dos Santos, C. C., Wargo, M. J., Cruz, F. F., Loi, R., DeSarno, M., Ashikaga, T., Antunes, M. A., Rocco, P. R. M., Liu, K. D., Lee, J.-W., Matthay, M. A., McKenna, D. H., & Weiss, D. J. (2019a. Lung Inflammatory Environments Differentially Alter Mesenchymal Stromal Cell Behavior. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *317*(6), L823–L831. https://doi.org/10.1152/ajplung.00263.2019

Abreu, S. C., Xisto, D. G., Oliveira, T. B., Blanco, N. G., Castro, L. L., Kitoko, J. Z., Olsen, P. C., Lopes-Pacheco, M., Morales, M. M., Weiss, D. J., & Rocco, P. R. M. (2019). Serum from Asthmatic Mice Potentiates the Therapeutic Effects of Mesenchymal Stromal Cells in Experimental Allergic Asthma. *Stem Cells Translational Medicine*, *8*(3), 301–312. https://doi.org/10.1002/sctm.18-0056

Acevedo, N., Zakzuk, J., & Caraballo, L. (2019). House Dust Mite Allergy Under Changing Environments. *Allergy, Asthma & Immunology Research*, *11*(4), 450. https://doi.org/10.4168/aair.2019.11.4.450

Adamali, H., Armstrong, M. E., McLaughlin, A. M., Cooke, G., McKone, E., Costello, C. M., Gallagher, C. G., Leng, L., Baugh, J. A., Fingerle-Rowson, G., Bucala, R. J., McLoughlin, P., & Donnelly, S. C. (2012). Macrophage Migration Inhibitory Factor Enzymatic Activity, Lung Inflammation, and Cystic Fibrosis. *American Journal of Respiratory and Critical Care Medicine*, *186*(2), 162–169. https://doi.org/10.1164/rccm.201110-1864OC

Agache, I., Song, Y., Rocha, C., Beltran, J., Posso, M., Steiner, C., Alonso-Coello, P., Akdis, C., Akdis, M., Canonica, G. W., Casale, T., Chivato, T., Corren, J., Giacco, S., Eiwegger, T., Firinu, D., Gern, J. E., Hamelmann, E., Hanania, N., … Jutel, M. (2020). Efficacy And Safety Of Treatment With Dupilumab For Severe Asthma: A Systematic Review Of The EAACI Guidelines—Recommendations On The Use Of Biologicals In Severe Asthma. *Allergy*, *75*(5), 1058–1068. https://doi.org/10.1111/all.14268

Ahmad, T., Mukherjee, S., Pattnaik, B., Kumar, M., Singh, S., Kumar, M., Rehman, R., Tiwari, B. K., Jha, K. A., Barhanpurkar, A. P., Wani, M. R., Roy, S. S., Mabalirajan, U., Ghosh, B., & Agrawal, A. (2014). Miro1 Regulates Intercellular Mitochondrial Transport &Amp; Enhances Mesenchymal Stem Cell Rescue Efficacy. *The EMBO Journal*, n/a-n/a. https://doi.org/10.1002/embj.201386030

Akdis, C. A. (2021). Does The Epithelial Barrier Hypothesis Explain The Increase In Allergy, Autoimmunity And Other Chronic Conditions? *Nature Reviews Immunology*, *21*(11), 739–751. https://doi.org/10.1038/s41577-021-00538-7

Akhter, W., Nakhle, J., Vaillant, L., Garcin, G., Le Saout, C., Simon, M., Crozet, C., Djouad, F., Jorgensen, C., Vignais, M.-L., & Hernandez, J. (2023). Transfer Of Mesenchymal Stem Cell Mitochondria To CD4+ T Cells Contributes To Repress Th1 Differentiation By Downregulating

T-Bet Expression. *Stem Cell Research & Therapy*, *14*(1), 12. https://doi.org/10.1186/s13287-022-03219-x

Aksakal, A., Kerget, B., Kerget, F., & Aşkın, S. (2021). Evaluation Of The Relationship Between Macrophage Migration Inhibitory Factor Level And Clinical Course In Patients With COVID-19 Pneumonia. *Journal of Medical Virology*, *93*(12), 6519–6524. https://doi.org/10.1002/jmv.27189

Al-Abed, Y., Dabideen, D., Aljabari, B., Valster, A., Messmer, D., Ochani, M., Tanovic, M., Ochani, K., Bacher, M., Nicoletti, F., Metz, C., Pavlov, V. A., Miller, E. J., & Tracey, K. J. (2005). ISO-1 Binding to the Tautomerase Active Site of MIF Inhibits Its Pro-inflammatory Activity and Increases Survival in Severe Sepsis. *Journal of Biological Chemistry*, *280*(44), 36541–36544. https://doi.org/10.1074/jbc.C500243200

Alampour-Rajabi, S., El Bounkari, O., Rot, A., Müller-Newen, G., Bachelerie, F., Gawaz, M., Weber, C., Schober, A., & Bernhagen, J. (2015). MIF Interacts With CXCR7 To Promote Receptor Internalization, ERK1/2 And ZAP-70 Signaling, And Lymphocyte Chemotaxis. *The FASEB Journal*, *29*(11), 4497–4511. https://doi.org/10.1096/fj.15-273904

Albers, G. J., Ogger, P. P., Gray, R., Halket, J. M., Gauvreau, G., O'Byrne, P., Lloyd, C. M., & Byrne, A. J. (2022). Allergen Exposure Induces Airway Macrophage Metabolic Reprogramming. *Airway Cell Biology and Immunopathology*, 43. https://doi.org/10.1183/23120541.LSC-2022.43

Allam, V. S. R. R., Pavlidis, S., Liu, G., Kermani, N. Z., Simpson, J., To, J., Donnelly, S., Guo, Y.-K., Hansbro, P. M., Phipps, S., Morand, E. F., Djukanovic, R., Sterk, P., Chung, K. F., Adcock, I., Harris, J., & Sukkar, M. B. (2022). Macrophage Migration Inhibitory Factor Promotes Glucocorticoid Resistance Of Neutrophilic Inflammation In A Murine Model Of Severe Asthma. *Thorax*, thoraxjnl-2021-218555. https://doi.org/10.1136/thorax-2021-218555

Al-Shuweli, S., Landt, E., Ellervik, C., Poulsen, H. E., Ramar, M., Dahl, M., & Fedulov, A. V. (2023). Risk Of Asthma In Offspring Of Asthmatic Fathers Versus Mothers: A Population-Based Study Of 21,000 Individuals In Denmark. *Respiratory Medicine*, *207*, 107116. https://doi.org/10.1016/j.rmed.2023.107116

Altorki, N. K., Markowitz, G. J., Gao, D., Port, J. L., Saxena, A., Stiles, B., McGraw, T., & Mittal, V. (2019). The Lung Microenvironment: An Important Regulator Of Tumour Growth And Metastasis. *Nature Reviews Cancer*, *19*(1), 9–31. https://doi.org/10.1038/s41568-018-0081-9

Amano, T., Nishihira, J., & Miki, I. (2007). Blockade Of Macrophage Migration Inhibitory Factor (MIF) Prevents The Antigen-Induced Response In A Murine Model Of Allergic Airway Inflammation. *Inflammation Research*, *56*(1), 24–31. https://doi.org/10.1007/s00011-007-5184-9

Amin, M. A., Haas, C. S., Zhu, K., Mansfield, P. J., Kim, M. J., Lackowski, N. P., & Koch, A. E. (2006). Migration Inhibitory Factor Up-Regulates Vascular Cell Adhesion Molecule-1 And Intercellular Adhesion Molecule-1 Via Src, PI3 Kinase, And Nfκb. *Blood*, *107*(6), 2252–2261. https://doi.org/10.1182/blood-2005-05-2011

Ankrum, J. A., Dastidar, R. G., Ong, J. F., Levy, O., & Karp, J. M. (2014). Performance-Enhanced Mesenchymal Stem Cells Via Intracellular Delivery Of Steroids. *Scientific Reports*, *4*(1), 4645. https://doi.org/10.1038/srep04645

Ankrum, J. A., Ong, J. F., & Karp, J. M. (2014). Mesenchymal Stem Cells: Immune Evasive, Not Immune Privileged. *Nature Biotechnology*, *32*(3), 252–260. https://doi.org/10.1038/nbt.2816

Arenberg, D., Luckhardt, T. R., Carskadon, S., Zhao, L., Amin, M. A., & Koch, A. E. (2010). Macrophage Migration Inhibitory Factor Promotes Tumor Growth in the Context of Lung Injury and Repair. *American Journal of Respiratory and Critical Care Medicine*, *182*(8), 1030–1037. https://doi.org/10.1164/rccm.201001-0120OC

Arutyunyan, I., Fatkhudinov, T., Kananykhina, E., Usman, N., Elchaninov, A., Makarov, A., Bolshakova, G., Goldshtein, D., & Sukhikh, G. (2016). Role Of VEGF-A In Angiogenesis Promoted By Umbilical Cord-Derived Mesenchymal Stromal/Stem Cells: In Vitro Study. *Stem Cell Research & Therapy*, *7*(1), 46. https://doi.org/10.1186/s13287-016-0305-4

Atanasova, E., Milosevic, D., Bornschlegl, S., Krucker, K. P., Jacob, E. K., Carmona Porquera, E. M., Anderson, D. K., Egan, A. M., Limper, A. H., & Dietz, A. B. (2022). Normal Ex Vivo Mesenchymal Stem Cell Function Combined With Abnormal Immune Profiles Sets The Stage For Informative Cell Therapy Trials In Idiopathic Pulmonary Fibrosis Patients. *Stem Cell Research & Therapy*, *13*(1), 45. https://doi.org/10.1186/s13287-021-02692-0

Attar-Zadeh, D., Capstick, T., Leese, D., Arnetorp, S., Rapsomaniki, E., Da Costa, K. P., Maslova, E., Xu, Y., Gibson, D., & Quint, J. K. (2023). Healthcare Costs Associated With Short-Acting B $_2$ -Agonists In Asthma: Observational UK SABINA Study. *BJGP Open*, BJGPO.2023.0015. https://doi.org/10.3399/BJGPO.2023.0015

Austin, C. D., Gonzalez Edick, M., Ferrando, R. E., Solon, M., Baca, M., Mesh, K., Bradding, P., Gauvreau, G. M., Sumino, K., FitzGerald, J. M., Israel, E., Bjermer, L., Bourdin, A., Arron, J. R., Choy, D. F., Olsson, J. K., Abreu, F., Howard, M., Wong, K., Woodruff, P. G. (2020). A Randomized, Placebo-Controlled Trial Evaluating Effects Of Lebrikizumab On Airway Eosinophilic Inflammation And Remodelling In Uncontrolled Asthma (CLAVIER). *Clinical & Experimental Allergy*, *50*(12), 1342–1351. https://doi.org/10.1111/cea.13731

Awandare, G. A., Martinson, J. J., Were, T., Ouma, C., Davenport, G. C., Ong'echa, J. M., Wang, W., Leng, L., Ferrell, R. E., Bucala, R., & Perkins, D. J. (2009). *MIF* (Macrophage Migration Inhibitory Factor) Promoter Polymorphisms and Susceptibility to Severe Malarial Anemia. *The Journal of Infectious Diseases*, *200*(4), 629–637. https://doi.org/10.1086/600894

Bacher, M., Metz, C. N., Calandra, T., Mayer, K., Chesney, J., Lohoff, M., Gemsa, D., Donnelly, T., & Bucala, R. (1996). An Essential Regulatory Role For Macrophage Migration Inhibitory Factor In T-Cell Activation. *Proceedings of the National Academy of Sciences*, *93*(15), 7849–7854. https://doi.org/10.1073/pnas.93.15.7849

Bakakos, P., Tryfon, S., Palamidas, A., Mathioudakis, N., & Galanakis, P. (2023). Patient Characteristics And Eligibility For Biologics In Severe Asthma: Results From The Greek Cohort Of The RECOGNISE "Real World" Study. *Respiratory Medicine*, *210*, 107170. https://doi.org/10.1016/j.rmed.2023.107170

Bandeira, E., Jang, S. C., Lässer, C., Johansson, K., Rådinger, M., & Park, K.-S. (2023). Effects Of Mesenchymal Stem Cell-Derived Nanovesicles In Experimental Allergic Airway Inflammation. *Respiratory Research*, *24*(1), 3. https://doi.org/10.1186/s12931-023-02310-y

Bargagli, E., Olivieri, C., Nikiforakis, N., Cintorino, M., Magi, B., Perari, M. G., Vagaggini, C., Spina, D., Prasse, A., & Rottoli, P. (2009). Analysis Of Macrophage Migration Inhibitory Factor (MIF) In Patients With Idiopathic Pulmonary Fibrosis. *Respiratory Physiology & Neurobiology*, *167*(3), 261–267. https://doi.org/10.1016/j.resp.2009.05.004

Baugh, J. A., Chitnis, S., Donnelly, S. C., Monteiro, J., Lin, X., Plant, B. J., Wolfe, F., Gregersen, P. K., & Bucala, R. (2002). A Functional Promoter Polymorphism In The Macrophage Migration Inhibitory Factor (MIF) Gene Associated With Disease Severity In Rheumatoid Arthritis. *Genes & Immunity*, *3*(3), 170–176. https://doi.org/10.1038/sj.gene.6363867

Bekkering, S., Blok, B. A., Joosten, L. A. B., Riksen, N. P., van Crevel, R., & Netea, M. G. (2016). *In Vitro* Experimental Model of Trained Innate Immunity in Human Primary Monocytes. *Clinical and Vaccine Immunology*, *23*(12), 926–933. https://doi.org/10.1128/CVI.00349-16

Bel, E. H. (2017). Moving Upstream — Anti-TSLP in Persistent Uncontrolled Asthma. *New England Journal of Medicine*, *377*(10), 989–991. https://doi.org/10.1056/NEJMe1709519

Bendrat, K., Al-Abed, Y., Callaway, D. J. E., Peng, T., Calandra, T., Metz, C. N., & Bucala, R. (1997). Biochemical and Mutational Investigations of the Enzymatic Activity of Macrophage Migration Inhibitory Factor. *Biochemistry*, *36*(49), 15356–15362. https://doi.org/10.1021/bi971153a

Berg, M. K., Yu, Q., Salvador, C. E., Melani, I., & Kitayama, S. (2020). Mandated Bacillus Calmette-Guérin (BCG) Vaccination Predicts Flattened Curves For The Spread Of COVID-19. *Science Advances*, *6*(32). https://doi.org/10.1126/sciadv.abc1463

Bernhagen, J., Calandra, T., & Bucala, R. (1994). The Emerging Role Of MIF In Septic Shock And Infection. *Biotherapy*, *8*(2), 123–127. https://doi.org/10.1007/BF01878495

Bernhagen, J., Calandra, T., Mitchell, R. A., Martin, S. B., Tracey, K. J., Voelter, W., Manogue, K. R., Cerami, A., & Bucala, R. (1993). MIF Is A Pituitary-Derived Cytokine That Potentiates Lethal Endotoxaemia. *Nature*, *365*(6448), 756–759. https://doi.org/10.1038/365756a0

Bernhagen, J., Krohn, R., Lue, H., Gregory, J. L., Zernecke, A., Koenen, R. R., Dewor, M., Georgiev, I., Schober, A., Leng, L., Kooistra, T., Fingerle-Rowson, G., Ghezzi, P., Kleemann, R., McColl, S. R., Bucala, R., Hickey, M. J., & Weber, C. (2007). MIF Is A Noncognate Ligand Of CXC Chemokine Receptors In Inflammatory And Atherogenic Cell Recruitment. *Nature Medicine*, *13*(5), 587–596. https://doi.org/10.1038/nm1567

Bernhagen, J., Mitchell, R. A., Calandra, T., Voelter, W., Cerami, A., & Bucala, R. (1994). Purification, Bioactivity, and Secondary Structure Analysis of Mouse and Human Macrophage Migration Inhibitory Factor (MIF). *Biochemistry*, *33*(47), 14144–14155. https://doi.org/10.1021/bi00251a025

Bilsborrow, J. B., Doherty, E., Tilstam, P. V., & Bucala, R. (2019). Macrophage Migration Inhibitory Factor (MIF) As A Therapeutic Target For Rheumatoid Arthritis And Systemic Lupus Erythematosus. *Expert Opinion on Therapeutic Targets*, *23*(9), 733–744. https://doi.org/10.1080/14728222.2019.1656718

Biswas, S., Mandal, G., Roy Chowdhury, S., Purohit, S., Payne, K. K., Anadon, C., Gupta, A., Swanson, P., Yu, X., Conejo-Garcia, J. R., & Bhattacharyya, A. (2019). Exosomes Produced by Mesenchymal Stem Cells Drive Differentiation of Myeloid Cells into Immunosuppressive M2-Polarized Macrophages in Breast Cancer. *The Journal of Immunology*, *203*(12), 3447–3460. https://doi.org/10.4049/jimmunol.1900692

Blauvelt, A., Thyssen, J. P., Guttman-Yassky, E., Bieber, T., Serra-Baldrich, E., Simpson, E., Rosmarin, D., Elmaraghy, H., Meskimen, E., Natalie, C. R., Liu, Z., Xu, C., Pierce, E., Morgan-Cox, M., Garcia Gil, E., & Silverberg, J. I. (2023). Efficacy And Safety Of Lebrikizumab In Moderate-To-Severe Atopic Dermatitis: 52-Week Results Of Two Randomized Double-Blinded Placebo-Controlled Phase III Trials. *British Journal of Dermatology*. https://doi.org/10.1093/bjd/ljad022

Bloom, J., Metz, C., Nalawade, S., Casabar, J., Cheng, K. F., He, M., Sherry, B., Coleman, T., Forsthuber, T., & Al-Abed, Y. (2016). Identification of Iguratimod as an Inhibitor of Macrophage Migration Inhibitory Factor (MIF) with Steroid-sparing Potential. *Journal of Biological Chemistry*, *291*(51), 26502–26514. https://doi.org/10.1074/jbc.M116.743328

Bogdan, C., Röllinghoff, M., & Diefenbach, A. (2000). The Role Of Nitric Oxide In Innate Immunity. *Immunological Reviews*, *173*(1), 17–26. https://doi.org/10.1034/j.1600-065X.2000.917307.x

Boland, L., Bitterlich, L. M., Hogan, A. E., Ankrum, J. A., & English, K. (2022). Translating MSC Therapy in the Age of Obesity. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.943333

Boland, L., Burand, A. J., Brown, A. J., Boyt, D., Lira, V. A., & Ankrum, J. A. (2018). IFN-γ and TNF-α Pre-licensing Protects Mesenchymal Stromal Cells from the Pro-inflammatory Effects of Palmitate. *Molecular Therapy*, *26*(3), 860–873. https://doi.org/10.1016/j.ymthe.2017.12.013

Bonfield, T. L., Sutton, M. T., Fletcher, D. R., Reese-Koc, J., Roesch, E. A., Lazarus, H. M., Chmiel, J. F., & Caplan, A. I. (2023). Human Mesenchymal Stem Cell (hMSC) Donor Potency Selection for the "First in Cystic Fibrosis" Phase I Clinical Trial (CEASE-CF). *Pharmaceuticals*, *16*(2), 220. https://doi.org/10.3390/ph16020220

Boonpiyathad, T., Sözener, Z. C., Satitsuksanoa, P., & Akdis, C. A. (2019). Immunologic Mechanisms In Asthma. *Seminars in Immunology*, *46*, 101333. https://doi.org/10.1016/j.smim.2019.101333

Bošnjak, B., Kazemi, S., Altenburger, L. M., Mokrović, G., & Epstein, M. M. (2019). Th2-TRMs Maintain Life-Long Allergic Memory in Experimental Asthma in Mice. *Frontiers in Immunology*, *10*. https://doi.org/10.3389/fimmu.2019.00840

Bourgoin-Heck, M., Amat, F., Trouvé, C., Bernard, A., Magny, J.-P., Lambert, N., & Just, J. (2018). Omalizumab Could Be Effective In Children With Severe Eosinophilic Non-Allergic Asthma. *Pediatric Allergy and Immunology*, *29*(1), 90–93. https://doi.org/10.1111/pai.12813

Boyt, D. T., Boland, L. K., Burand, A. J., Brown, A. J., & Ankrum, J. A. (2020). Dose And Duration Of Interferon Gamma Pre-Licensing Interact With Donor Characteristics To Influence The Expression And Function Of Indoleamine-2,3-Dioxygenase In Mesenchymal Stromal Cells. *Journal of The Royal Society Interface*, *17*(167), 20190815. https://doi.org/10.1098/rsif.2019.0815

Bozza, F. A., Gomes, R. N., Japiass, A. M., Soares, M., Castro-Faria-Neto, H. C., Bozza, P. T., & Bozza, M. T. (2004). Macrophage Migration Inhibitory Factor Levels Correlate With Fatal Outcome In Sepsis. *Shock*, *22*(4), 309–313. Https://Doi.Org/10.1097/01.Shk.0000140305.01641.C8

Bozza, M. T., Lintomen, L., Kitoko, J. Z., Paiva, C. N., & Olsen, P. C. (2020). The Role of MIF on Eosinophil Biology and Eosinophilic Inflammation. *Clinical Reviews in Allergy & Immunology*, *58*(1), 15–24. https://doi.org/10.1007/s12016-019-08726-z

Branchett, W. J., Cook, J., Oliver, R. A., Bruno, N., Walker, S. A., Stölting, H., Mack, M., O'Garra, A., Saglani, S., & Lloyd, C. M. (2021). Airway Macrophage-Intrinsic TGF-B1 Regulates Pulmonary

Immunity During Early-Life Allergen Exposure. *Journal of Allergy and Clinical Immunology*, *147*(5), 1892–1906. https://doi.org/10.1016/j.jaci.2021.01.026

Braza, F., Dirou, S., Forest, V., Sauzeau, V., Hassoun, D., Chesné, J., Cheminant-Muller, M.-A., Sagan, C., Magnan, A., & Lemarchand, P. (2016). Mesenchymal Stem Cells Induce Suppressive Macrophages Through Phagocytosis in a Mouse Model of Asthma. *Stem Cells*, *34*(7), 1836–1845. https://doi.org/10.1002/stem.2344

Brichkina, A., & Simon, H.-U. (2023). A Novel Role For Mitochondrial Fission In Macrophages: Trained Innate Immunity Induced By Beta-Glucan. *Cellular & Molecular Immunology*. https://doi.org/10.1038/s41423-023-01017-w

Bucala, R. (2013). MIF, MIF Alleles, and Prospects for Therapeutic Intervention in Autoimmunity. *Journal of Clinical Immunology*, *33*(S1), 72–78. https://doi.org/10.1007/s10875-012-9781-1

Burand, A. J., Di, L., Boland, L. K., Boyt, D. T., Schrodt, M. V., Santillan, D. A., & Ankrum, J. A. (2020). Aggregation of Human Mesenchymal Stromal Cells Eliminates Their Ability to Suppress Human T Cells. *Frontiers in Immunology*, *11*. https://doi.org/10.3389/fimmu.2020.00143

Bush, A. (2023). Differing Effects Of Mepolizumab Across The Life Course. *The Lancet Respiratory Medicine*, *11*(2), 123–125. https://doi.org/10.1016/S2213-2600(23)00004-8

Busse, W. W., Bleecker, E. R., FitzGerald, J. M., Ferguson, G. T., Barker, P., Sproule, S., Olsson, R. F., Martin, U. J., Goldman, M., Yañez, A., Fernández, M., Tolcachier, A., Belloni, J., Taborda, J., De Salvo, M., Maspero, J., Victorio, C., Navarta, M. C., Grilli, M., Le, L. (2019). Long-Term Safety And Efficacy Of Benralizumab In Patients With Severe, Uncontrolled Asthma: 1-Year Results From The BORA Phase 3 Extension Trial. *The Lancet Respiratory Medicine*, *7*(1), 46–59. https://doi.org/10.1016/S2213-2600(18)30406-5

Butala, S., Castelo-Soccio, L., Seshadri, R., Simpson, E. L., O'Shea, J. J., Bieber, T., & Paller, A. S. (2023). Biologic Versus Small Molecule Therapy for Treating Moderate to Severe Atopic Dermatitis: Clinical Considerations. *The Journal of Allergy and Clinical Immunology: In Practice*. https://doi.org/10.1016/j.jaip.2023.03.011

Byers, D. E. (2019). Linking VEGF Deficiency and IL-33 Upregulation in Chronic Obstructive Pulmonary Disease. *American Journal of Respiratory Cell and Molecular Biology*, *61*(5), 550–551. https://doi.org/10.1165/rcmb.2019-0175ED

Cahill, E. F., Kennelly, H., Carty, F., Mahon, B. P., & English, K. (2016). Hepatocyte Growth Factor Is Required for Mesenchymal Stromal Cell Protection Against Bleomycin-Induced Pulmonary Fibrosis. *Stem Cells Translational Medicine*, *5*(10), 1307–1318. https://doi.org/10.5966/sctm.2015-0337

Cahill, E. F., Tobin, L. M., Carty, F., Mahon, B. P., & English, K. (2015). Jagged-1 Is Required For The Expansion Of CD4+ CD25+ Foxp3+ Regulatory T Cells And Tolerogenic Dendritic Cells By Murine Mesenchymal Stromal Cells. *Stem Cell Research & Therapy*, *6*(1), 19. https://doi.org/10.1186/s13287-015-0021-5

Calandra, T., Bernhagen, J., Metz, C. N., Spiegel, L. A., Bacher, M., Donnelly, T., Cerami, A., & Bucala, R. (1995). MIF As A Glucocorticoid-Induced Modulator Of Cytokine Production. *Nature*, *377*(6544), 68–71. https://doi.org/10.1038/377068a0

Calandra, T., Bernhagen, J., Mitchell, R. A., & Bucala, R. (1994). The Macrophage Is An Important And Previously Unrecognized Source Of Macrophage Migration Inhibitory Factor. *Journal of Experimental Medicine*, *179*(6), 1895–1902. https://doi.org/10.1084/jem.179.6.1895

Calandra, T., Echtenacher, B., Roy, D. Le, Pugin, J., Metz, C. N., Hültner, L., Heumann, D., Männel, D., Bucala, R., & Glauser, M. P. (2000). Protection From Septic Shock By Neutralization Of Macrophage Migration Inhibitory Factor. *Nature Medicine*, *6*(2), 164–170. https://doi.org/10.1038/72262

Calandra, T., & Roger, T. (2003). Macrophage Migration Inhibitory Factor: A Regulator Of Innate Immunity. *Nature Reviews Immunology*, *3*(10), 791–800. https://doi.org/10.1038/nri1200

Camarinha, C., Fernandes, M., Alarc.úo, V., Franco, J., Mana.ºas, M. E., B.írbara, C., & Nicola, P. J. (2023). Determinants Associated With Uncontrolled Asthma In Portugal: A National Population-Based Study. *Pulmonology*, *29*(1), 29–41. https://doi.org/10.1016/j.pulmoe.2020.02.014

Carty, F., Corbett, J. M., Cunha, J. P. M. C. M., Reading, J. L., Tree, T. I. M., Ting, A. E., Stubblefield, S. R., & English, K. (2018). Multipotent Adult Progenitor Cells Suppress T Cell Activation in In

Vivo Models of Homeostatic Proliferation in a Prostaglandin E2-Dependent Manner. *Frontiers in Immunology*, *9*. https://doi.org/10.3389/fimmu.2018.00645

Carty, F., Dunbar, H., Hawthorne, I. J., Ting, A. E., Stubblefield, S. R., Van't Hof, W., & English, K. (2021). IFN-γ and PPARδ Influence the Efficacy and Retention of Multipotent Adult Progenitor Cells in Graft vs Host Disease. *Stem Cells Translational Medicine*, *10*(11), 1561–1574. https://doi.org/10.1002/sctm.21-0008

Carty, F., Mahon, B. P., & English, K. (2017). The Influence Of Macrophages On Mesenchymal Stromal Cell Therapy: Passive Or Aggressive Agents? *Clinical and Experimental Immunology*, *188*(1), 1–11. https://doi.org/10.1111/cei.12929

Carvalho, A. É. S.-, Sousa, M. R. R., Alencar-Silva, T., Carvalho, J. L., & Saldanha-Araujo, F. (2019). Mesenchymal Stem Cells Immunomodulation: The Road To IFN-Γ Licensing And The Path Ahead. *Cytokine & Growth Factor Reviews*, *47*, 32–42. https://doi.org/10.1016/j.cytogfr.2019.05.006

Casale, T. B., Luskin, A. T., Busse, W., Zeiger, R. S., Trzaskoma, B., Yang, M., Griffin, N. M., & Chipps, B. E. (2019). Omalizumab Effectiveness by Biomarker Status in Patients with Asthma: Evidence From PROSPERO, A Prospective Real-World Study. *The Journal of Allergy and Clinical Immunology: In Practice*, *7*(1), 156-164.e1. https://doi.org/10.1016/j.jaip.2018.04.043

Castro, B. A., Flanigan, P., Jahangiri, A., Hoffman, D., Chen, W., Kuang, R., De Lay, M., Yagnik, G., Wagner, J. R., Mascharak, S., Sidorov, M., Shrivastav, S., Kohanbash, G., Okada, H., & Aghi, M. K. (2017). Macrophage Migration Inhibitory Factor Downregulation: A Novel Mechanism Of Resistance To Anti-Angiogenic Therapy. *Oncogene*, *36*(26), 3749–3759. https://doi.org/10.1038/onc.2017.1

Castro, L. L., Kitoko, J. Z., Xisto, D. G., Olsen, P. C., Guedes, H. L. M., Morales, M. M., Lopes-Pacheco, M., Cruz, F. F., & Rocco, P. R. M. (2020). Multiple Doses Of Adipose Tissue-Derived Mesenchymal Stromal Cells Induce Immunosuppression In Experimental Asthma. *Stem Cells Translational Medicine*, *9*(2), 250–260. https://doi.org/10.1002/sctm.19-0120

Castro, M., Corren, J., Pavord, I. D., Maspero, J., Wenzel, S., Rabe, K. F., Busse, W. W., Ford, L., Sher, L., FitzGerald, J. M., Katelaris, C., Tohda, Y., Zhang, B., Staudinger, H., Pirozzi, G., Amin, N., Ruddy, M., Akinlade, B., Khan, A., Teper, A. (2018). Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma. *New England Journal of Medicine*, *378*(26), 2486–2496. https://doi.org/10.1056/NEJMoa1804092

Cereta, A. D., Oliveira, V. R., Costa, I. P., Afonso, J. P. R., Fonseca, A. L., de Souza, A. R. T., Silva, G. A. M., Mello, D. A. C. P. G., Oliveira, L. V. F. de, & da Palma, R. K. (2021). Emerging Cell-Based Therapies in Chronic Lung Diseases: What About Asthma? *Frontiers in Pharmacology*, *12*. https://doi.org/10.3389/fphar.2021.648506

Chapman, M. D., Pomés, A., Breiteneder, H., & Ferreira, F. (2007). Nomenclature And Structural Biology Of Allergens. *Journal of Allergy and Clinical Immunology*, *119*(2), 414–420. https://doi.org/10.1016/j.jaci.2006.11.001

Chen, H., Xia, W., & Hou, M. (2020). Lncrna-NEAT1 From The Competing Endogenous RNA Network Promotes Cardioprotective Efficacy Of Mesenchymal Stem Cell-Derived Exosomes Induced By Macrophage Migration Inhibitory Factor Via The Mir-142-3p/FOXO1 Signaling Pathway. *Stem Cell Research & Therapy*, *11*(1), 31. https://doi.org/10.1186/s13287-020-1556-7

Chen, J., Gao, L., Wu, X., Fan, Y., Liu, M., Peng, L., Song, J., Li, B., Liu, A., & Bao, F. (2023). BCG-Induced Trained Immunity: History, Mechanisms And Potential Applications. *Journal of Translational Medicine*, *21*(1), 106. https://doi.org/10.1186/s12967-023-03944-8

Chen, P.-F., Luo, Y., Wang, W., Wang, J., Lai, W., Hu, S., Cheng, K. F., & Al-Abed, Y. (2010). ISO-1, a Macrophage Migration Inhibitory Factor Antagonist, Inhibits Airway Remodeling in a Murine Model of Chronic Asthma. *Molecular Medicine*, *16*(9–10), 400–408. https://doi.org/10.2119/molmed.2009.00128

Cheng, H.-Y., Anggelia, M. R., Lin, C.-H., & Lin, C.-F. (2021). Preconditioned Mesenchymal Stromal Cells to Improve Allotransplantation Outcome. *Cells*, *10*(9), 2325. https://doi.org/10.3390/cells10092325

Chinnadurai, R., Copland, I. B., Patel, S. R., & Galipeau, J. (2014). IDO-Independent Suppression of T Cell Effector Function by IFN-γ–Licensed Human Mesenchymal Stromal Cells. *The Journal of Immunology*, *192*(4), 1491–1501. https://doi.org/10.4049/jimmunol.1301828

Chiossone, L., Conte, R., Spaggiari, G. M., Serra, M., Romei, C., Bellora, F., Becchetti, F., Andaloro, A., Moretta, L., & Bottino, C. (2016). Mesenchymal Stromal Cells Induce Peculiar Alternatively Activated Macrophages Capable of Dampening Both Innate and Adaptive Immune Responses. *Stem Cells*, *34*(7), 1909–1921. https://doi.org/10.1002/stem.2369

Choi, J. Y., Hur, J., Jeon, S., Jung, C. K., & Rhee, C. K. (2022). Effects Of Human Adipose Tissue- And Bone Marrow-Derived Mesenchymal Stem Cells On Airway Inflammation And Remodeling In A Murine Model Of Chronic Asthma. *Scientific Reports*, *12*(1), 12032. https://doi.org/10.1038/s41598-022-16165-8

Christ, A., & Latz, E. (2019). The Western Lifestyle Has Lasting Effects On Metaflammation. *Nature Reviews Immunology*, *19*(5), 267–268. https://doi.org/10.1038/s41577-019-0156-1

Chupp, G., Heaney, L., Price, R., Brusselle, G., Gardiner, F., Raimondi, A., Chaudhuri, R., & Howarth, P. (2023). Real-World Impact of Mepolizumab On The Oral Corticosteroid Freedom Component Of Clinical Remission In Severe Eosinophilic Asthma: International, Prospective REALITI-A Study. *Journal of Allergy and Clinical Immunology*, *151*(2), AB20. https://doi.org/10.1016/j.jaci.2022.12.066

Cisneros, J. A., Robertson, M. J., Valhondo, M., & Jorgensen, W. L. (2016). Irregularities In Enzyme Assays: The Case Of Macrophage Migration Inhibitory Factor. *Bioorganic & Medicinal Chemistry Letters*, *26*(12), 2764–2767. https://doi.org/10.1016/j.bmcl.2016.04.074

Coleman, A. M., Rendon, B. E., Zhao, M., Qian, M.-W., Bucala, R., Xin, D., & Mitchell, R. A. (2008). Cooperative Regulation Of Non-Small Cell Lung Carcinoma Angiogenic Potential By Macrophage Migration Inhibitory Factor And Its Homolog, <Scp>D</Scp> -Dopachrome Tautomerase. *The Journal of Immunology*, *181*(4), 2330–2337. https://doi.org/10.4049/jimmunol.181.4.2330

Conrath, U., Beckers, G. J. M., Langenbach, C. J. G., & Jaskiewicz, M. R. (2015). Priming for Enhanced Defense. *Annual Review of Phytopathology*, *53*(1), 97–119. https://doi.org/10.1146/annurev-phyto-080614-120132

Corbett, J. M., Hawthorne, I., Dunbar, H., Coulter, I., Chonghaile, M. N., Flynn, C. M., & English, K. (2021). Cyclosporine A and IFNγ Licencing Enhances Human Mesenchymal Stromal Cell Potency In A Humanised Mouse Model Of Acute Graft Versus Host Disease. *Stem Cell Research & Therapy*, *12*(1), 238. https://doi.org/10.1186/s13287-021-02309-6

Corren, J., Parnes, J. R., Wang, L., Mo, M., Roseti, S. L., Griffiths, J. M., & van der Merwe, R. (2017). Tezepelumab in Adults with Uncontrolled Asthma. *New England Journal of Medicine*, *377*(10), 936–946. https://doi.org/10.1056/NEJMoa1704064

Costa, L. A., Eiro, N., Fraile, M., Gonzalez, L. O., Saá, J., Garcia-Portabella, P., Vega, B., Schneider, J., & Vizoso, F. J. (2021). Functional Heterogeneity Of Mesenchymal Stem Cells From Natural Niches To Culture Conditions: Implications For Further Clinical Uses. *Cellular and Molecular Life Sciences*, *78*(2), 447–467. https://doi.org/10.1007/s00018-020-03600-0

Cotzomi-Ortega, I., Nieto-Yañez, O., Juárez-Avelar, I., Rojas-Sanchez, G., Montes-Alvarado, J. B., Reyes-Leyva, J., Aguilar-Alonso, P., Rodriguez-Sosa, M., & Maycotte, P. (2021). Autophagy Inhibition In Breast Cancer Cells Induces ROS-Mediated MIF Expression And M1 Macrophage Polarization. *Cellular Signalling*, *86*, 110075. https://doi.org/10.1016/j.cellsig.2021.110075

Covián, C., Retamal-Díaz, A., Bueno, S. M., & Kalergis, A. M. (2020). Could BCG Vaccination Induce Protective Trained Immunity for SARS-CoV-2? *Frontiers in Immunology*, *11*. https://doi.org/10.3389/fimmu.2020.00970

Creese, H., Lai, E., Mason, K., Schlüter, D. K., Saglani, S., Taylor-Robinson, D., & Saxena, S. (2022). Disadvantage In Early-Life And Persistent Asthma In Adolescents: A UK Cohort Study. *Thorax*, *77*(9), 854–864. https://doi.org/10.1136/thoraxjnl-2021-217312

Cruz, F. F., Borg, Z. D., Goodwin, M., Sokocevic, D., Wagner, D. E., Coffey, A., Antunes, M., Robinson, K. L., Mitsialis, S. A., Kourembanas, S., Thane, K., Hoffman, A. M., McKenna, D. H., Rocco, P. R. M., & Weiss, D. J. (2015). Systemic Administration of Human Bone Marrow-Derived Mesenchymal Stromal Cell Extracellular Vesicles Ameliorates *Aspergillus* Hyphal Extract-Induced Allergic Airway Inflammation in Immunocompetent Mice. *Stem Cells Translational Medicine*, *4*(11), 1302–1316. https://doi.org/10.5966/sctm.2014-0280

Cruz, F. F., Borg, Z. D., Goodwin, M., Sokocevic, D., Wagner, D., McKenna, D. H., Rocco, P. R. M., & Weiss, D. J. (2015). Freshly Thawed And Continuously Cultured Human Bone Marrow-Derived

Mesenchymal Stromal Cells Comparably Ameliorate Allergic Airways Inflammation In Immunocompetent Mice. *Stem Cells Translational Medicine*, *4*(6), 615–624. https://doi.org/10.5966/sctm.2014-0268

Cruz, F. F., Rocco, P. R. M., & Weiss, D. J. (2018). Hmscs As An Alternative Therapeutic Option For Asthma With Neutrophil Mediated Inflammation. *Experimental & Molecular Medicine*, *50*(6), 1–2. https://doi.org/10.1038/s12276-018-0072-7

Cunningham, K. T., Finlay, C. M., & Mills, K. H. G. (2021). Helminth Imprinting of Hematopoietic Stem Cells Sustains Anti-Inflammatory Trained Innate Immunity That Attenuates Autoimmune Disease. *The Journal of Immunology*, *206*(7), 1618–1630. https://doi.org/10.4049/jimmunol.2001225

Cvetkovic, I., Al-Abed, Y., Miljkovic, D., Maksimovic-Ivanic, D., Roth, J., Bacher, M., Lan, H. Y., Nicoletti, F., & Stosic-Grujicic, S. (2005). Critical Role of Macrophage Migration Inhibitory Factor Activity in Experimental Autoimmune Diabetes. *Endocrinology*, *146*(7), 2942–2951. https://doi.org/10.1210/en.2004-1393

Dagenais, A., Villalba-Guerrero, C., & Olivier, M. (2023). Trained Immunity: A "New" Weapon In The Fight Against Infectious Diseases. *Frontiers in Immunology*, *14*. https://doi.org/10.3389/fimmu.2023.1147476

D'Amato-Brito, C., Cipriano, D., Colin, D. J., Germain, S., Seimbille, Y., Robert, J. H., Triponez, F., & Serre-Beinier, V. (2016). Role Of MIF/CD74 Signaling Pathway In The Development Of Pleural Mesothelioma. *Oncotarget*, *7*(10), 11512–11525. https://doi.org/10.18632/oncotarget.7314

Dameshghi, S., Zavaran-Hosseini, A., Soudi, S., Shirazi, F. J., Nojehdehi, S., & Hashemi, S. M. (2016). Mesenchymal Stem Cells Alter Macrophage Immune Responses To Leishmania Major Infection In Both Susceptible And Resistance Mice. *Immunology Letters*, *170*, 15–26. https://doi.org/10.1016/j.imlet.2015.12.002

Das, R., Koo, M.-S., Kim, B. H., Jacob, S. T., Subbian, S., Yao, J., Leng, L., Levy, R., Murchison, C., Burman, W. J., Moore, C. C., Scheld, W. M., David, J. R., Kaplan, G., MacMicking, J. D., & Bucala, R. (2013). Macrophage Migration Inhibitory Factor (MIF) Is A Critical Mediator Of The Innate Immune Response To *Mycobacterium Tuberculosis*. *Proceedings of the National Academy of Sciences*, *110*(32). https://doi.org/10.1073/pnas.1301128110

Das, R., Moss, J. E., Robinson, E., Roberts, S., Levy, R., Mizue, Y., Leng, L., McDonald, C., Tigelaar, R. E., Herrick, C. A., & Bucala, R. (2011). Role of Macrophage Migration Inhibitory Factor in the Th2 Immune Response to Epicutaneous Sensitization. *Journal of Clinical Immunology*, *31*(4), 666–680. https://doi.org/10.1007/s10875-011-9541-7

David, J. R. (1966). Delayed Hypersensitivity In Vitro: Its Mediation By Cell-Free Substances Formed By Lymphoid Cell-Antigen Interaction. *Proceedings of the National Academy of Sciences*, *56*(1), 72–77. https://doi.org/10.1073/pnas.56.1.72

de Araujo Farias, V., O'Valle, F., Serrano-Saenz, S., Anderson, P., Andrés, E., López-Peñalver, J., Tovar, I., Nieto, A., Santos, A., Martín, F., Expósito, J., Oliver, F. J., & de Almodóvar, J. M. R. (2018). Exosomes Derived From Mesenchymal Stem Cells Enhance Radiotherapy-Induced Cell Death In Tumor And Metastatic Tumor Foci. *Molecular Cancer*, *17*(1), 122. https://doi.org/10.1186/s12943-018-0867-0

de Castro, L. L., Xisto, D. G., Kitoko, J. Z., Cruz, F. F., Olsen, P. C., Redondo, P. A. G., Ferreira, T. P. T., Weiss, D. J., Martins, M. A., Morales, M. M., & Rocco, P. R. M. (2017). Human Adipose Tissue Mesenchymal Stromal Cells And Their Extracellular Vesicles Act Differentially On Lung Mechanics And Inflammation In Experimental Allergic Asthma. *Stem Cell Research & Therapy*, *8*(1), 151. https://doi.org/10.1186/s13287-017-0600-8

de las Vecillas, L., & Quirce, S. (2023). Landscape Of Short-Acting Beta-Agonists (<Scp>SABA</Scp>) Overuse In Europe. *Clinical & Experimental Allergy*, *53*(2), 132–144. https://doi.org/10.1111/cea.14250

de Souza, H. S., Tortori, C. A., Lintomen, L., Figueiredo, R. T., Bernardazzi, C., Leng, L., Bucala, R., Madi, K., Buongusto, F., Elia, C. C. S., Castelo-Branco, M. T. L., & Bozza, M. T. (2015). Macrophage Migration Inhibitory Factor Promotes Eosinophil Accumulation And Tissue Remodeling In Eosinophilic Esophagitis. *Mucosal Immunology*, *8*(5), 1154–1165. https://doi.org/10.1038/mi.2015.6

Deniz, I. A., Karbanová, J., Wobus, M., Bornhäuser, M., Wimberger, P., Kuhlmann, J. D., & Corbeil, D. (2023). Mesenchymal Stromal Cell-Associated Migrasomes: A New Source Of Chemoattractant For Cells Of Hematopoietic Origin. *Cell Communication and Signaling*, *21*(1), 36. https://doi.org/10.1186/s12964-022-01028-6

Ding, C., Shrestha, R., Zhu, X., Geller, A. E., Wu, S., Woeste, M. R., Li, W., Wang, H., Yuan, F., Xu, R., Chariker, J. H., Hu, X., Li, H., Tieri, D., Zhang, H.-G., Rouchka, E. C., Mitchell, R., Siskind, L. J., Zhang, X., … Yan, J. (2023). Inducing Trained Immunity In Pro-Metastatic Macrophages To Control Tumor Metastasis. *Nature Immunology*, *24*(2), 239–254. https://doi.org/10.1038/s41590-022-01388-8

Dios, A., Mitchell, R. A., Aljabari, B., Lubetsky, J., O'Connor, K., Liao, H., Senter, P. D., Manogue, K. R., Lolis, E., Metz, C., Bucala, R., Callaway, D. J. E., & Al-Abed, Y. (2002). Inhibition of MIF Bioactivity by Rational Design of Pharmacological Inhibitors of MIF Tautomerase Activity. *Journal of Medicinal Chemistry*, *45*(12), 2410–2416. https://doi.org/10.1021/jm010534q

DiPietro, L. A. (2016). Angiogenesis And Wound Repair: When Enough Is Enough. *Journal of Leukocyte Biology*, *100*(5), 979–984. https://doi.org/10.1189/jlb.4MR0316-102R

Divangahi, M., Aaby, P., Khader, S. A., Barreiro, L. B., Bekkering, S., Chavakis, T., van Crevel, R., Curtis, N., DiNardo, A. R., Dominguez-Andres, J., Duivenvoorden, R., Fanucchi, S., Fayad, Z., Fuchs, E., Hamon, M., Jeffrey, K. L., Khan, N., Joosten, L. A. B., Kaufmann, E., Netea, M. G. (2021). Trained Immunity, Tolerance, Priming And Differentiation: Distinct Immunological Processes. *Nature Immunology*, *22*(1), 2–6. https://doi.org/10.1038/s41590-020-00845-6

Doherty, D. F., Roets, L., & Krasnodembskaya, A. D. (2023). The Role of Lung Resident Mesenchymal Stromal Cells in the Pathogenesis and Repair of Chronic Lung Disease. *Stem Cells*. https://doi.org/10.1093/stmcls/sxad014

Domínguez-Andrés, J., dos Santos, J. C., Bekkering, S., Mulder, W. J. M., van der Meer, J. W. M., Riksen, N. P., Joosten, L. A. B., & Netea, M. G. (2023). Trained Immunity: Adaptation Within Innate Immune Mechanisms. *Physiological Reviews*, *103*(1), 313–346. https://doi.org/10.1152/physrev.00031.2021

Domínguez-Andrés, J., Ferreira, A. V, Jansen, T., Smithers, N., Prinjha, R. K., Furze, R. C., & Netea, M. G. (2019). Bromodomain Inhibitor I-BET151 Suppresses Immune Responses During Fungal–Immune Interaction. *European Journal of Immunology*, *49*(11), 2044–2050. https://doi.org/10.1002/eji.201848081

Donn, R. P., Shelley, E., Ollier, W. E., Thomson, W., & British Paediatric Rheumatology Study Group. (2001). A Novel 5'-Flanking Region Polymorphism Of Macrophage Migration Inhibitory Factor Is Associated With Systemic-Onset Juvenile Idiopathic Arthritis. *Arthritis and Rheumatism*, *44*(8), 1782–1785. https://doi.org/10.1002/1529-0131(200108)44:8<1782::AID-ART314>3.0.CO;2-#

Donnelly, S. C. (2023). Macrophage Migration Inhibitory Factor (MIF) And Severe Acute COVID-19 Infection. *QJM: An International Journal of Medicine*, *116*(3), 159–160. https://doi.org/10.1093/qjmed/hcad033

Donnelly, S. C., Haslett, C., Reid, P. T., Grant, I. S., Wallace, W. A. H., Metz, C. N., Bruce, L. J., & Bucala, R. (1997). Regulatory Role For Macrophage Migration Inhibitory Factor In Acute Respiratory Distress Syndrome. *Nature Medicine*, *3*(3), 320–323. https://doi.org/10.1038/nm0397-320

Doroudian, M., O'Neill, A., O'Reilly, C., Tynan, A., Mawhinney, L., McElroy, A., Webster, S. S., MacLoughlin, R., Volkov, Y., E Armstrong, M., A. O'Toole, G., Prina-Mello, A., & C Donnelly, S. (2020). Aerosolized Drug-Loaded Nanoparticles Targeting Migration Inhibitory Factors Inhibit *Pseudomonas Aeruginosa* -Induced Inflammation And Biofilm Formation. *Nanomedicine*, *15*(30), 2933–2953. https://doi.org/10.2217/nnm-2020-0344

Drake, S. M., Simpson, A., & Fowler, S. J. (2019). Asthma Diagnosis: The Changing Face of Guidelines. *Pulmonary Therapy*, *5*(2), 103–115. https://doi.org/10.1007/s41030-019-0093-y

Du, G.-L., Luo, J.-Y., Wang, D., Li, Y.-H., Fang, B.-B., Li, X.-M., Gao, X.-M., & Yang, Y.-N. (2020). MIF Gene Rs755622 Polymorphism Positively Associated With Acute Coronary Syndrome In Chinese Han Population: Case–Control Study. *Scientific Reports*, *10*(1), 140. https://doi.org/10.1038/s41598-019-56949-z

Duffy, M. M., Pindjakova, J., Hanley, S. A., McCarthy, C., Weidhofer, G. A., Sweeney, E. M., English, K., Shaw, G., Murphy, J. M., Barry, F. P., Mahon, B. P., Belton, O., Ceredig, R., & Griffin, M. D.

(2011). Mesenchymal Stem Cell Inhibition Of T-Helper 17 Cell- Differentiation Is Triggered By Cell-Cell Contact And Mediated By Prostaglandin E2 Via The EP4 Receptor. *European Journal of Immunology*, *41*(10), 2840–2851. https://doi.org/10.1002/eji.201141499

Dullaers, M., Schuijs, M. J., Willart, M., Fierens, K., Van Moorleghem, J., Hammad, H., & Lambrecht, B. N. (2017). House Dust Mite–Driven Asthma And Allergen-Specific T Cells Depend On B Cells When The Amount Of Inhaled Allergen Is Limiting. *Journal of Allergy and Clinical Immunology*, *140*(1), 76-88.e7. https://doi.org/10.1016/j.jaci.2016.09.020

Dunbar, H., Hawthorne, I. J., Tunstead, C., Armstrong, M. E., Donnelly, S. C., & English, K. (2023). Blockade Of <Scp>MIF</Scp> Biological Activity Ameliorates House Dust Mite-Induced Allergic Airway Inflammation In Humanized <Scp>MIF</Scp> Mice. *The FASEB Journal*, *37*(8). https://doi.org/10.1096/fj.202300787R

Dunbar, H., Weiss, D. J., Rolandsson Enes, S., Laffey, J. G., & English, K. (2021). The Inflammatory Lung Microenvironment; a Key Mediator in MSC Licensing. *Cells*, *10*(11), 2982. https://doi.org/10.3390/cells10112982

Dunican, E. M., Elicker, B. M., Gierada, D. S., Nagle, S. K., Schiebler, M. L., Newell, J. D., Raymond, W. W., Lachowicz-Scroggins, M. E., Di Maio, S., Hoffman, E. A., Castro, M., Fain, S. B., Jarjour, N. N., Israel, E., Levy, B. D., Erzurum, S. C., Wenzel, S. E., Meyers, D. A., Bleecker, E. R., … Fahy, J. V. (2018). Mucus Plugs In Patients With Asthma Linked To Eosinophilia And Airflow Obstruction. *Journal of Clinical Investigation*, *128*(3), 997–1009. https://doi.org/10.1172/JCI95693

Duong, K. M., Arikkatt, J., Ullah, M. A., Lynch, J. P., Zhang, V., Atkinson, K., Sly, P. D., & Phipps, S. (2015). Immunomodulation of Airway Epithelium Cell Activation by Mesenchymal Stromal Cells Ameliorates House Dust Mite–Induced Airway Inflammation in Mice. *American Journal of Respiratory Cell and Molecular Biology*, *53*(5), 615–624. https://doi.org/10.1165/rcmb.2014-0431OC

Eggenhofer, E., Benseler, V., Kroemer, A., Popp, F. C., Geissler, E. K., Schlitt, H. J., Baan, C. C., Dahlke, M. H., & Hoogduijn, M. J. (2012). Mesenchymal Stem Cells Are Short-Lived And Do Not Migrate Beyond The Lungs After Intravenous Infusion. *Frontiers in Immunology*, *3*. https://doi.org/10.3389/fimmu.2012.00297

Ehrentraut, H., Clambey, E. T., McNamee, E. N., Brodsky, K. S., Ehrentraut, S. F., Poth, J. M., Riegel, A. K., Westrich, J. A., Colgan, S. P., & Eltzschig, H. K. (2013). CD73 $^{+}$ Regulatory T Cells Contribute To Adenosine-Mediated Resolution Of Acute Lung Injury. *The FASEB Journal*, *27*(6), 2207–2219. https://doi.org/10.1096/fj.12-225201

Eisenbarth, S. C., Piggott, D. A., Huleatt, J. W., Visintin, I., Herrick, C. A., & Bottomly, K. (2002). Lipopolysaccharide-enhanced, Toll-like Receptor 4–dependent T Helper Cell Type 2 Responses to Inhaled Antigen. *Journal of Experimental Medicine*, *196*(12), 1645–1651. https://doi.org/10.1084/jem.20021340

El-Zein, M., Parent, M. E., Benedetti, A., & Rousseau, M. C. (2010). Does BCG Vaccination Protect Against The Development Of Childhood Asthma? A Systematic Review And Meta-Analysis Of Epidemiological Studies. *International Journal of Epidemiology*, *39*(2), 469–486. https://doi.org/10.1093/ije/dyp307

English, K. (2013). Mechanisms Of Mesenchymal Stromal Cell Immunomodulation. *Immunology & Cell Biology*, *91*(1), 19–26. https://doi.org/10.1038/icb.2012.56

English, K., Barry, F. P., Field-Corbett, C. P., & Mahon, B. P. (2007). IFN-γ and TNF-α Differentially Regulate Immunomodulation By Murine Mesenchymal Stem Cells. *Immunology Letters*, *110*(2), 91–100. https://doi.org/10.1016/j.imlet.2007.04.001

English, K., Ryan, J. M., Tobin, L., Murphy, M. J., Barry, F. P., & Mahon, B. P. (2009). Cell Contact, Prostaglandin E2 And Transforming Growth Factor Beta 1 Play Non-Redundant Roles In Human Mesenchymal Stem Cell Induction Of CD4+CD25Highforkhead Box P3+ Regulatory T Cells. *Clinical and Experimental Immunology*, *156*(1), 149–160. https://doi.org/10.1111/j.1365-2249.2009.03874.x

English, K., Tonlorenzi, R., Cossu, G., & Wood, K. J. (2013). Mesoangioblasts Suppress T Cell Proliferation Through IDO and PGE-2-Dependent Pathways. *Stem Cells and Development*, *22*(3), 512–523. https://doi.org/10.1089/scd.2012.0386

English, K., & Wood, K. J. (2013). Mesenchymal Stromal Cells in Transplantation Rejection and Tolerance. *Cold Spring Harbor Perspectives in Medicine*, *3*(5), a015560–a015560. https://doi.org/10.1101/cshperspect.a015560

Espagnolle, N., Balguerie, A., Arnaud, E., Sensebé, L., & Varin, A. (2017). CD54-Mediated Interaction with Pro-inflammatory Macrophages Increases the Immunosuppressive Function of Human Mesenchymal Stromal Cells. *Stem Cell Reports*, *8*(4), 961–976. https://doi.org/10.1016/j.stemcr.2017.02.008

Esumi, N., Budarf †, ‡, Lawrence Ci, Marcia, & Wistow, G. (1998). Conserved Gene Structure And Genomic Linkage For D-Dopachrome Tautomerase (DDT) And MIF. *Mammalian Genome*, *9*(9), 753–757. https://doi.org/10.1007/s003359900858

Fahy, J. V. (2015). Type 2 Inflammation In Asthma — Present In Most, Absent In Many. *Nature Reviews Immunology*, *15*(1), 57–65. https://doi.org/10.1038/nri3786

Falcone, F. H., Loke, P., Zang, X., MacDonald, A. S., Maizels, R. M., & Allen, J. E. (2001). A *Brugia malayi* Homolog of Macrophage Migration Inhibitory Factor Reveals an Important Link Between Macrophages and Eosinophil Recruitment During Nematode Infection. *The Journal of Immunology*, *167*(9), 5348–5354. https://doi.org/10.4049/jimmunol.167.9.5348

Fang, S.-B., Zhang, H.-Y., Jiang, A.-Y., Fan, X.-L., Lin, Y.-D., Li, C.-L., Wang, C., Meng, X.-C., & Fu, Q.-L. (2018). Human Ipsc-Mscs Prevent Steroid-Resistant Neutrophilic Airway Inflammation Via Modulating Th17 Phenotypes. *Stem Cell Research & Therapy*, *9*(1), 147. https://doi.org/10.1186/s13287-018-0897-y

Fang, S.-B., Zhang, H.-Y., Meng, X.-C., Wang, C., He, B.-X., Peng, Y.-Q., Xu, Z.-B., Fan, X.-L., Wu, Z.-J., Wu, Z.-C., Zheng, S.-G., & Fu, Q.-L. (2020). Small Extracellular Vesicles Derived From Human Mscs Prevent Allergic Airway Inflammation Via Immunomodulation On Pulmonary Macrophages. *Cell Death & Disease*, *11*(6), 409. https://doi.org/10.1038/s41419-020-2606-x

Faverio, P., Ronco, R., Monzio Compagnoni, M., Franchi, M., Franco, G., Bonaiti, G., Bonifazi, M., Mei, F., Luppi, F., Pesci, A., & Corrao, G. (2023). Effectiveness And Economic Impact Of Dupilumab In Asthma: A Population-Based Cohort Study. *Respiratory Research*, *24*(1), 70. https://doi.org/10.1186/s12931-023-02372-y

Feng, G., Xu, Y., Ma, N., Midorikawa, K., Oikawa, S., Kobayashi, H., Nakamura, S., Ishinaga, H., Zhang, Z., Huang, G., Takeuchi, K., & Murata, M. (2021). Influence Of Epstein–Barr Virus And Human Papillomavirus Infection On Macrophage Migration Inhibitory Factor And Macrophage Polarization In Nasopharyngeal Carcinoma. *BMC Cancer*, *21*(1), 929. https://doi.org/10.1186/s12885-021-08675-x

Ferrini, E., Stellari, F. F., Franceschi, V., Macchi, F., Russo, L., Murgia, A., Grisendi, G., Villetti, G., Dominici, M., & Donofrio, G. (2021). Persistency of Mesenchymal Stromal/Stem Cells in Lungs. *Frontiers in Cell and Developmental Biology*, *9*. https://doi.org/10.3389/fcell.2021.709225

Fichtel, P., von Bonin, M., Kuhnert, R., Möbus, K., Bornhäuser, M., & Wobus, M. (2022). Mesenchymal Stromal Cell-Derived Extracellular Vesicles Modulate Hematopoietic Stem and Progenitor Cell Viability and the Expression of Cell Cycle Regulators in an Age-dependent Manner. *Frontiers in Bioengineering and Biotechnology*, *10*. https://doi.org/10.3389/fbioe.2022.892661

Filbey, K. J., Varyani, F., Harcus, Y., Hewitson, J. P., Smyth, D. J., McSorley, H. J., Ivens, A., Nylén, S., Rottenberg, M., Löser, S., & Maizels, R. M. (2019). Macrophage Migration Inhibitory Factor (MIF) Is Essential for Type 2 Effector Cell Immunity to an Intestinal Helminth Parasite. *Frontiers in Immunology*, *10*. https://doi.org/10.3389/fimmu.2019.02375

Fingerle-Rowson, G., Petrenko, O., Metz, C. N., Forsthuber, T. G., Mitchell, R., Huss, R., Moll, U., Müller, W., & Bucala, R. (2003). The P53-Dependent Effects Of Macrophage Migration Inhibitory Factor Revealed By Gene Targeting. *Proceedings of the National Academy of Sciences*, *100*(16), 9354–9359. https://doi.org/10.1073/pnas.1533295100

Finlay, C. M., Stefanska, A. M., Walsh, K. P., Kelly, P. J., Boon, L., Lavelle, E. C., Walsh, P. T., & Mills, K. H. G. (2016). Helminth Products Protect against Autoimmunity via Innate Type 2 Cytokines IL-5 and IL-33, Which Promote Eosinophilia. *The Journal of Immunology*, *196*(2), 703–714. https://doi.org/10.4049/jimmunol.1501820

Flores, M., Saavedra, R., Bautista, R., Viedma, R., Tenorio, E. P., Leng, L., Sanchez, Y., Juárez, I., Satoskar, A. A., Shenoy, A. S., Terrazas, L. I., Bucala, R., Barbi, J., Satoskar, A. R., & Rodriguez-Sosa, M. (2008). Macrophage Migration Inhibitory Factor (MIF) Is Critical For The Host

Resistance Against *Toxoplasma Gondii*. *The FASEB Journal*, *22*(10), 3661–3671. https://doi.org/10.1096/fj.08-111666

Florez-Sampedro, L., Brandsma, C.-A., de Vries, M., Timens, W., Bults, R., Vermeulen, C. J., van den Berge, M., Obeidat, M., Joubert, P., Nickle, D. C., Poelarends, G. J., Faiz, A., & Melgert, B. N. (2020). Genetic Regulation Of Gene Expression Of MIF Family Members In Lung Tissue. *Scientific Reports*, *10*(1), 16980. https://doi.org/10.1038/s41598-020-74121-w

Florez-Sampedro, L., Soto-Gamez, A., Poelarends, G. J., & Melgert, B. N. (2020). The Role Of MIF In Chronic Lung Diseases: Looking Beyond Inflammation. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *318*(6), L1183–L1197. https://doi.org/10.1152/ajplung.00521.2019

Fonseca, W., Rasky, A. J., Ptaschinski, C., Morris, S. H., Best, S. K. K., Phillips, M., Malinczak, C.-A., & Lukacs, N. W. (2019). Group 2 Innate Lymphoid Cells (ILC2) Are Regulated By Stem Cell Factor During Chronic Asthmatic Disease. *Mucosal Immunology*, *12*(2), 445–456. https://doi.org/10.1038/s41385-018-0117-1

Foudah, D., Redaelli, S., Donzelli, E., Bentivegna, A., Miloso, M., Dalprà, L., & Tredici, G. (2009). Monitoring The Genomic Stability Of In Vitro Cultured Rat Bone-Marrow-Derived Mesenchymal Stem Cells. *Chromosome Research*, *17*(8), 1025. https://doi.org/10.1007/s10577-009-9090-6

François, M., Romieu-Mourez, R., Li, M., & Galipeau, J. (2012). Human MSC Suppression Correlates With Cytokine Induction of Indoleamine 2,3-Dioxygenase and Bystander M2 Macrophage Differentiation. *Molecular Therapy*, *20*(1), 187–195. https://doi.org/10.1038/mt.2011.189

Fujio, Y., & Walsh, K. (1999). Akt Mediates Cytoprotection of Endothelial Cells by Vascular Endothelial Growth Factor in an Anchorage-dependent Manner. *Journal of Biological Chemistry*, *274*(23), 16349–16354. https://doi.org/10.1074/jbc.274.23.16349

Funes, S. C., Rios, M., Fernández-Fierro, A., Di Genaro, M. S., & Kalergis, A. M. (2022). Trained Immunity Contribution to Autoimmune and Inflammatory Disorders. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.868343

Furlani, D., Ugurlucan, M., Ong, L., Bieback, K., Pittermann, E., Westien, I., Wang, W., Yerebakan, C., Li, W., Gaebel, R., Li, R., Vollmar, B., Steinhoff, G., & Ma, N. (2009). Is The Intravascular Administration Of Mesenchymal Stem Cells Safe? *Microvascular Research*, *77*(3), 370–376. https://doi.org/10.1016/j.mvr.2009.02.001

Galipeau, J. (2021). Macrophages at the Nexus of Mesenchymal Stromal Cell Potency: The Emerging Role of Chemokine Cooperativity. *Stem Cells*, *39*(9), 1145–1154. https://doi.org/10.1002/stem.3380

Galipeau, J., & Sensébé, L. (2018). Mesenchymal Stromal Cells: Clinical Challenges and Therapeutic Opportunities. *Cell Stem Cell*, *22*(6), 824–833. https://doi.org/10.1016/j.stem.2018.05.004

Gall, X. M., Domínguez-Ortega, J., Pascual, S., Cabrera López, C., Gustavo Resler, Nuevo, J., & Monteagudo, G. (2023). Clinical Burden Related To Oral Corticosteroid Treatment Of Severe Asthma In Spain: LEVANTE Study. *Journal of Asthma*, *60*(5), 890–899. https://doi.org/10.1080/02770903.2022.2103428

Galleu, A., Riffo-Vasquez, Y., Trento, C., Lomas, C., Dolcetti, L., Cheung, T. S., von Bonin, M., Barbieri, L., Halai, K., Ward, S., Weng, L., Chakraverty, R., Lombardi, G., Watt, F. M., Orchard, K., Marks, D. I., Apperley, J., Bornhauser, M., Walczak, H., … Dazzi, F. (2017). Apoptosis In Mesenchymal Stromal Cells Induces In Vivo Recipient-Mediated Immunomodulation. *Science Translational Medicine*, *9*(416). https://doi.org/10.1126/scitranslmed.aam7828

Gao, Q., Liu, Y., Wu, Y., Zhao, Q., Wang, L., Gao, S., Wen, W., Zhang, W., Guo, N., Zhou, J., & Yuan, Z. (2016). IL-17 Intensifies IFN-Γ-Induced NOS2 Upregulation In RAW 264.7 Cells By Further Activating STAT1 And NF-Kb. *International Journal of Molecular Medicine*, *37*(2), 347–358. https://doi.org/10.3892/ijmm.2015.2433

Garcia, J., Hurwitz, H. I., Sandler, A. B., Miles, D., Coleman, R. L., Deurloo, R., & Chinot, O. L. (2020). Bevacizumab (Avastin®) In Cancer Treatment: A Review Of 15 Years Of Clinical Experience And Future Outlook. *Cancer Treatment Reviews*, *86*, 102017. https://doi.org/10.1016/j.ctrv.2020.102017

García-Marcos, L., Asher, M. I., Pearce, N., Ellwood, E., Bissell, K., Chiang, C.-Y., El Sony, A., Ellwood, P., Marks, G. B., Mortimer, K., Martínez-Torres, A. E., Morales, E., Perez-Fernandez, V., Robertson, S., Rutter, C. E., Silverwood, R. J., & Strachan, D. P. (2022). The Burden Of

Asthma, Hay Fever And Eczema In Children In 25 Countries: GAN Phase I Study. *European Respiratory Journal*, *60*(3), 2102866. https://doi.org/10.1183/13993003.02866-2021

Gautier, C., & Charpin, D. (2017). Environmental Triggers And Avoidance In The Management Of Asthma. *Journal of Asthma and Allergy*, *Volume10*, 47–56. https://doi.org/10.2147/JAA.S121276

Ge, Q., Zhang, H., Hou, J., Wan, L., Cheng, W., Wang, X., Dong, D., Chen, C., Xia, J., Guo, J., Chen, X., & Wu, X. (2017). VEGF Secreted By Mesenchymal Stem Cells Mediates The Differentiation Of Endothelial Progenitor Cells Into Endothelial Cells Via Paracrine Mechanisms. *Molecular Medicine Reports*. https://doi.org/10.3892/mmr.2017.8059

George, M., & Vaughan, J. H. (1962). In vitro Cell Migration as a Model for Delayed Hypersensitivity. *Experimental Biology and Medicine*, *111*(2), 514–521. https://doi.org/10.3181/00379727-111-27841

Gerber, H.-P., Dixit, V., & Ferrara, N. (1998). Vascular Endothelial Growth Factor Induces Expression of the Antiapoptotic Proteins Bcl-2 and A1 in Vascular Endothelial Cells. *Journal of Biological Chemistry*, *273*(21), 13313–13316. https://doi.org/10.1074/jbc.273.21.13313

Gerber, H.-P., McMurtrey, A., Kowalski, J., Yan, M., Keyt, B. A., Dixit, V., & Ferrara, N. (1998). Vascular Endothelial Growth Factor Regulates Endothelial Cell Survival through the Phosphatidylinositol 3′-Kinase/Akt Signal Transduction Pathway. *Journal of Biological Chemistry*, *273*(46), 30336–30343. https://doi.org/10.1074/jbc.273.46.30336

Ghalehbandi, S., Yuzugulen, J., Pranjol, M. Z. I., & Pourgholami, M. H. (2023). The Role Of VEGF In Cancer-Induced Angiogenesis And Research Progress Of Drugs Targeting VEGF. *European Journal of Pharmacology*, 175586. https://doi.org/10.1016/j.ejphar.2023.175586

Girdlestone, J., Pido-Lopez, J., Srivastava, S., Chai, J., Leaver, N., Galleu, A., Lombardi, G., & Navarrete, C. V. (2015). Enhancement Of The Immunoregulatory Potency Of Mesenchymal Stromal Cells By Treatment With Immunosuppressive Drugs. *Cytotherapy*, *17*(9), 1188–1199. https://doi.org/10.1016/j.jcyt.2015.05.009

Giri, J., & Galipeau, J. (2020). Mesenchymal Stromal Cell Therapeutic Potency Is Dependent Upon Viability, Route Of Delivery, And Immune Match. *Blood Advances*, *4*(9), 1987–1997. https://doi.org/10.1182/bloodadvances.2020001711

Gore, Y., Starlets, D., Maharshak, N., Becker-Herman, S., Kaneyuki, U., Leng, L., Bucala, R., & Shachar, I. (2008). Macrophage Migration Inhibitory Factor Induces B Cell Survival by Activation of a CD74-CD44 Receptor Complex. *Journal of Biological Chemistry*, *283*(5), 2784–2792. https://doi.org/10.1074/jbc.M703265200

Gorgun, C., Ceresa, D., Lesage, R., Villa, F., Reverberi, D., Balbi, C., Santamaria, S., Cortese, K., Malatesta, P., Geris, L., Quarto, R., & Tasso, R. (2021). Dissecting The Effects Of Preconditioning With Inflammatory Cytokines And Hypoxia On The Angiogenic Potential Of Mesenchymal Stromal Cell (MSC)-Derived Soluble Proteins And Extracellular Vesicles (EVs). *Biomaterials*, *269*, 120633. https://doi.org/10.1016/j.biomaterials.2020.120633

Gorman, E., Millar, J., McAuley, D., & O'Kane, C. (2021). Mesenchymal Stromal Cells For Acute Respiratory Distress Syndrome (ARDS), Sepsis, And COVID-19 Infection: Optimizing The Therapeutic Potential. *Expert Review of Respiratory Medicine*, *15*(3), 301–324. https://doi.org/10.1080/17476348.2021.1848555

Gourbal, B., Pinaud, S., Beckers, G. J. M., Van Der Meer, J. W. M., Conrath, U., & Netea, M. G. (2018). Innate Immune Memory: An Evolutionary Perspective. *Immunological Reviews*, *283*(1), 21–40. https://doi.org/10.1111/imr.12647

Gregory, L. G., & Lloyd, C. M. (2011). Orchestrating house dust mite-associated allergy in the lung. *Trends in Immunology*, *32*(9), 402–411. https://doi.org/10.1016/j.it.2011.06.006

Greven, D., Leng, L., & Bucala, R. (2010). Autoimmune Diseases: MIF As A Therapeutic Target. *Expert Opinion on Therapeutic Targets*, *14*(3), 253–264. https://doi.org/10.1517/14728220903551304

Gunawardena, T. N. A., Rahman, M. T., Abdullah, B. J. J., & Abu Kasim, N. H. (2019). Conditioned Media Derived From Mesenchymal Stem Cell Cultures: The Next Generation For Regenerative Medicine. *Journal of Tissue Engineering and Regenerative Medicine*, *13*(4), 569–586. https://doi.org/10.1002/term.2806

Günther, S., Bordenave, J., Hua-Huy, T., Nicco, C., Cumont, A., Thuillet, R., Tu, L., Quatremarre, T., Guilbert, T., Jalce, G., Batteux, F., Humbert, M., Savale, L., Guignabert, C., & Dinh-Xuan, A.-T. (2018). Macrophage Migration Inhibitory Factor (MIF) Inhibition in a Murine Model of

Bleomycin-Induced Pulmonary Fibrosis. *International Journal of Molecular Sciences*, *19*(12), 4105. https://doi.org/10.3390/ijms19124105

Guo, Y., Hou, J., Luo, Y., & Wang, D. (2013). Functional Disruption Of Macrophage Migration Inhibitory Factor (MIF) Suppresses Proliferation Of Human H460 Lung Cancer Cells By Caspase-Dependent Apoptosis. *Cancer Cell International*, *13*(1), 28. https://doi.org/10.1186/1475-2867-13-28

Guo, Y.-C., Chiu, Y.-H., Chen, C.-P., & Wang, H.-S. (2018). Interleukin-1β Induces CXCR3-Mediated Chemotaxis To Promote Umbilical Cord Mesenchymal Stem Cell Transendothelial Migration. *Stem Cell Research & Therapy*, *9*(1), 281. https://doi.org/10.1186/s13287-018-1032-9

Hackel, A., Vollmer, S., Bruderek, K., Lang, S., & Brandau, S. (2023). Immunological Priming Of Mesenchymal Stromal/Stem Cells And Their Extracellular Vesicles Augments Their Therapeutic Benefits In Experimental Graft-Versus-Host Disease Via Engagement Of PD-1 Ligands. *Frontiers in Immunology*, *14*. https://doi.org/10.3389/fimmu.2023.1078551

Haimerl, P., Bernhardt, U., Schindela, S., Henkel, F. D. R., Lechner, A., Zissler, U. M., Pastor, X., Thomas, D., Cecil, A., Ge, Y., Haid, M., Prehn, C., Tokarz, J., Heinig, M., Adamski, J., Schmidt-Weber, C. B., Chaker, A. M., & Esser-von Bieren, J. (2021). Inflammatory Macrophage Memory In Nonsteroidal Anti-Inflammatory Drug–Exacerbated Respiratory Disease. *Journal of Allergy and Clinical Immunology*, *147*(2), 587–599. https://doi.org/10.1016/j.jaci.2020.04.064

Hale, E. Mac, Greene, G., Mulvey, C., Mokoka, M. C., van Boven, J. F. M., Cushen, B., Sulaiman, I., Brennan, V., Lombard, L., Walsh, J., Plunkett, S., McCartan, T. A., Kerr, P. J., Reilly, R. B., Hughes, C., Kent, B. D., Jackson, D. J., Butler, M., Counihan, I., … McCartan, T. A. (2023). Use Of Digital Measurement Of Medication Adherence And Lung Function To Guide The Management Of Uncontrolled Asthma (INCA Sun): A Multicentre, Single-Blinded, Randomised Clinical Trial. *The Lancet Respiratory Medicine*. https://doi.org/10.1016/S2213-2600(22)00534-3

Hall, I., Walker, S., & Holgate, S. T. (2022). Respiratory research in the UK: investing for the next 10 years. *Thorax*, *77*(9), 851–853. https://doi.org/10.1136/thoraxjnl-2021-218459

Hammad, H., Chieppa, M., Perros, F., Willart, M. A., Germain, R. N., & Lambrecht, B. N. (2009). House Dust Mite Allergen Induces Asthma Via Toll-Like Receptor 4 Triggering Of Airway Structural Cells. *Nature Medicine*, *15*(4), 410–416. https://doi.org/10.1038/nm.1946

Hammad, H., & Lambrecht, B. N. (2021). The Basic Immunology Of Asthma. *Cell*, *184*(6), 1469–1485. https://doi.org/10.1016/j.cell.2021.02.016

Hammad, H., Plantinga, M., Deswarte, K., Pouliot, P., Willart, M. A. M., Kool, M., Muskens, F., & Lambrecht, B. N. (2010). Inflammatory Dendritic Cells—Not Basophils—Are Necessary And Sufficient For Induction Of Th2 Immunity To Inhaled House Dust Mite Allergen. *Journal of Experimental Medicine*, *207*(10), 2097–2111. https://doi.org/10.1084/jem.20101563

Han, Y., Yang, J., Fang, J., Zhou, Y., Candi, E., Wang, J., Hua, D., Shao, C., & Shi, Y. (2022). The Secretion Profile Of Mesenchymal Stem Cells And Potential Applications In Treating Human Diseases. *Signal Transduction and Targeted Therapy*, *7*(1), 92. https://doi.org/10.1038/s41392-022-00932-0

Harada, N., Makita, N., Fukui, K., Nishida, K., Oneda, K., & Tashiro, N. (2023). A Retrospective Claims Database Study to Clarify Disease Burden of Severe Asthma Patients with Type 2 High or Low Inflammation. *Journal of Asthma and Allergy*, *Volume 16*, 83–93. https://doi.org/10.2147/JAA.S378505

Harris, J., VanPatten, S., Deen, N. S., Al-Abed, Y., & Morand, E. F. (2019). Rediscovering MIF: New Tricks for an Old Cytokine. *Trends in Immunology*, *40*(5), 447–462. https://doi.org/10.1016/j.it.2019.03.002

Hart, S. P., Rossi, A. G., & Dransfield, I. (1998). You Can Lead A Macrophage To An Inflammatory Site But Can You Make It Eat? *Biochemical Society Transactions*, *26*(4), 650–652. https://doi.org/10.1042/bst0260650

Hartung, F., & Esser-von Bieren, J. (2022). Trained Immunity In Type 2 Immune Responses. *Mucosal Immunology*, *15*(6), 1158–1169. https://doi.org/10.1038/s41385-022-00557-0

Hattner, R., & Frost, H. M. (1963). An Equation For The Mesenchymal Cell Activation Function In The Lamellar Bone Remodelling Equations. *Henry Ford Hospital Medical Bulletin*, *11*(4), 455–473.

Healy, M. E., Bergin, R., Mahon, B. P., & English, K. (2015). Mesenchymal Stromal Cells Protect Against Caspase 3-Mediated Apoptosis of CD19 $^{+}$ Peripheral B Cells Through Contact-Dependent Upregulation of VEGF. *Stem Cells and Development*, *24*(20), 2391–2402. https://doi.org/10.1089/scd.2015.0089

Hekking, P.-P. W., Wener, R. R., Amelink, M., Zwinderman, A. H., Bouvy, M. L., & Bel, E. H. (2015). The Prevalence Of Severe Refractory Asthma. *Journal of Allergy and Clinical Immunology*, *135*(4), 896–902. https://doi.org/10.1016/j.jaci.2014.08.042

Henkel, F. D. R., Friedl, A., Haid, M., Thomas, D., Bouchery, T., Haimerl, P., de los Reyes Jiménez, M., Alessandrini, F., Schmidt-Weber, C. B., Harris, N. L., Adamski, J., & Esser-von Bieren, J. (2018). House Dust Mite Drives Pro-Inflammatory Eicosanoid Reprogramming And Macrophage Effector Functions. *Allergy*, all.13700. https://doi.org/10.1111/all.13700

Hillyer, P., Shepard, R., Uehling, M., Krenz, M., Sheikh, F., Thayer, K. R., Huang, L., Yan, L., Panda, D., Luongo, C., Buchholz, U. J., Collins, P. L., Donnelly, R. P., & Rabin, R. L. (2018). Differential Responses by Human Respiratory Epithelial Cell Lines to Respiratory Syncytial Virus Reflect Distinct Patterns of Infection Control. *Journal of Virology*, *92*(15). https://doi.org/10.1128/JVI.02202-17

Hizawa, N., Yamaguchi, E., Takahashi, D., Nishihira, J., & Nishimura, M. (2004). Functional Polymorphisms in the Promoter Region of Macrophage Migration Inhibitory Factor and Atopy. *American Journal of Respiratory and Critical Care Medicine*, *169*(9), 1014–1018. https://doi.org/10.1164/rccm.200307-933OC

Hoang, D. M., Pham, P. T., Bach, T. Q., Ngo, A. T. L., Nguyen, Q. T., Phan, T. T. K., Nguyen, G. H., Le, P. T. T., Hoang, V. T., Forsyth, N. R., Heke, M., & Nguyen, L. T. (2022). Stem Cell-Based Therapy For Human Diseases. *Signal Transduction and Targeted Therapy*, *7*(1), 272. https://doi.org/10.1038/s41392-022-01134-4

Hoffman, S. M., Tully, J. E., Nolin, J. D., Lahue, K. G., Goldman, D. H., Daphtary, N., Aliyeva, M., Irvin, C. G., Dixon, A. E., Poynter, M. E., & Anathy, V. (2013). Endoplasmic Reticulum Stress Mediates House Dust Mite-Induced Airway Epithelial Apoptosis And Fibrosis. *Respiratory Research*, *14*(1), 141. https://doi.org/10.1186/1465-9921-14-141

Holcomb, I. N., Kabakoff, R. C., Chan, B., Baker, T. W., Gurney, A., Henzel, W., Nelson, C., Lowman, H. B., Wright, B. D., Skelton, N. J., Frantz, G. D., Tumas, D. B., Peale, F. V., Shelton, D. L., & Hébert, C. C. (2000). FIZZ1, A Novel Cysteine-Rich Secreted Protein Associated With Pulmonary Inflammation, Defines A New Gene Family. *The EMBO Journal*, *19*(15), 4046–4055. https://doi.org/10.1093/emboj/19.15.4046

Hsieh, A., Assadinia, N., & Hackett, T.-L. (2023). Airway Remodeling Heterogeneity In Asthma And Its Relationship To Disease Outcomes. *Frontiers in Physiology*, *14*. https://doi.org/10.3389/fphys.2023.1113100

Hsu, P., Santner-Nanan, B., Hu, M., Skarratt, K., Lee, C. H., Stormon, M., Wong, M., Fuller, S. J., & Nanan, R. (2015). IL-10 Potentiates Differentiation of Human Induced Regulatory T Cells via STAT3 and Foxo1. *The Journal of Immunology*, *195*(8), 3665–3674. https://doi.org/10.4049/jimmunol.1402898

Huber, H. L. (1922). The Pathology Of Bronchial Asthma. *Archives of Internal Medicine*, *30*(6), 689. https://doi.org/10.1001/archinte.1922.00110120002001

Humbert, M., Taillé, C., Mala, L., Le Gros, V., Just, J., & Molimard, M. (2018). Omalizumab Effectiveness In Patients With Severe Allergic Asthma According To Blood Eosinophil Count: The STELLAIR Study. *European Respiratory Journal*, *51*(5), 1702523. https://doi.org/10.1183/13993003.02523-2017

Husebø, G. R., Bakke, P. S., Grønseth, R., Hardie, J. A., Ueland, T., Aukrust, P., & Eagan, T. M. L. (2016). Macrophage Migration Inhibitory Factor, A Role In COPD. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *311*(1), L1–L7. https://doi.org/10.1152/ajplung.00461.2015

Ionescu, L., Byrne, R. N., van Haaften, T., Vadivel, A., Alphonse, R. S., Rey-Parra, G. J., Weissmann, G., Hall, A., Eaton, F., & Thébaud, B. (2012). Stem Cell Conditioned Medium Improves Acute Lung Injury In Mice: In Vivo Evidence For Stem Cell Paracrine Action. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *303*(11), L967–L977. https://doi.org/10.1152/ajplung.00144.2011

Jackson, M. V., Morrison, T. J., Doherty, D. F., McAuley, D. F., Matthay, M. A., Kissenpfennig, A., O'Kane, C. M., & Krasnodembskaya, A. D. (2016). Mitochondrial Transfer via Tunneling Nanotubes is an Important Mechanism by Which Mesenchymal Stem Cells Enhance Macrophage Phagocytosis in the In Vitro and In Vivo Models of ARDS. *Stem Cells*, *34*(8), 2210–2223. https://doi.org/10.1002/stem.2372

Jacquet, A. (2013). Innate Immune Responses in House Dust Mite Allergy. *ISRN Allergy*, *2013*, 1–18. https://doi.org/10.1155/2013/735031

Jakwerth, C. A., Ordovas-Montanes, J., Blank, S., Schmidt-Weber, C. B., & Zissler, U. M. (2022). Role of Respiratory Epithelial Cells in Allergic Diseases. *Cells*, *11*(9), 1387. https://doi.org/10.3390/cells11091387

Jalce, G., & Guignabert, C. (2020). Multiple Roles Of Macrophage Migration Inhibitory Factor In Pulmonary Hypertension. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *318*(1), L1–L9. https://doi.org/10.1152/ajplung.00234.2019

Jiang, M., Fang, Y., Li, Y., Huang, H., Wei, Z., Gao, X., Sung, H.-K., Hu, J., Qiang, L., Ruan, J., Chen, Q., Jiang, D., Whitsett, J. A., Ai, X., & Que, J. (2021). VEGF Receptor 2 (KDR) Protects Airways From Mucus Metaplasia Through A Sox9-Dependent Pathway. *Developmental Cell*, *56*(11), 1646-1660.e5. https://doi.org/10.1016/j.devcel.2021.04.027

Johnson, K. E., & Wilgus, T. A. (2014). Vascular Endothelial Growth Factor and Angiogenesis in the Regulation of Cutaneous Wound Repair. *Advances in Wound Care*, *3*(10), 647–661. https://doi.org/10.1089/wound.2013.0517

Jüttner, S., Bernhagen, J., Metz, C. N., Röllinghoff, M., Bucala, R., & Gessner, A. (1998). Migration Inhibitory Factor Induces Killing Of Leishmania Major By Macrophages: Dependence On Reactive Nitrogen Intermediates And Endogenous TNF-Alpha. *Journal of Immunology (Baltimore, Md. : 1950)*, *161*(5), 2383–2390.

Kanannejad, Z., Soleimanian, S., Ghahramani, Z., Sepahi, N., Mohkam, M., Alyasin, S., & Kheshtchin, N. (2023). Immune Checkpoint Molecules In Prevention And Development Of Asthma. *Frontiers in Immunology*, *14*. https://doi.org/10.3389/fimmu.2023.1070779

Kang, I., & Bucala, R. (2019). The Immunobiology Of MIF: Function, Genetics And Prospects For Precision Medicine. *Nature Reviews Rheumatology*, *15*(7), 427–437. https://doi.org/10.1038/s41584-019-0238-2

Kang, Q., Li, L., Pang, Y., Zhu, W., & Meng, L. (2022). An Update On Ym1 And Its Immunoregulatory Role In Diseases. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.891220

Kang, S., & Kumanogoh, A. (2020). The Spectrum Of Macrophage Activation By Immunometabolism. *International Immunology*, *32*(7), 467–473. https://doi.org/10.1093/intimm/dxaa017

Kanniess, F., Tamasi, L., Mroz, R. R., Bakakos, P., Tubis, S., Resler Plat, G., & Shavit, A. (2021). Real-World Patient Characteristics And Eligibility For Biologics In Severe Asthma (RECOGNISE). *Airway Pharmacology and Treatment*, PA3729. https://doi.org/10.1183/13993003.congress-2021.PA3729

Kantomaa, M. T., Tolvanen, M., Halonen, M., Svanes, C., Järvelin, M.-R., & Sebert, S. (2023). Influence of Farm Environment on Asthma during the Life Course: A Population-Based Birth Cohort Study in Northern Finland. *International Journal of Environmental Research and Public Health*, *20*(3), 2128. https://doi.org/10.3390/ijerph20032128

Kaufmann, E., Khan, N., Tran, K. A., Ulndreaj, A., Pernet, E., Fontes, G., Lupien, A., Desmeules, P., McIntosh, F., Abow, A., Moorlag, S. J. C. F. M., Debisarun, P., Mossman, K., Banerjee, A., Karo-Atar, D., Sadeghi, M., Mubareka, S., Vinh, D. C., King, I. L., Divangahi, M. (2022). BCG Vaccination Provides Protection Against IAV But Not SARS-Cov-2. *Cell Reports*, *38*(10), 110502. https://doi.org/10.1016/j.celrep.2022.110502

Ke, X., Do, D. C., Li, C., Zhao, Y., Kollarik, M., Fu, Q., Wan, M., & Gao, P. (2019). Ras Homolog Family Member A/Rho-Associated Protein Kinase 1 Signaling Modulates Lineage Commitment Of Mesenchymal Stem Cells In Asthmatic Patients Through Lymphoid Enhancer–Binding Factor 1. *Journal of Allergy and Clinical Immunology*, *143*(4), 1560-1574.e6. https://doi.org/10.1016/j.jaci.2018.08.023

Kelsen, S. G., Agache, I. O., Soong, W., Israel, E., Chupp, G. L., Cheung, D. S., Theess, W., Yang, X., Staton, T. L., Choy, D. F., Fong, A., Dash, A., Dolton, M., Pappu, R., & Brightling, C. E. (2021). Astegolimab (Anti-ST2) Efficacy And Safety In Adults With Severe Asthma: A Randomized

Clinical Trial. *Journal of Allergy and Clinical Immunology*, *148*(3), 790–798. https://doi.org/10.1016/j.jaci.2021.03.044

Kennelly, H., Mahon, B. P., & English, K. (2016). Human Mesenchymal Stromal Cells Exert HGF Dependent Cytoprotective Effects In A Human Relevant Pre-Clinical Model Of COPD. *Scientific Reports*, *6*(1), 38207. https://doi.org/10.1038/srep38207

Kevill, K. A., Bhandari, V., Kettunen, M., Leng, L., Fan, J., Mizue, Y., Dzuira, J. D., Reyes-Mugica, M., McDonald, C. L., Baugh, J. A., O'Connor, C. L., Aghai, Z. H., Donnelly, S. C., Bazzy-Asaad, A., & Bucala, R. J. (2008). A Role for Macrophage Migration Inhibitory Factor in the Neonatal Respiratory Distress Syndrome. *The Journal of Immunology*, *180*(1), 601–608. https://doi.org/10.4049/jimmunol.180.1.601

Kim, H.-R., Park, M.-K., Cho, M.-L., Yoon, C.-H., Lee, S.-H., Park, S.-H., Leng, L., Bucala, R., Kang, I., Choe, J., & Kim, H.-Y. (2007). Macrophage Migration Inhibitory Factor Upregulates Angiogenic Factors And Correlates With Clinical Measures In Rheumatoid Arthritis. *The Journal of Rheumatology*, *34*(5), 927–936.

Kim, J., & Hematti, P. (2009). Mesenchymal Stem Cell–Educated Macrophages: A Novel Type Of Alternatively Activated Macrophages. *Experimental Hematology*, *37*(12), 1445–1453. https://doi.org/10.1016/j.exphem.2009.09.004

Kim, K.-W., & Kim, H.-R. (2016). Macrophage Migration Inhibitory Factor: A Potential Therapeutic Target For Rheumatoid Arthritis. *The Korean Journal of Internal Medicine*, *31*(4), 634–642. https://doi.org/10.3904/kjim.2016.098

Kim, R. L., Bang, J.-Y., Kim, J., Mo, Y., Kim, Y., Lee, C.-G., Elias, J. A., Kim, H. Y., & Kang, H.-R. (2022). Mesenchymal Stem Cells Exert Their Anti-Asthmatic Effects Through Macrophage Modulation In A Murine Chronic Asthma Model. *Scientific Reports*, *12*(1), 9811. https://doi.org/10.1038/s41598-022-14027-x

Kim, S.-Y., Lee, J.-H., Kim, H. J., Park, M. K., Huh, J. W., Ro, J. Y., Oh, Y.-M., Lee, S.-D., & Lee, Y.-S. (2012). Mesenchymal Stem Cell-Conditioned Media Recovers Lung Fibroblasts From Cigarette Smoke-Induced Damage. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *302*(9), L891–L908. https://doi.org/10.1152/ajplung.00288.2011

Kobayashi, M. (2006). Role Of Macrophage Migration Inhibitory Factor In Ovalbumin-Induced Airway Inflammation In Rats. *European Respiratory Journal*, *27*(4), 726–734. https://doi.org/10.1183/09031936.06.00107004

Koebernick, H., Grode, L., David, J. R., Rohde, W., Rolph, M. S., Mittrücker, H.-W., & Kaufmann, S. H. E. (2002). Macrophage Migration Inhibitory Factor (MIF) Plays A Pivotal Role In Immunity Against *Salmonella Typhimurium*. *Proceedings of the National Academy of Sciences*, *99*(21), 13681–13686. https://doi.org/10.1073/pnas.212488699

Kok, T., Wasiel, A. A., Cool, R. H., Melgert, B. N., Poelarends, G. J., & Dekker, F. J. (2018). Small-Molecule Inhibitors Of Macrophage Migration Inhibitory Factor (MIF) As An Emerging Class Of Therapeutics For Immune Disorders. *Drug Discovery Today*, *23*(11), 1910–1918. https://doi.org/10.1016/j.drudis.2018.06.017

Kok, T., Wasiel, A. A., Cool, R. H., Melgert, B. N., Poelarends, G. J., & Dekker, F. J. (2018). Small-Molecule Inhibitors Of Macrophage Migration Inhibitory Factor (MIF) As An Emerging Class Of Therapeutics For Immune Disorders. *Drug Discovery Today*, *23*(11), 1910–1918. https://doi.org/10.1016/j.drudis.2018.06.017

Korf, H., Breser, L., Van Hoeck, J., Godoy, J., Cook, D. P., Stijlemans, B., De Smidt, E., Moyson, C., Monteiro Carvalho Mori Cunha, J. P., Rivero, V., Gysemans, C., & Mathieu, C. (2017). MIF Inhibition Interferes With The Inflammatory And T Cell-Stimulatory Capacity Of NOD Macrophages And Delays Autoimmune Diabetes Onset. *PLOS ONE*, *12*(11), e0187455. https://doi.org/10.1371/journal.pone.0187455

Kozhukharova, I., Minkevich, N., Alekseenko, L., Domnina, A., & Lyublinskaya, O. (2022). Paracrine and Autocrine Effects of VEGF Are Enhanced in Human eMSC Spheroids. *International Journal of Molecular Sciences*, *23*(22), 14324. https://doi.org/10.3390/ijms232214324

Krammer, C., Yang, B., Reichl, S., Besson-Girard, S., Ji, H., Bolini, V., Schulte, C., Noels, H., Schlepckow, K., Jocher, G., Werner, G., Willem, M., El Bounkari, O., Kapurniotu, A., Gokce, O., Weber, C., Mohanta, S., & Bernhagen, J. (2023). Pathways Linking Aging And Atheroprotection

In *Mif* -Deficient Atherosclerotic Mice. *The FASEB Journal*, *37*(3). https://doi.org/10.1096/fj.202200056R

Kroes, J. A., Alfonso-Cristancho, R., Bansal, A. T., Berret, E., Bieksiene, K., Bourdin, A., Brussino, L., Canhoto, D., Cardini, C., Celik, G., Csoma, Z., Dahlén, B., Damadoglu, E., Eger, K., Gauquelin, L., Gemicioglu, B., Goksel, O., Graff, S., Heffler, E.,Ten Brinke, A. (2023). Evaluation Of Real-World Mepolizumab Use In Severe Asthma Across Europe: The SHARP Experience With Privacy-Preserving Federated Analysis. *ERJ Open Research*, *9*(2), 00745–02022. https://doi.org/10.1183/23120541.00745-2022

Kruk, D. M. L. W., Wisman, M., Noordhoek, J. A., Nizamoglu, M., Jonker, M. R., de Bruin, H. G., Arevalo Gomez, K., ten Hacken, N. H. T., Pouwels, S. D., & Heijink, I. H. (2021). Paracrine Regulation of Alveolar Epithelial Damage and Repair Responses by Human Lung-Resident Mesenchymal Stromal Cells. *Cells*, *10*(11), 2860. https://doi.org/10.3390/cells10112860

Kubo, S., Takahashi, H. K., Takei, M., Iwagaki, H., Yoshino, T., Tanaka, N., Mori, S., & Nishibori, M. (2004). E-Prostanoid (EP)2/EP4 Receptor-Dependent Maturation of Human Monocyte-Derived Dendritic Cells and Induction of Helper T2 Polarization. *Journal of Pharmacology and Experimental Therapeutics*, *309*(3), 1213–1220. https://doi.org/10.1124/jpet.103.062646

Kulesza, A., Paczek, L., & Burdzinska, A. (2023). The Role of COX-2 and PGE2 in the Regulation of Immunomodulation and Other Functions of Mesenchymal Stromal Cells. *Biomedicines*, *11*(2), 445. https://doi.org/10.3390/biomedicines11020445

Kurtz, J. (2005). Specific Memory Within Innate Immune Systems. *Trends in Immunology*, *26*(4), 186–192. https://doi.org/10.1016/j.it.2005.02.001

Lambrecht, B. N., & Hammad, H. (2017). The immunology of the allergy epidemic and the hygiene hypothesis. *Nature Immunology*, *18*(10), 1076–1083. https://doi.org/10.1038/ni.3829

Lambrecht, B. N., Hammad, H., & Fahy, J. V. (2019). The Cytokines of Asthma. *Immunity*, *50*(4), 975–991. https://doi.org/10.1016/j.immuni.2019.03.018

Lan, H., Luo, L., Chen, Y., Wang, M., Yu, Z., & Gong, Y. (2020). MIF Signaling Blocking Alleviates Airway Inflammation And Airway Epithelial Barrier Disruption In A HDM-Induced Asthma Model. *Cellular Immunology*, *347*, 103965. https://doi.org/10.1016/j.cellimm.2019.103965

Lang, T., Foote, A., Lee, J. P. W., Morand, E. F., & Harris, J. (2015). MIF: Implications in the Pathoetiology of Systemic Lupus Erythematosus. *Frontiers in Immunology*, *6*. https://doi.org/10.3389/fimmu.2015.00577

Lang, T., Lee, J. P. W., Elgass, K., Pinar, A. A., Tate, M. D., Aitken, E. H., Fan, H., Creed, S. J., Deen, N. S., Traore, D. A. K., Mueller, I., Stanisic, D., Baiwog, F. S., Skene, C., Wilce, M. C. J., Mansell, A., Morand, E. F., & Harris, J. (2018). Macrophage Migration Inhibitory Factor Is Required For NLRP3 Inflammasome Activation. *Nature Communications*, *9*(1), 2223. https://doi.org/10.1038/s41467-018-04581-2

Laorden, D., Hernández, I., Domínguez-Ortega, J., Romero, D., Álvarez-Sala, R., & Quirce, S. (2023). A Real Life Cohort Of Mepolizumab Treatment In Severe Eosinophilic Asthma. *European Annals of Allergy and Clinical Immunology*, *online first*. https://doi.org/10.23822/EurAnnACI.1764-1489.289

Lazarus, H. M., Haynesworth, S. E., Gerson, S. L., Rosenthal, N. S., & Caplan, A. I. (1995). Ex Vivo Expansion And Subsequent Infusion Of Human Bone Marrow-Derived Stromal Progenitor Cells (Mesenchymal Progenitor Cells): Implications For Therapeutic Use. *Bone Marrow Transplantation*, *16*(4), 557–564.

Le Floc'h, A., Allinne, J., Nagashima, K., Scott, G., Birchard, D., Asrat, S., Bai, Y., Lim, W. K., Martin, J., Huang, T., Potocky, T. B., Kim, J. H., Rafique, A., Papadopoulos, N. J., Stahl, N., Yancopoulos, G. D., Murphy, A. J., Sleeman, M. A., & Orengo, J. M. (2020). Dual Blockade Of IL-4 And IL-13 With Dupilumab, An IL-4Rα Antibody, Is Required To Broadly Inhibit Type 2 Inflammation. *Allergy*, *75*(5), 1188–1204. https://doi.org/10.1111/all.14151

Lechner, A., Henkel, F. D. R., Hartung, F., Bohnacker, S., Alessandrini, F., Gubernatorova, E. O., Drutskaya, M. S., Angioni, C., Schreiber, Y., Haimerl, P., Ge, Y., Thomas, D., Kabat, A. M., Pearce, E. J., Ohnmacht, C., Nedospasov, S. A., Murray, P. J., Chaker, A. M., Schmidt-Weber, C. B., & Esser-von Bieren, J. (2022). Macrophages Acquire A TNF-Dependent Inflammatory Memory In Allergic Asthma. *Journal of Allergy and Clinical Immunology*, *149*(6), 2078–2090. https://doi.org/10.1016/j.jaci.2021.11.026

Lee, H., & Hong, I. (2017). Double-Edged Sword Of Mesenchymal Stem Cells: Cancer-Promoting Versus Therapeutic Potential. *Cancer Science*, *108*(10), 1939–1946. https://doi.org/10.1111/cas.13334

Lee, J., Ryu, H., Ferrante, R. J., Morris, S. M., & Ratan, R. R. (2003). Translational Control Of Inducible Nitric Oxide Synthase Expression By Arginine Can Explain The Arginine Paradox. *Proceedings of the National Academy of Sciences*, *100*(8), 4843–4848. https://doi.org/10.1073/pnas.0735876100

Lee, J.-U., Chang, H. S., Lee, H. J., Jung, C. A., Bae, D. J., Song, H. J., Park, J. S., Uh, S.-T., Kim, Y. H., Seo, K.-H., & Park, C.-S. (2017). Upregulation Of Interleukin-33 And Thymic Stromal Lymphopoietin Levels In The Lungs Of Idiopathic Pulmonary Fibrosis. *BMC Pulmonary Medicine*, *17*(1), 39. https://doi.org/10.1186/s12890-017-0380-z

Leentjens, J., Kox, M., Stokman, R., Gerretsen, J., Diavatopoulos, D. A., van Crevel, R., Rimmelzwaan, G. F., Pickkers, P., & Netea, M. G. (2015). BCG Vaccination Enhances the Immunogenicity of Subsequent Influenza Vaccination in Healthy Volunteers: A Randomized, Placebo-Controlled Pilot Study. *Journal of Infectious Diseases*, *212*(12), 1930–1938. https://doi.org/10.1093/infdis/jiv332

Leibacher, J., & Henschler, R. (2016). Biodistribution, Migration And Homing Of Systemically Applied Mesenchymal Stem/Stromal Cells. *Stem Cell Research & Therapy*, *7*(1), 7. https://doi.org/10.1186/s13287-015-0271-2

Leng, L., & Bucala, R. (2006). Insight Into The Biology Of Macrophage Migration Inhibitory Factor (MIF) Revealed By The Cloning Of Its Cell Surface Receptor. *Cell Research*, *16*(2), 162–168. https://doi.org/10.1038/sj.cr.7310022

Leng, L., Metz, C. N., Fang, Y., Xu, J., Donnelly, S., Baugh, J., Delohery, T., Chen, Y., Mitchell, R. A., & Bucala, R. (2003). MIF Signal Transduction Initiated by Binding to CD74. *Journal of Experimental Medicine*, *197*(11), 1467–1476. https://doi.org/10.1084/jem.20030286

Li, B. W. S., de Bruijn, M. J. W., Lukkes, M., Nimwegen, M., Bergen, I. M., KleinJan, A., GeurtsvanKessel, C. H., Andeweg, A., Rimmelzwaan, G. F., & Hendriks, R. W. (2019). T Cells And ILC2s Are Major Effector Cells In Influenza-Induced Exacerbation Of Allergic Airway Inflammation In Mice. *European Journal of Immunology*, *49*(1), 144–156. https://doi.org/10.1002/eji.201747421

Li, C., Cheung, M. K. H., Han, S., Zhang, Z., Chen, L., Chen, J., Zeng, H., & Qiu, J. (2019). Mesenchymal Stem Cells And Their Mitochondrial Transfer: A Double-Edged Sword. *Bioscience Reports*, *39*(5). https://doi.org/10.1042/BSR20182417

Li, C.-L., Xu, Z.-B., Fan, X.-L., Chen, H.-X., Yu, Q.-N., Fang, S.-B., Wang, S.-Y., Lin, Y.-D., & Fu, Q.-L. (2018). MicroRNA-21 Mediates the Protective Effects of Mesenchymal Stem Cells Derived from iPSCs to Human Bronchial Epithelial Cell Injury Under Hypoxia. *Cell Transplantation*, *27*(3), 571–583. https://doi.org/10.1177/0963689718767159

Li, J., Zhang, J., Xie, F., Peng, J., & Wu, X. (2017). Macrophage Migration Inhibitory Factor Promotes Warburg Effect Via Activation Of The NF-Kb/HIF-1α Pathway In Lung Cancer. *International Journal of Molecular Medicine*. https://doi.org/10.3892/ijmm.2017.3277

Li, R., Li, D., Wang, H., Chen, K., Wang, S., Xu, J., & Ji, P. (2022). Exosomes From Adipose-Derived Stem Cells Regulate M1/M2 Macrophage Phenotypic Polarization To Promote Bone Healing Via Mir-451a/MIF. *Stem Cell Research & Therapy*, *13*(1), 149. https://doi.org/10.1186/s13287-022-02823-1

Li, R., Wang, F., Wei, J., Lin, Y., Tang, G., Rao, L., Ma, L., Xu, Q., Wu, J., Lv, Q., Zhou, R., Lei, H., Zhao, X., Yao, D., Xiao, B., Huang, H., Zhang, J., & Mo, B. (2021). The Role of Macrophage Migration Inhibitory Factor (MIF) in Asthmatic Airway Remodeling. *Allergy, Asthma & Immunology Research*, *13*(1), 88. https://doi.org/10.4168/aair.2021.13.1.88

Li, T., Sun, H., Li, Y., Su, L., Jiang, J., Liu, Y., Jiang, N., Huang, R., Zhang, J., & Peng, Z. (2022). Downregulation Of Macrophage Migration Inhibitory Factor Attenuates NLRP3 Inflammasome Mediated Pyroptosis In Sepsis-Induced AKI. *Cell Death Discovery*, *8*(1), 61. https://doi.org/10.1038/s41420-022-00859-z

Li, X., An, G., Wang, Y., Liang, D., Zhu, Z., & Tian, L. (2018). Targeted Migration Of Bone Marrow Mesenchymal Stem Cells Inhibits Silica-Induced Pulmonary Fibrosis In Rats. *Stem Cell Research & Therapy*, *9*(1), 335. https://doi.org/10.1186/s13287-018-1083-y

Li, X., Wang, H., Yu, X., Saha, G., Kalafati, L., Ioannidis, C., Mitroulis, I., Netea, M. G., Chavakis, T., & Hajishengallis, G. (2022). Maladaptive Innate Immune Training Of Myelopoiesis Links Inflammatory Comorbidities. *Cell*, *185*(10), 1709-1727.e18. https://doi.org/10.1016/j.cell.2022.03.043

Li, Y., Wang, W., Lv, Z., Li, Y., Chen, Y., Huang, K., Corrigan, C. J., & Ying, S. (2018). Elevated Expression of IL-33 and TSLP in the Airways of Human Asthmatics In Vivo: A Potential Biomarker of Severe Refractory Disease. *The Journal of Immunology*, *200*(7), 2253–2262. https://doi.org/10.4049/jimmunol.1701455

Liesveld, J. L., Sharma, N., & Aljitawi, O. S. (2020). Stem Cell Homing: From Physiology To Therapeutics. *Stem Cells*, *38*(10), 1241–1253. https://doi.org/10.1002/stem.3242

Lintomen, L., Kluppel, L. M., Kitoko, J. Z., Montes-Cobos, E., Vidal, V. M., Tan, L. B., de Farias, J. N., de Souza, H. S., Olsen, P. C., & Bozza, M. T. (2023). MIF Is Essential To The Establishment Of House Dust Mite-Induced Airway Inflammation And Tissue Remodeling In Mice. *European Journal of Immunology*. https://doi.org/10.1002/eji.202250016

Liu, C., Xiao, K., & Xie, L. (2022). Advances In Mesenchymal Stromal Cell Therapy For Acute Lung Injury/Acute Respiratory Distress Syndrome. *Frontiers in Cell and Developmental Biology*, *10*. https://doi.org/10.3389/fcell.2022.951764

Liu, J., Qiu, X., Lv, Y., Zheng, C., Dong, Y., Dou, G., Zhu, B., Liu, A., Wang, W., Zhou, J., Liu, S., Liu, S., Gao, B., & Jin, Y. (2020). Apoptotic Bodies Derived From Mesenchymal Stem Cells Promote Cutaneous Wound Healing Via Regulating The Functions Of Macrophages. *Stem Cell Research & Therapy*, *11*(1), 507. https://doi.org/10.1186/s13287-020-02014-w

Liu, M., Lu, J., Zhang, Q., Zhang, Y., & Guo, Z. (2021). Clara Cell 16 Kda Protein Mitigates House Dust Mite-Induced Airway Inflammation And Damage Via Regulating Airway Epithelial Cell Apoptosis In A Manner Dependent On HMGB1-Mediated Signaling Inhibition. *Molecular Medicine*, *27*(1), 11. https://doi.org/10.1186/s10020-021-00277-4

Liu, S., Verma, M., Michalec, L., Liu, W., Sripada, A., Rollins, D., Good, J., Ito, Y., Chu, H., Gorska, M. M., Martin, R. J., & Alam, R. (2018). Steroid Resistance Of Airway Type 2 Innate Lymphoid Cells From Patients With Severe Asthma: The Role Of Thymic Stromal Lymphopoietin. *Journal of Allergy and Clinical Immunology*, *141*(1), 257-268.e6. https://doi.org/10.1016/j.jaci.2017.03.032

Liu, X., Li, X., Zhu, W., Zhang, Y., Hong, Y., Liang, X., Fan, B., Zhao, H., He, H., & Zhang, F. (2020). Exosomes From Mesenchymal Stem Cells Overexpressing MIF Enhance Myocardial Repair. *Journal of Cellular Physiology*, *235*(11), 8010–8022. https://doi.org/10.1002/jcp.29456

Lloyd, C. (2002). Chemokines in allergic lung inflammation. *Immunology*, *105*(2), 144–154. https://doi.org/10.1046/j.1365-2567.2002.01344.x

Locati, M., Curtale, G., & Mantovani, A. (2020). Diversity, Mechanisms, and Significance of Macrophage Plasticity. *Annual Review of Pathology: Mechanisms of Disease*, *15*(1), 123–147. https://doi.org/10.1146/annurev-pathmechdis-012418-012718

Loli-Ausejo, D., Perdomo, D., Mascaró, B., Martínez-Olondris, P., Sánchez-Fernández, M., Mullol, J., Valero, A., Arismendi, E., & Bobolea, I. (2023). Mepolizumab Treatment For Severe Eosinophilic Asthma: A 5-Years Real-Life Experience. *Journal of Investigational Allergology and Clinical Immunology*, *33*(6). https://doi.org/10.18176/jiaci.0898

López-García, L., & Castro-Manrreza, M. E. (2021). TNF-α and IFN-γ Participate in Improving the Immunoregulatory Capacity of Mesenchymal Stem/Stromal Cells: Importance of Cell–Cell Contact and Extracellular Vesicles. *International Journal of Molecular Sciences*, *22*(17), 9531. https://doi.org/10.3390/ijms22179531

López-Tiro, J., Contreras-Contreras, A., Rodríguez-Arellano, M. E., & Costa-Urrutia, P. (2022). Economic Burden Of Severe Asthma Treatment: A Real-Life Study. *World Allergy Organization Journal*, *15*(7), 100662. https://doi.org/10.1016/j.waojou.2022.100662

Lourenco, S., Teixeira, V., Kalber, T., Thakrar, R., Floto, A., & Janes, S. (2014). MIF As The Key Regulator For Mesenchymal Stem Cells Homing To Tumours By 3D And In Vivo Lung Metastasis Models. *Thorax*, *69*(Suppl 2), A58–A58. https://doi.org/10.1136/thoraxjnl-2014-206260.114

Lubetsky, J. B., Swope, M., Dealwis, C., Blake, P., & Lolis, E. (1999). Pro-1 of Macrophage Migration Inhibitory Factor Functions as a Catalytic Base in the Phenylpyruvate Tautomerase Activity ˙. *Biochemistry*, *38*(22), 7346–7354. https://doi.org/10.1021/bi990306m

Lue, H., Thiele, M., Franz, J., Dahl, E., Speckgens, S., Leng, L., Fingerle-Rowson, G., Bucala, R., Lüscher, B., & Bernhagen, J. (2007). Macrophage Migration Inhibitory Factor (MIF) Promotes Cell Survival By Activation Of The Akt Pathway And Role For CSN5/JAB1 In The Control Of Autocrine MIF Activity. *Oncogene*, *26*(35), 5046–5059. https://doi.org/10.1038/sj.onc.1210318

Lundahl, M. LE, Mitermite, M., Ryan, D. G., Case, S., Williams, N. C., Yang, M., Lynch, R. I., Lagan, E., Lebre, F. M., Gorman, A. L., Stojkovic, B., Bracken, A. P., Frezza, C., Sheedy, F. J., Scanlan, E. M., O'Neill, L. A., Gordon, S. V, & Lavelle, E. C. (2022). Macrophage Innate Training Induced By IL-4 And IL-13 Activation Enhances OXPHOS Driven Anti-Mycobacterial Responses. *ELife*, *11*. https://doi.org/10.7554/eLife.74690

Luo, Y., Yi, H., Huang, X., Lin, G., Kuang, Y., Guo, Y., & Xie, C. (2021). Inhibition Of Macrophage Migration Inhibitory Factor (MIF) As A Therapeutic Target In Bleomycin-Induced Pulmonary Fibrosis Rats. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *321*(1), L6–L16. https://doi.org/10.1152/ajplung.00288.2020

Luque-Campos, N., Bustamante-Barrientos, F. A., Pradenas, C., García, C., Araya, M. J., Bohaud, C., Contreras-López, R., Elizondo-Vega, R., Djouad, F., Luz-Crawford, P., & Vega-Letter, A. M. (2021). The Macrophage Response Is Driven by Mesenchymal Stem Cell-Mediated Metabolic Reprogramming. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.624746

Lynch, K., Treacy, O., Chen, X., Murphy, N., Lohan, P., Islam, M. N., Donohoe, E., Griffin, M. D., Watson, L., McLoughlin, S., O'Malley, G., Ryan, A. E., & Ritter, T. (2020). TGF-β1-Licensed Murine MSCs Show Superior Therapeutic Efficacy in Modulating Corneal Allograft Immune Rejection In Vivo. *Molecular Therapy*, *28*(9), 2023–2043. https://doi.org/10.1016/j.ymthe.2020.05.023

Ma, F., Kouzoukas, D. E., Meyer-Siegler, K. L., Hunt, D. E., Leng, L., Bucala, R., & Vera, P. L. (2019). MIF Mediates Bladder Pain, Not Inflammation, In Cyclophosphamide Cystitis. *Cytokine: X*, *1*(1), 100003. https://doi.org/10.1016/j.cytox.2019.100003

Ma, S.-Y., Zhao, N., Cui, L., Li, Y., Zhang, H., Wang, J., Pang, J., Wang, M., Wang, C., Ying, S., Jin, Z.-B., & Zhang, L. (2022). Therapeutic Effects of Human Pluripotent Stem Cell-Derived Mesenchymal Stem Cells on a Murine Model of Acute Type-2-Dominated Airway Inflammation. *Stem Cell Reviews and Reports*, *18*(8), 2939–2951. https://doi.org/10.1007/s12015-022-10389-x

Magalhães, E. S., Mourao-Sa, D. S., Vieira-de-Abreu, A., Figueiredo, R. T., Pires, A. L., Farias-Filho, F. A., Fonseca, B. P. F., Viola, J. P. B., Metz, C., Martins, M. A., Castro-Faria-Neto, H. C., Bozza, P. T., & Bozza, M. T. (2007). Macrophage Migration Inhibitory Factor Is Essential For Allergic Asthma But Not For Th2 Differentiation. *European Journal of Immunology*, *37*(4), 1097–1106. https://doi.org/10.1002/eji.200635968

Magalhaes, E. S., Paiva, C. N., Souza, H. S. P., Pyrrho, A. S., Mourao-Sá, D., Figueiredo, R. T., Vieira-de-Abreu, A., Dutra, H. S., Silveira, M. S., Gaspar-Elsas, M. I. C., Xavier-Elsas, P., Bozza, P. T., & Bozza, M. T. (2009). Macrophage Migration Inhibitory Factor Is Critical To Interleukin-5-Driven Eosinophilopoiesis And Tissue Eosinophilia Triggered By *Schistosoma Mansoni* Infection. *The FASEB Journal*, *23*(4), 1262–1271. https://doi.org/10.1096/fj.08-124248

Mahalingam, D., Patel, M. R., Sachdev, J. C., Hart, L. L., Halama, N., Ramanathan, R. K., Sarantopoulos, J., Völkel, D., Youssef, A., Jong, F. A., & Tsimberidou, A. M. (2020). Phase I Study Of Imalumab (BAX69), A Fully Human Recombinant Antioxidized Macrophage Migration Inhibitory Factor Antibody In Advanced Solid Tumours. *British Journal of Clinical Pharmacology*, *86*(9), 1836–1848. https://doi.org/10.1111/bcp.14289

Maio, S., Fasola, S., Marcon, A., Angino, A., Baldacci, S., Bilò, M. B., Bono, R., La Grutta, S., Marchetti, P., Sarno, G., Squillacioti, G., Stanisci, I., Pirina, P., Tagliaferro, S., Verlato, G., Villani, S., Gariazzo, C., Stafoggia, M., & Viegi, G. (2023). Relationship Of Long-Term Air Pollution Exposure With Asthma And Rhinitis In Italy: An Innovative Multipollutant Approach. *Environmental Research*, *224*, 115455. https://doi.org/10.1016/j.envres.2023.115455

Mäkikyrö, E. M. S., Jaakkola, M. S., Lajunen, T. K., Malmberg, L. P., & Jaakkola, J. J. K. (2023). Subtypes of Adult-Onset Asthma at the Time of Diagnosis: A Latent Class Analysis. *International Journal of Environmental Research and Public Health*, *20*(4), 3072. https://doi.org/10.3390/ijerph20043072

Mariñas-Pardo, L., Mirones, I., Amor-Carro, Ó., Fraga-Iriso, R., Lema-Costa, B., Cubillo, I., Rodríguez Milla, M. Á., García-Castro, J., & Ramos-Barbón, D. (2014). Mesenchymal Stem Cells Regulate

Airway Contractile Tissue Remodeling In Murine Experimental Asthma. *Allergy*, *69*(6), 730–740. https://doi.org/10.1111/all.12392

Marks, G. B., Ng, K., Zhou, J., Toelle, B. G., Xuan, W., Belousova, E. G., MApplSc, & Britton, W. J. (2003). The Effect Of Neonatal BCG Vaccination On Atopy And Asthma At Age 7 To 14 Years: An Historical Cohort Study In A Community With A Very Low Prevalence Of Tuberculosis Infection And A High Prevalence Of Atopic Disease. *Journal of Allergy and Clinical Immunology*, *111*(3), 541–549. https://doi.org/10.1067/mai.2003.171

Marsh, L. M., Cakarova, L., Kwapiszewska, G., von Wulffen, W., Herold, S., Seeger, W., & Lohmeyer, J. (2009). Surface Expression Of CD74 By Type II Alveolar Epithelial Cells: A Potential Mechanism For Macrophage Migration Inhibitory Factor-Induced Epithelial Repair. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *296*(3), L442–L452. https://doi.org/10.1152/ajplung.00525.2007

Martiney, J. A., Sherry, B., Metz, C. N., Espinoza, M., Ferrer, A. S., Calandra, T., Broxmeyer, H. E., & Bucala, R. (2000). Macrophage Migration Inhibitory Factor Release by Macrophages after Ingestion of *Plasmodium chabaudi* -Infected Erythrocytes: Possible Role in the Pathogenesis of Malarial Anemia. *Infection and Immunity*, *68*(4), 2259–2267. https://doi.org/10.1128/IAI.68.4.2259-2267.2000

Martinez, F. O., Helming, L., Milde, R., Varin, A., Melgert, B. N., Draijer, C., Thomas, B., Fabbri, M., Crawshaw, A., Ho, L. P., Ten Hacken, N. H., Cobos Jiménez, V., Kootstra, N. A., Hamann, J., Greaves, D. R., Locati, M., Mantovani, A., & Gordon, S. (2013). Genetic Programs Expressed In Resting And IL-4 Alternatively Activated Mouse And Human Macrophages: Similarities And Differences. *Blood*, *121*(9), e57–e69. https://doi.org/10.1182/blood-2012-06-436212

Mathias, L. J., Khong, S. M. L., Spyroglou, L., Payne, N. L., Siatskas, C., Thorburn, A. N., Boyd, R. L., & Heng, T. S. P. (2013). Alveolar Macrophages Are Critical for the Inhibition of Allergic Asthma by Mesenchymal Stromal Cells. *The Journal of Immunology*, *191*(12), 5914–5924. https://doi.org/10.4049/jimmunol.1300667

Matthay, M. A., Calfee, C. S., Zhuo, H., Thompson, B. T., Wilson, J. G., Levitt, J. E., Rogers, A. J., Gotts, J. E., Wiener-Kronish, J. P., Bajwa, E. K., Donahoe, M. P., McVerry, B. J., Ortiz, L. A., Exline, M., Christman, J. W., Abbott, J., Delucchi, K. L., Caballero, L., McMillan, M., Liu, K. D. (2019). Treatment With Allogeneic Mesenchymal Stromal Cells For Moderate To Severe Acute Respiratory Distress Syndrome (START Study): A Randomised Phase 2a Safety Trial. *The Lancet Respiratory Medicine*, *7*(2), 154–162. https://doi.org/10.1016/S2213-2600(18)30418-1

Mawhinney, L., Armstrong, M. E., O'Reilly, C., Bucala, R., Leng, L., Fingerle-Rowson, G., Fayne, D., Keane, M. P., Tynan, A., Maher, L., Cooke, G., Lloyd, D., Conroy, H., & Donnelly, S. C. (2014). Macrophage Migration Inhibitory Factor (MIF) Enzymatic Activity and Lung Cancer. *Molecular Medicine*, *20*(1), 729–735. https://doi.org/10.2119/molmed.2014.00136

Melo, M. M., Cruz, F. F., & Rocco, P. R. M. (2023). Mesenchymal Stromal Cell Therapy For Chronic Lung Diseases: Experimental And Clinical Evidence. *Expert Review of Respiratory Medicine*. https://doi.org/10.1080/17476348.2023.2196015

Melotti, P., Mafficini, A., Lebecque, P., Ortombina, M., Leal, T., Pintani, E., Pepermans, X., Sorio, C., & Assael, B. M. (2014). Impact of MIF Gene Promoter Polymorphism on F508del Cystic Fibrosis Patients. *PLoS ONE*, *9*(12), e114274. https://doi.org/10.1371/journal.pone.0114274

Menzies-Gow, A., Wechsler, M. E., Brightling, C. E., Korn, S., Corren, J., Israel, E., Chupp, G., Bednarczyk, A., Ponnarambil, S., Caveney, S., Almqvist, G., Gołąbek, M., Simonsson, L., Lawson, K., Bowen, K., Colice, G., Hetzel, J. L., Fiterman, J., Souza Machado, A., … Nguyen, T. T. D. (2023). Long-Term Safety And Efficacy Of Tezepelumab In People With Severe, Uncontrolled Asthma (DESTINATION): A Randomised, Placebo-Controlled Extension Study. *The Lancet Respiratory Medicine*, *11*(5), 425–438. https://doi.org/10.1016/S2213-2600(22)00492-1

Merk, M., Zierow, S., Leng, L., Das, R., Du, X., Schulte, W., Fan, J., Lue, H., Chen, Y., Xiong, H., Chagnon, F., Bernhagen, J., Lolis, E., Mor, G., Lesur, O., & Bucala, R. (2011). The *D* -Dopachrome Tautomerase (*DDT*) Gene Product Is A Cytokine And Functional Homolog Of Macrophage Migration Inhibitory Factor (MIF). *Proceedings of the National Academy of Sciences*, *108*(34). https://doi.org/10.1073/pnas.1102941108

Mezey, É. (2022). Human Mesenchymal Stem/Stromal Cells in Immune Regulation and Therapy. *Stem Cells Translational Medicine*, *11*(2), 114–134. https://doi.org/10.1093/stcltm/szab020

Miller, E. J., Li, J., Leng, L., McDonald, C., Atsumi, T., Bucala, R., & Young, L. H. (2008). Macrophage Migration Inhibitory Factor Stimulates AMP-Activated Protein Kinase In The Ischaemic Heart. *Nature*, *451*(7178), 578–582. https://doi.org/10.1038/nature06504

Miller, M. H., Swaby, L. G., Vailoces, V. S., LaFratta, M., Zhang, Y., Zhu, X., Hitchcock, D. J., Jewett, T. J., Zhang, B., & Tigno-Aranjuez, J. T. (2023). LMAN1 Is A Receptor For House Dust Mite Allergens. *Cell Reports*, *42*(3), 112208. https://doi.org/10.1016/j.celrep.2023.112208

Mitchell, R. A., Liao, H., Chesney, J., Fingerle-Rowson, G., Baugh, J., David, J., & Bucala, R. (2002). Macrophage Migration Inhibitory Factor (MIF) Sustains Macrophage Proinflammatory Function By Inhibiting P53: Regulatory Role In The Innate Immune Response. *Proceedings of the National Academy of Sciences*, *99*(1), 345–350. https://doi.org/10.1073/pnas.012511599

Mitchell, R. A., Metz, C. N., Peng, T., & Bucala, R. (1999). Sustained Mitogen-activated Protein Kinase (MAPK) and Cytoplasmic Phospholipase A2 Activation by Macrophage Migration Inhibitory Factor (MIF). *Journal of Biological Chemistry*, *274*(25), 18100–18106. https://doi.org/10.1074/jbc.274.25.18100

Miura, M., Miura, Y., Padilla-Nash, H. M., Molinolo, A. A., Fu, B., Patel, V., Seo, B.-M., Sonoyama, W., Zheng, J. J., Baker, C. C., Chen, W., Ried, T., & Shi, S. (2006). Accumulated Chromosomal Instability in Murine Bone Marrow Mesenchymal Stem Cells Leads to Malignant Transformation. *Stem Cells*, *24*(4), 1095–1103. https://doi.org/10.1634/stemcells.2005-0403

Mizue, Y., Ghani, S., Leng, L., McDonald, C., Kong, P., Baugh, J., Lane, S. J., Craft, J., Nishihira, J., Donnelly, S. C., Zhu, Z., & Bucala, R. (2005). Role For Macrophage Migration Inhibitory Factor In Asthma. *Proceedings of the National Academy of Sciences*, *102*(40), 14410–14415. https://doi.org/10.1073/pnas.0507189102

Mo, Y., Kang, H., Bang, J.-Y., Shin, J. W., Kim, H. Y., Cho, S.-H., & Kang, H.-R. (2022). Intratracheal Administration Of Mesenchymal Stem Cells Modulates Lung Macrophage Polarization And Exerts Anti-Asthmatic Effects. *Scientific Reports*, *12*(1), 11728. https://doi.org/10.1038/s41598-022-14846-y

Mo, Y., Kim, Y., Bang, J.-Y., Jung, J., Lee, C.-G., Elias, J. A., & Kang, H.-R. (2022). Mesenchymal Stem Cells Attenuate Asthmatic Inflammation and Airway Remodeling by Modulating Macrophages/Monocytes in the IL-13-Overexpressing Mouse Model. *Immune Network*, *22*(5). https://doi.org/10.4110/in.2022.22.e40

Mohammadalipour, A., Dumbali, S. P., & Wenzel, P. L. (2020). Mitochondrial Transfer and Regulators of Mesenchymal Stromal Cell Function and Therapeutic Efficacy. *Frontiers in Cell and Developmental Biology*, *8*. https://doi.org/10.3389/fcell.2020.603292

Moll, G., Ankrum, J. A., Olson, S. D., & Nolta, J. A. (2022). Improved MSC Minimal Criteria to Maximize Patient Safety: A Call to Embrace Tissue Factor and Hemocompatibility Assessment of MSC Products. *Stem Cells Translational Medicine*, *11*(1), 2–13. https://doi.org/10.1093/stcltm/szab005

Mönch, D., Reinders, M. E. J., Dahlke, M. H., & Hoogduijn, M. J. (2022). How to Make Sense out of 75,000 Mesenchymal Stromal Cell Publications? *Cells*, *11*(9), 1419. https://doi.org/10.3390/cells11091419

Mönch, D., Reinders, M. E. J., Hoogduijn, M. J., & Dahlke, M.-H. (2023). Mesenchymal Stromal Cell-Based Therapy. *Cells*, *12*(4), 559. https://doi.org/10.3390/cells12040559

Mondru, A. K., Aljasir, M. A., Alrumayh, A., Nithianandarajah, G. N., Ahmed, K., Muller, J., Goldring, C. E. P., Wilm, B., & Cross, M. J. (2023). VEGF Stimulates Activation of ERK5 in the Absence of C-Terminal Phosphorylation Preventing Nuclear Localization and Facilitating AKT Activation in Endothelial Cells. *Cells*, *12*(6), 967. https://doi.org/10.3390/cells12060967

Moorlag, S. J. C. F. M., Taks, E., ten Doesschate, T., van der Vaart, T. W., Janssen, A. B., Müller, L., Ostermann, P., Dijkstra, H., Lemmers, H., Simonetti, E., Mazur, M., Schaal, H., ter Heine, R., van de Veerdonk, F. L., Bleeker-Rovers, C. P., van Crevel, R., ten Oever, J., de Jonge, M. I., Bonten, M. J., … Netea, M. G. (2022). Efficacy of BCG Vaccination Against Respiratory Tract Infections in Older Adults During the Coronavirus Disease 2019 Pandemic. *Clinical Infectious Diseases*, *75*(1), e938–e946. https://doi.org/10.1093/cid/ciac182

Morand, E. F., Leech, M., & Bernhagen, J. (2006). MIF: A New Cytokine Link Between Rheumatoid Arthritis And Atherosclerosis. *Nature Reviews Drug Discovery*, *5*(5), 399–411. https://doi.org/10.1038/nrd2029

Moreira, Naqvi, & Hall. (2020). Effects Of Mesenchymal Stromal Cell-Conditioned Media On Measures Of Lung Structure And Function: A Systematic Review And Meta-Analysis Of Preclinical Studies. *Stem Cell Res Ther* .

Morrison, T. J., Jackson, M. V., Cunningham, E. K., Kissenpfennig, A., McAuley, D. F., O'Kane, C. M., & Krasnodembskaya, A. D. (2017). Mesenchymal Stromal Cells Modulate Macrophages in Clinically Relevant Lung Injury Models by Extracellular Vesicle Mitochondrial Transfer. *American Journal of Respiratory and Critical Care Medicine*, *196*(10), 1275–1286. https://doi.org/10.1164/rccm.201701-0170OC

Mukherjee, M., Forero, D. F., Tran, S., Boulay, M.-E., Bertrand, M., Bhalla, A., Cherukat, J., Al-Hayyan, H., Ayoub, A., Revill, S. D., Javkar, T., Radford, K., Kjarsgaard, M., Huang, C., Dvorkin-Gheva, A., Ask, K., Olivenstein, R., Dendukuri, N., Lemiere, C., … Nair, P. (2020). Suboptimal Treatment Response To Anti-IL-5 Monoclonal Antibodies In Severe Eosinophilic Asthmatics With Airway Autoimmune Phenomena. *European Respiratory Journal*, *56*(4), 2000117. https://doi.org/10.1183/13993003.00117-2020

Mulder, W. J. M., Ochando, J., Joosten, L. A. B., Fayad, Z. A., & Netea, M. G. (2019). Therapeutic Targeting Of Trained Immunity. *Nature Reviews Drug Discovery*, *18*(7), 553–566. https://doi.org/10.1038/s41573-019-0025-4

Mura, M., Han, B., Andrade, C. F., Seth, R., Hwang, D., Waddell, T. K., Keshavjee, S., & Liu, M. (2006). The Early Responses Of VEGF And Its Receptors During Acute Lung Injury: Implication Of VEGF In Alveolar Epithelial Cell Survival. *Critical Care*, *10*(5), R130. https://doi.org/10.1186/cc5042

Murray, L. A., Habiel, D. M., Hohmann, M., Camelo, A., Shang, H., Zhou, Y., Coelho, A. L., Peng, X., Gulati, M., Crestani, B., Sleeman, M. A., Mustelin, T., Moore, M. W., Ryu, C., Osafo-Addo, A. D., Elias, J. A., Lee, C. G., Hu, B., Herazo-Maya, J. D., Herzog, E. L. (2017). Antifibrotic Role Of Vascular Endothelial Growth Factor In Pulmonary Fibrosis. *JCI Insight*, *2*(16). https://doi.org/10.1172/jci.insight.92192

Műzes, G., & Sipos, F. (2022). Mesenchymal Stem Cell-Derived Secretome: A Potential Therapeutic Option for Autoimmune and Immune-Mediated Inflammatory Diseases. *Cells*, *11*(15), 2300. https://doi.org/10.3390/cells11152300

Nelson, R. P., DiNicolo, R., Fernández-Caldas, E., Seleznick, M. J., Lockey, R. F., & Good, R. A. (1996). Allergen-Specific Ige Levels And Mite Allergen Exposure In Children With Acute Asthma First Seen In An Emergency Department And In Nonasthmatic Control Subjects. *Journal of Allergy and Clinical Immunology*, *98*(2), 258–263. https://doi.org/10.1016/S0091-6749(96)70148-3

Németh, K., Leelahavanichkul, A., Yuen, P. S. T., Mayer, B., Parmelee, A., Doi, K., Robey, P. G., Leelahavanichkul, K., Koller, B. H., Brown, J. M., Hu, X., Jelinek, I., Star, R. A., & Mezey, É. (2009). Bone Marrow Stromal Cells Attenuate Sepsis Via Prostaglandin E2–Dependent Reprogramming Of Host Macrophages To Increase Their Interleukin-10 Production. *Nature Medicine*, *15*(1), 42–49. https://doi.org/10.1038/nm.1905

Netea, M. G., Domínguez-Andrés, J., Barreiro, L. B., Chavakis, T., Divangahi, M., Fuchs, E., Joosten, L. A. B., van der Meer, J. W. M., Mhlanga, M. M., Mulder, W. J. M., Riksen, N. P., Schlitzer, A., Schultze, J. L., Stabell Benn, C., Sun, J. C., Xavier, R. J., & Latz, E. (2020). Defining Trained Immunity And Its Role In Health And Disease. *Nature Reviews Immunology*, *20*(6), 375–388. https://doi.org/10.1038/s41577-020-0285-6

Netea, M. G., Joosten, L. A. B., Latz, E., Mills, K. H. G., Natoli, G., Stunnenberg, H. G., O'Neill, L. A. J., & Xavier, R. J. (2016). Trained Immunity: A Program Of Innate Immune Memory In Health And Disease. *Science*, *352*(6284). https://doi.org/10.1126/science.aaf1098

Ng, K. S., Kuncewicz, T. M., & Karp, J. M. (2015). Beyond Hit-and-Run: Stem Cells Leave a Lasting Memory. *Cell Metabolism*, *22*(4), 541–543. https://doi.org/10.1016/j.cmet.2015.09.019

Ni, X., Ou, C., Guo, J., Liu, B., Zhang, J., Wu, Z., Li, H., & Chen, M. (2017). Lentiviral Vector-Mediated Co-Overexpression Of VEGF And Bcl-2 Improves Mesenchymal Stem Cell Survival And

Enhances Paracrine Effects In Vitro. *International Journal of Molecular Medicine*, *40*(2), 418–426. https://doi.org/10.3892/ijmm.2017.3019

Nör, J. E., Christensen, J., Mooney, D. J., & Polverini, P. J. (1999). Vascular Endothelial Growth Factor (VEGF)-Mediated Angiogenesis Is Associated with Enhanced Endothelial Cell Survival and Induction of Bcl-2 Expression. *The American Journal of Pathology*, *154*(2), 375–384. https://doi.org/10.1016/S0002-9440(10)65284-4

Noronha, N. de C., Mizukami, A., Caliári-Oliveira, C., Cominal, J. G., Rocha, J. L. M., Covas, D. T., Swiech, K., & Malmegrim, K. C. R. (2019). Priming Approaches To Improve The Efficacy Of Mesenchymal Stromal Cell-Based Therapies. *Stem Cell Research & Therapy*, *10*(1), 131. https://doi.org/10.1186/s13287-019-1224-y

Ochando, J., Mulder, W. J. M., Madsen, J. C., Netea, M. G., & Duivenvoorden, R. (2023). Trained Immunity — Basic Concepts And Contributions To Immunopathology. *Nature Reviews Nephrology*, *19*(1), 23–37. https://doi.org/10.1038/s41581-022-00633-5

Ohta, S., Misawa, A., Fukaya, R., Inoue, S., Kanemura, Y., Okano, H., Kawakami, Y., & Toda, M. (2012). Macrophage Migration Inhibitory Factor (MIF) Promotes Cell Survival And Proliferation Of Neural Stem/Progenitor Cells. *Journal of Cell Science*. https://doi.org/10.1242/jcs.102210

Ohwada, A. (2003). VEGF Regulates The Proliferation Of Acid-Exposed Alveolar Lining Epithelial Cells. *Thorax*, *58*(4), 328–332. https://doi.org/10.1136/thorax.58.4.328

Oliver, J., Marquez, A., Gomez-Garcia, M., Martinez, A., Mendoza, J. L., Vilchez, J. R., Lopez-Nevot, M. A., Pinero, A., de la Concha, E. G., Nieto, A., Urcelay, E., & Martin, D. J. (2007). Association Of The Macrophage Migration Inhibitory Factor Gene Polymorphisms With Inflammatory Bowel Disease. *Gut*, *56*(1), 150–151. https://doi.org/10.1136/gut.2006.107649

Olivieri, C., Bargagli, E., Inghilleri, S., Campo, I., Cintorino, M., & Rottoli, P. (2016). Macrophage Migration Inhibitory Factor In Lung Tissue Of Idiopathic Pulmonary Fibrosis Patients. *Experimental Lung Research*, *42*(5), 263–266. https://doi.org/10.1080/01902148.2016.1199744

O'Neill, L. A. J., & Netea, M. G. (2020). BCG-Induced Trained Immunity: Can It Offer Protection Against COVID-19? *Nature Reviews Immunology*, *20*(6), 335–337. https://doi.org/10.1038/s41577-020-0337-y

Ortega, H. G., Liu, M. C., Pavord, I. D., Brusselle, G. G., FitzGerald, J. M., Chetta, A., Humbert, M., Katz, L. E., Keene, O. N., Yancey, S. W., & Chanez, P. (2014). Mepolizumab Treatment in Patients with Severe Eosinophilic Asthma. *New England Journal of Medicine*, *371*(13), 1198–1207. https://doi.org/10.1056/NEJMoa1403290

Palumbo, S., Tsai, T.-L., & Li, W.-J. (2014). Macrophage Migration Inhibitory Factor Regulates AKT Signaling in Hypoxic Culture to Modulate Senescence of Human Mesenchymal Stem Cells. *Stem Cells and Development*, *23*(8), 852–865. https://doi.org/10.1089/scd.2013.0294

Pang, S. H. M., D'Rozario, J., Mendonca, S., Bhuvan, T., Payne, N. L., Zheng, D., Hisana, A., Wallis, G., Barugahare, A., Powell, D., Rautela, J., Huntington, N. D., Dewson, G., Huang, D. C. S., Gray, D. H. D., & Heng, T. S. P. (2021). Mesenchymal Stromal Cell Apoptosis Is Required For Their Therapeutic Function. *Nature Communications*, *12*(1), 6495. https://doi.org/10.1038/s41467-021-26834-3

Park, J., Kim, S., Lim, H., Liu, A., Hu, S., Lee, J., Zhuo, H., Hao, Q., Matthay, M. A., & Lee, J.-W. (2019). Therapeutic Effects Of Human Mesenchymal Stem Cell Microvesicles In An Ex Vivo Perfused Human Lung Injured With Severe *E. Coli* Pneumonia. *Thorax*, *74*(1), 43–50. https://doi.org/10.1136/thoraxjnl-2018-211576

Pascoe, C. D., Jha, A., Basu, S., Mahood, T., Lee, A., Hinshaw, S., Falsafi, R., Hancock, R. E. W., Mookherjee, N., & Halayko, A. J. (2020). The Importance Of Reporting House Dust Mite Endotoxin Abundance: Impact On The Lung Transcriptome. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *318*(6), L1229–L1236. https://doi.org/10.1152/ajplung.00103.2020

Pati, S., Khakoo, A. Y., Zhao, J., Jimenez, F., Gerber, M. H., Harting, M., Redell, J. B., Grill, R., Matsuo, Y., Guha, S., Cox, C. S., Reitz, M. S., Holcomb, J. B., & Dash, P. K. (2011). Human Mesenchymal Stem Cells Inhibit Vascular Permeability by Modulating Vascular Endothelial Cadherin/β-Catenin Signaling. *Stem Cells and Development*, *20*(1), 89–101. https://doi.org/10.1089/scd.2010.0013

Patil, M., Brooks, E. G., Henry, M., & Akopian, A. (2013). House Dust Mite Group 2 Allergen Modulates LPS-Induced Responses in Airway Sensory Neurons. *Journal of Allergy and Clinical Immunology*, *131*(2), AB14. https://doi.org/10.1016/j.jaci.2012.12.726

Pavord, I. D., Beasley, R., Agusti, A., Anderson, G. P., Bel, E., Brusselle, G., Cullinan, P., Custovic, A., Ducharme, F. M., Fahy, J. V, Frey, U., Gibson, P., Heaney, L. G., Holt, P. G., Humbert, M., Lloyd, C. M., Marks, G., Martinez, F. D., Sly, P. D., Bush, A. (2018). After Asthma: Redefining Airways Diseases. *The Lancet*, *391*(10118), 350–400. https://doi.org/10.1016/S0140-6736(17)30879-6

Pavord, I. D., Korn, S., Howarth, P., Bleecker, E. R., Buhl, R., Keene, O. N., Ortega, H., & Chanez, P. (2012). Mepolizumab For Severe Eosinophilic Asthma (DREAM): A Multicentre, Double-Blind, Placebo-Controlled Trial. *The Lancet*, *380*(9842), 651–659. https://doi.org/10.1016/S0140-6736(12)60988-X

Pavord, I. D., Tran, T. N., Jones, R. C., Nuevo, J., van den Berge, M., Brusselle, G. G., Menzies-Gow, A. N., Skinner, D., Carter, V., Kocks, J. W. H., & Price, D. B. (2023). Effect of Stepping Up to High-Dose Inhaled Corticosteroids in Patients With Asthma: UK Database Study. *The Journal of Allergy and Clinical Immunology: In Practice*, *11*(2), 532–543. https://doi.org/10.1016/j.jaip.2022.10.040

Pavord, I., Gardiner, F., Heaney, L. G., Domingo, C., Price, R. G., Pullan, A., Oppenheimer, J., Brusselle, G., Nagase, H., Chupp, G., Pizzichini, E., Bañas-Conejero, D., & Howarth, P. (2023). Remission Outcomes In Severe Eosinophilic Asthma With Mepolizumab Therapy: Analysis Of The REDES Study. *Frontiers in Immunology*, *14*. https://doi.org/10.3389/fimmu.2023.1150162

Pelaia, C., Heffler, E., Crimi, C., Maglio, A., Vatrella, A., Pelaia, G., & Canonica, G. W. (2022). Interleukins 4 and 13 in Asthma: Key Pathophysiologic Cytokines and Druggable Molecular Targets. *Frontiers in Pharmacology*, *13*. https://doi.org/10.3389/fphar.2022.851940

Percopo, C. M., Dyer, K. D., Ochkur, S. I., Luo, J. L., Fischer, E. R., Lee, J. J., Lee, N. A., Domachowske, J. B., & Rosenberg, H. F. (2014). Activated Mouse Eosinophils Protect Against Lethal Respiratory Virus Infection. *Blood*, *123*(5), 743–752. https://doi.org/10.1182/blood-2013-05-502443

Pesce, J. T., Ramalingam, T. R., Mentink-Kane, M. M., Wilson, M. S., El Kasmi, K. C., Smith, A. M., Thompson, R. W., Cheever, A. W., Murray, P. J., & Wynn, T. A. (2009). Arginase-1–Expressing Macrophages Suppress Th2 Cytokine–Driven Inflammation and Fibrosis. *PLoS Pathogens*, *5*(4), e1000371. https://doi.org/10.1371/journal.ppat.1000371

Phipps, S., Lam, C. E., Kaiko, G. E., Foo, S. Y., Collison, A., Mattes, J., Barry, J., Davidson, S., Oreo, K., Smith, L., Mansell, A., Matthaei, K. I., & Foster, P. S. (2009). Toll/IL-1 Signaling Is Critical for House Dust Mite–specific Th1 and Th2 Responses. *American Journal of Respiratory and Critical Care Medicine*, *179*(10), 883–893. https://doi.org/10.1164/rccm.200806-974OC

Pidgeon, G. P., Barr, M. P., Harmey, J. H., Foley, D. A., & Bouchier-Hayes, D. J. (2001). Vascular Endothelial Growth Factor (VEGF) Upregulates BCL-2 And Inhibits Apoptosis In Human And Murine Mammary Adenocarcinoma Cells. *British Journal of Cancer*, *85*(2), 273–278. https://doi.org/10.1054/bjoc.2001.1876

Pittet, L. F., Messina, N. L., Orsini, F., Moore, C. L., Abruzzo, V., Barry, S., Bonnici, R., Bonten, M., Campbell, J., Croda, J., Dalcolmo, M., Gardiner, K., Gell, G., Germano, S., Gomes-Silva, A., Goodall, C., Gwee, A., Jamieson, T., Jardim, B., … Curtis, N. (2023). Randomized Trial of BCG Vaccine to Protect against Covid-19 in Health Care Workers. *New England Journal of Medicine*, *388*(17), 1582–1596. https://doi.org/10.1056/NEJMoa2212616

Plant, B. J., Gallagher, C. G., Bucala, R., Baugh, J. A., Chappell, S., Morgan, L., O'Connor, C. M., Morgan, K., & Donnelly, S. C. (2005). Cystic Fibrosis, Disease Severity, and a Macrophage Migration Inhibitory Factor Polymorphism. *American Journal of Respiratory and Critical Care Medicine*, *172*(11), 1412–1415. https://doi.org/10.1164/rccm.200412-1714OC

Plant, B. J., Ghani, S., O'Mahony, M. J., Morgan, L., O'Connor, C. M., Morgan, K., Baugh, J. A., & Donnelly, S. C. (2006). Sarcoidosis And MIF Gene Polymorphism: A Case-Control Study In An Irish Population. *European Respiratory Journal*, *29*(2), 325–329. https://doi.org/10.1183/09031936.00129905

Porsbjerg, C., Melén, E., Lehtimäki, L., & Shaw, D. (2023). Asthma. *The Lancet*, *401*(10379), 858–873. https://doi.org/10.1016/S0140-6736(22)02125-0

Poston, R. N., Chanez, P., Lacoste, J. Y., Litchfield, T., Lee, T. H., & Bousquet, J. (1992). Immunohistochemical Characterization of the Cellular Infiltration in Asthmatic Bronchi. *American Review of Respiratory Disease*, *145*(4_pt_1), 918–921. https://doi.org/10.1164/ajrccm/145.4_Pt_1.918

Price, D., Fletcher, M., & van der Molen, T. (2014). Asthma Control And Management In 8,000 European Patients: The Recognise Asthma And Link To Symptoms And Experience (REALISE) Survey. *Npj Primary Care Respiratory Medicine*, *24*(1), 14009. https://doi.org/10.1038/npjpcrm.2014.9

Prieto-Lafuente, L., Gregory, W. F., Allen, J. E., & Maizels, R. M. (2009). MIF Homologues From A Filarial Nematode Parasite Synergize With IL-4 To Induce Alternative Activation Of Host Macrophages. *Journal of Leukocyte Biology*, *85*(5), 844–854. https://doi.org/10.1189/jlb.0808459

Pronier, E., Imanci, A., Selimoglu-Buet, D., Badaoui, B., Itzykson, R., Roger, T., Jego, C., Naimo, A., Francillette, M., Breckler, M., Wagner-Ballon, O., Figueroa, M. E., Aglave, M., Gautheret, D., Porteu, F., Bernard, O. A., Vainchenker, W., Delhommeau, F., Solary, E., & Droin, N. M. (2022). Macrophage Migration Inhibitory Factor Is Overproduced Through EGR1 In TET2low Resting Monocytes. *Communications Biology*, *5*(1), 110. https://doi.org/10.1038/s42003-022-03057-w

Quinn, S. M., Cunningham, K., Raverdeau, M., Walsh, R. J., Curham, L., Malara, A., & Mills, K. H. G. (2019). Anti-inflammatory Trained Immunity Mediated by Helminth Products Attenuates the Induction of T Cell-Mediated Autoimmune Disease. *Frontiers in Immunology*, *10*. https://doi.org/10.3389/fimmu.2019.01109

Rabani, R., Volchuk, A., Jerkic, M., Ormesher, L., Garces-Ramirez, L., Canton, J., Masterson, C., Gagnon, S., Tatham, K. C., Marshall, J., Grinstein, S., Laffey, J. G., Szaszi, K., & Curley, G. F. (2018). Mesenchymal Stem Cells Enhance NOX2-Dependent Reactive Oxygen Species Production And Bacterial Killing In Macrophages During Sepsis. *European Respiratory Journal*, *51*(4), 1702021. https://doi.org/10.1183/13993003.02021-2017

Rabe, K. F., FitzGerald, J. M., Bateman, E. D., Castro, M., Pavord, I. D., Maspero, J. F., Busse, W. W., Izuhara, K., Daizadeh, N., Ortiz, B., Pandit-Abid, N., Rowe, P. J., & Deniz, Y. (2022). Dupilumab Is Effective in Patients With Moderate-to-Severe Uncontrolled GINA-Defined Type 2 Asthma Irrespective of an Allergic Asthma Phenotype. *The Journal of Allergy and Clinical Immunology: In Practice*, *10*(11), 2916-2924.e4. https://doi.org/10.1016/j.jaip.2022.06.036

Ramonell, R. P., Runnstrom, M. C., Yang, P., Schulman, D. A., & Lee, F. E.-H. (2020). Wiping Out Wheezing: Novel Therapeutic Targets for Patients with Severe Asthma. *American Journal of Respiratory and Critical Care Medicine*, *202*(11), 1576–1578. https://doi.org/10.1164/rccm.202005-1797RR

Rath, M., MÃ¼ller, I., Kropf, P., Closs, E. I., & Munder, M. (2014). Metabolism via Arginase or Nitric Oxide Synthase: Two Competing Arginine Pathways in Macrophages. *Frontiers in Immunology*, *5*. https://doi.org/10.3389/fimmu.2014.00532

Ravenel, M. P. (1928). La Vaccination Préventive Contre la Tuberculose par le "BCG." *American Journal of Public Health and the Nations Health*, *18*(8), 1075–1075. https://doi.org/10.2105/AJPH.18.8.1075-a

Reddel, H. K., FitzGerald, J. M., Bateman, E. D., Bacharier, L. B., Becker, A., Brusselle, G., Buhl, R., Cruz, A. A., Fleming, L., Inoue, H., Ko, F. W., Krishnan, J. A., Levy, M. L., Lin, J., Pedersen, S. E., Sheikh, A., Yorgancioglu, A., & Boulet, L.-P. (2019). GINA 2019: A Fundamental Change In Asthma Management. *European Respiratory Journal*, *53*(6), 1901046. https://doi.org/10.1183/13993003.01046-2019

Rendon, B. E., Roger, T., Teneng, I., Zhao, M., Al-Abed, Y., Calandra, T., & Mitchell, R. A. (2007). Regulation of Human Lung Adenocarcinoma Cell Migration and Invasion by Macrophage Migration Inhibitory Factor. *Journal of Biological Chemistry*, *282*(41), 29910–29918. https://doi.org/10.1074/jbc.M704898200

Renner, A., Marth, K., Breyer-Kohansal, R., & Pohl, W. (2023). Combined Analysis Of Five Non-Interventional Studies Of The Effectiveness, Tolerability, And Safety Of The Extrafine Fixed Dose Beclomethasone/Formoterol Combination In The Treatment Of Asthma In Austria. *Respiratory Medicine*, *207*, 107097. https://doi.org/10.1016/j.rmed.2022.107097

Reza, A. M. M. T., Choi, Y.-J., Yasuda, H., & Kim, J.-H. (2016). Human Adipose Mesenchymal Stem Cell-Derived Exosomal-Mirnas Are Critical Factors For Inducing Anti-Proliferation Signalling To

A2780 And SKOV-3 Ovarian Cancer Cells. *Scientific Reports*, *6*(1), 38498. https://doi.org/10.1038/srep38498

Ripamonti, C., Spadotto, V., Pozzi, P., Stevenazzi, A., Vergani, B., Marchini, M., Sandrone, G., Bonetti, E., Mazzarella, L., Minucci, S., Steinkühler, C., & Fossati, G. (2022). HDAC Inhibition as Potential Therapeutic Strategy to Restore the Deregulated Immune Response in Severe COVID-19. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.841716

Riquelme, P., Tomiuk, S., Kammler, A., Fändrich, F., Schlitt, H. J., Geissler, E. K., & Hutchinson, J. A. (2013). IFN-γ-induced iNOS Expression in Mouse Regulatory Macrophages Prolongs Allograft Survival in Fully Immunocompetent Recipients. *Molecular Therapy*, *21*(2), 409–422. https://doi.org/10.1038/mt.2012.168

Rodrigues, H. C., Martins, C., Fragoso, E., Lopes, C., & Azevedo, P. (2023). Mepolizumab In Severe Asthma Exacerbation In A Respiratory ICU—A Successful Off-Label Use. *Pulmonology*. https://doi.org/10.1016/j.pulmoe.2023.03.002

Rodrigues, S. de O., Cunha, C. M. C. da, Soares, G. M. V., Silva, P. L., Silva, A. R., & Gonçalves-de-Albuquerque, C. F. (2021). Mechanisms, Pathophysiology and Currently Proposed Treatments of Chronic Obstructive Pulmonary Disease. *Pharmaceuticals*, *14*(10), 979. https://doi.org/10.3390/ph14100979

Rodríguez-Sosa, M., Rosas, L. E., David, J. R., Bojalil, R., Satoskar, A. R., & Terrazas, L. I. (2003). Macrophage Migration Inhibitory Factor Plays a Critical Role in Mediating Protection against the Helminth Parasite *Taenia crassiceps*. *Infection and Immunity*, *71*(3), 1247–1254. https://doi.org/10.1128/IAI.71.3.1247-1254.2003

Roger, T., David, J., Glauser, M. P., & Calandra, T. (2001). MIF Regulates Innate Immune Responses Through Modulation Of Toll-Like Receptor 4. *Nature*, *414*(6866), 920–924. https://doi.org/10.1038/414920a

Roger, T., Froidevaux, C., Martin, C., & Calandra, T. (2003). Macrophage Migration Inhibitory Factor (MIF) Regulates Host Responses To Endotoxin Through Modulation Of Toll-Like Receptor 4 (TLR4). *Journal of Endotoxin Research*, *9*(2), 119–123. https://doi.org/10.1179/096805103125001513

Roger, T., Glauser, M. P., & Calandra, T. (2001). Macrophage Migration Inhibitory Factor (MIF) Modulates Innate Immune Responses Induced By Endotoxin And Gram-Negative Bacteria. *Journal of Endotoxin Research*, *7*(6), 456–460.

Roger, T., Lugrin, J., Le Roy, D., Goy, G., Mombelli, M., Koessler, T., Ding, X. C., Chanson, A.-L., Reymond, M. K., Miconnet, I., Schrenzel, J., François, P., & Calandra, T. (2011). Histone Deacetylase Inhibitors Impair Innate Immune Responses To Toll-Like Receptor Agonists And To Infection. *Blood*, *117*(4), 1205–1217. https://doi.org/10.1182/blood-2010-05-284711

Roger, T., Schneider, A., Weier, M., Sweep, F. C. G. J., Le Roy, D., Bernhagen, J., Calandra, T., & Giannoni, E. (2016). High Expression Levels Of Macrophage Migration Inhibitory Factor Sustain The Innate Immune Responses Of Neonates. *Proceedings of the National Academy of Sciences*, *113*(8). https://doi.org/10.1073/pnas.1514018113

Rolandsson Enes, S., Krasnodembskaya, A. D., English, K., Dos Santos, C. C., & Weiss, D. J. (2021). Research Progress on Strategies that can Enhance the Therapeutic Benefits of Mesenchymal Stromal Cells in Respiratory Diseases With a Specific Focus on Acute Respiratory Distress Syndrome and Other Inflammatory Lung Diseases. *Frontiers in Pharmacology*, *12*. https://doi.org/10.3389/fphar.2021.647652

Rosenberg, H. F., Dyer, K. D., & Foster, P. S. (2013). Eosinophils: Changing Perspectives In Health And Disease. *Nature Reviews Immunology*, *13*(1), 9–22. https://doi.org/10.1038/nri3341

Rosengren, E., Åman, P., Thelin, S., Hansson, C., Ahlfors, S., Björk, P., Jacobsson, L., & Rorsman, H. (1997). The Macrophage Migration Inhibitory Factor MIF Is A Phenylpyruvate Tautomerase. *FEBS Letters*, *417*(1), 85–88. https://doi.org/10.1016/S0014-5793(97)01261-1

Rossello-Gelabert, M., Gonzalez-Pujana, A., Igartua, M., Santos-Vizcaino, E., & Hernandez, R. M. (2022). Clinical Progress In MSC-Based Therapies For The Management Of Severe COVID-19. *Cytokine & Growth Factor Reviews*, *68*, 25–36. https://doi.org/10.1016/j.cytogfr.2022.07.002

Rossi, A. G., Haslett, C., Hirani, N., Greening, A. P., Rahman, I., Metz, C. N., Bucala, R., & Donnelly, S. C. (1998). Human Circulating Eosinophils Secrete Macrophage Migration Inhibitory Factor

(MIF). Potential Role In Asthma. *Journal of Clinical Investigation*, *101*(12), 2869–2874. https://doi.org/10.1172/JCI1524

Russell, K. E., Chung, K. F., Clarke, C. J., Durham, A. L., Mallia, P., Footitt, J., Johnston, S. L., Barnes, P. J., Hall, S. R., Simpson, K. D., Starkey, M. R., Hansbro, P. M., Adcock, I. M., & Wiegman, C. H. (2016). The MIF Antagonist ISO-1 Attenuates Corticosteroid-Insensitive Inflammation and Airways Hyperresponsiveness in an Ozone-Induced Model of COPD. *PLOS ONE*, *11*(1), e0146102. https://doi.org/10.1371/journal.pone.0146102

Sabogal Piñeros, Y. S., Bal, S. M., Dijkhuis, A., Majoor, C. J., Dierdorp, B. S., Dekker, T., Hoefsmit, E. P., Bonta, P. I., Picavet, D., van der Wel, N. N., Koenderman, L., Sterk, P. J., Ravanetti, L., & Lutter, R. (2019). Eosinophils Capture Viruses, A Capacity That Is Defective In Asthma. *Allergy*, *74*(10), 1898–1909. https://doi.org/10.1111/all.13802

Samarasinghe, A. E., Melo, R. C. N., Duan, S., LeMessurier, K. S., Liedmann, S., Surman, S. L., Lee, J. J., Hurwitz, J. L., Thomas, P. G., & McCullers, J. A. (2017). Eosinophils Promote Antiviral Immunity in Mice Infected with Influenza A Virus. *The Journal of Immunology*, *198*(8), 3214–3226. https://doi.org/10.4049/jimmunol.1600787

Samarelli, A. V., Tonelli, R., Heijink, I., Martin Medina, A., Marchioni, A., Bruzzi, G., Castaniere, I., Andrisani, D., Gozzi, F., Manicardi, L., Moretti, A., Cerri, S., Fantini, R., Tabbì, L., Nani, C., Mastrolia, I., Weiss, D. J., Dominici, M., & Clini, E. (2021). Dissecting the Role of Mesenchymal Stem Cells in Idiopathic Pulmonary Fibrosis: Cause or Solution. *Frontiers in Pharmacology*, *12*. https://doi.org/10.3389/fphar.2021.692551

Sampey, A. V., Hall, P. H., Mitchell, R. A., Metz, C. N., & Morand, E. F. (2001). Regulation Of Synoviocyte Phospholipase A2 And Cyclooxygenase 2 By Macrophage Migration Inhibitory Factor. *Arthritis & Rheumatism*, *44*(6), 1273–1280. https://doi.org/10.1002/1529-0131(200106)44:6<1273::AID-ART219>3.0.CO;2-8

Sánchez-Zamora, Y. I., & Rodriguez-Sosa, M. (2014). The Role of MIF in Type 1 and Type 2 Diabetes Mellitus. *Journal of Diabetes Research*, *2014*, 1–6. https://doi.org/10.1155/2014/804519

Sánchez-Zuno, G. A., Bucala, R., Hernández-Bello, J., Román-Fernández, I. V., García-Chagollán, M., Nicoletti, F., Matuz-Flores, M. G., García-Arellano, S., Esparza-Michel, J. A., Cerpa-Cruz, S., Pérez-Guerrero, E. E., & Muñoz-Valle, J. F. (2021). Canonical (CD74/CD44) and Non-Canonical (CXCR2, 4 and 7) MIF Receptors Are Differentially Expressed in Rheumatoid Arthritis Patients Evaluated by DAS28-ESR. *Journal of Clinical Medicine*, *11*(1), 120. https://doi.org/10.3390/jcm11010120

Sarwar, M. (2020). House Dust Mites: Ecology, Biology, Prevalence, Epidemiology and Elimination. In *Parasitology and Microbiology Research*. IntechOpen. https://doi.org/10.5772/intechopen.91891

Satoskar, A. R., Bozza, M., Rodriguez Sosa, M., Lin, G., & David, J. R. (2001). Migration-Inhibitory Factor Gene-Deficient Mice Are Susceptible to Cutaneous *Leishmania major* Infection. *Infection and Immunity*, *69*(2), 906–911. https://doi.org/10.1128/IAI.69.2.906-911.2001

Sauler, M., Leng, L., Trentalange, M., Haslip, M., Shan, P., Piecychna, M., Zhang, Y., Andrews, N., Mannam, P., Allore, H., Fried, T., Bucala, R., & Lee, P. J. (2014). Macrophage Migration Inhibitory Factor Deficiency In Chronic Obstructive Pulmonary Disease. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *306*(6), L487–L496. https://doi.org/10.1152/ajplung.00284.2013

Schlinkert, P., Casals, E., Boyles, M., Tischler, U., Hornig, E., Tran, N., Zhao, J., Himly, M., Riediker, M., Oostingh, G. J., Puntes, V., & Duschl, A. (2015). The Oxidative Potential Of Differently Charged Silver And Gold Nanoparticles On Three Human Lung Epithelial Cell Types. *Journal of Nanobiotechnology*, *13*(1), 1. https://doi.org/10.1186/s12951-014-0062-4

Schwartz, V., Lue, H., Kraemer, S., Korbiel, J., Krohn, R., Ohl, K., Bucala, R., Weber, C., & Bernhagen, J. (2009). A Functional Heteromeric MIF Receptor Formed By CD74 And CXCR4. *FEBS Letters*, *583*(17), 2749–2757. https://doi.org/10.1016/j.febslet.2009.07.058

Scott, G., Asrat, S., Allinne, J., Keat Lim, W., Nagashima, K., Birchard, D., Srivatsan, S., Ajithdoss, D. K., Oyejide, A., Ben, L.-H., Walls, J., Le Floc'h, A., Yancopoulos, G. D., Murphy, A. J., Sleeman, M. A., & Orengo, J. M. (2023). IL-4 And IL-13, Not Eosinophils, Drive Type 2 Airway Inflammation, Remodeling And Lung Function Decline. *Cytokine*, *162*, 156091. https://doi.org/10.1016/j.cyto.2022.156091

Selleri, S., Bifsha, P., Civini, S., Pacelli, C., Dieng, M. M., Lemieux, W., Jin, P., Bazin, R., Patey, N., Marincola, F. M., Moldovan, F., Zaouter, C., Trudeau, L.-E., Benabdhalla, B., Louis, I., Beauséjour, C., Stroncek, D., Le Deist, F., & Haddad, E. (2016). Human Mesenchymal Stromal Cell-Secreted Lactate Induces M2-Macrophage Differentiation By Metabolic Reprogramming. *Oncotarget*, *7*(21), 30193–30210. https://doi.org/10.18632/oncotarget.8623

Sensebé, L., & Fleury-Cappellesso, S. (2013). Biodistribution Of Mesenchymal Stem/Stromal Cells In A Preclinical Setting. *Stem Cells International*, *2013*, 1–5. https://doi.org/10.1155/2013/678063

Senter, P. D., Al-Abed, Y., Metz, C. N., Benigni, F., Mitchell, R. A., Chesney, J., Han, J., Gartner, C. G., Nelson, S. D., Todaro, G. J., & Bucala, R. (2002). Inhibition Of Macrophage Migration Inhibitory Factor (MIF) Tautomerase And Biological Activities By Acetaminophen Metabolites. *Proceedings of the National Academy of Sciences*, *99*(1), 144–149. https://doi.org/10.1073/pnas.011569399

Sethi, G. S., Gracias, D. T., Gupta, R. K., Carr, D., Miki, H., Da Silva Antunes, R., & Croft, M. (2023). <Scp>Anti-CD3</Scp> Inhibits Circulatory And Tissue-Resident Memory <Scp>CD4</Scp> T Cells That Drive Asthma Exacerbations In Mice. *Allergy*. https://doi.org/10.1111/all.15722

Shams, F., Moravvej, H., Hosseinzadeh, S., Mostafavi, E., Bayat, H., Kazemi, B., Bandehpour, M., Rostami, E., Rahimpour, A., & Moosavian, H. (2022). Overexpression Of VEGF In Dermal Fibroblast Cells Accelerates The Angiogenesis And Wound Healing Function: In Vitro And In Vivo Studies. *Scientific Reports*, *12*(1), 18529. https://doi.org/10.1038/s41598-022-23304-8

Shang, S., Wang, J., Chen, S., Tian, R., Zeng, H., Wang, L., Xia, M., Zhu, H., & Zuo, C. (2019). Exosomal Mirna-1231 Derived From Bone Marrow Mesenchymal Stem Cells Inhibits The Activity Of Pancreatic Cancer. *Cancer Medicine*, *8*(18), 7728–7740. https://doi.org/10.1002/cam4.2633

Shi, L., Han, Q., Hong, Y., Li, W., Gong, G., Cui, J., Mao, M., Liang, X., Hu, B., Li, X., Luo, Q., & Zhang, Y. (2021). Inhibition Of Mir-199a-5p Rejuvenates Aged Mesenchymal Stem Cells Derived From Patients With Idiopathic Pulmonary Fibrosis And Improves Their Therapeutic Efficacy In Experimental Pulmonary Fibrosis. *Stem Cell Research & Therapy*, *12*(1), 147. https://doi.org/10.1186/s13287-021-02215-x

Shi, L., Huang, H., Lu, X., Yan, X., Jiang, X., Xu, R., Wang, S., Zhang, C., Yuan, X., Xu, Z., Huang, L., Fu, J.-L., Li, Y., Zhang, Y., Yao, W.-Q., Liu, T., Song, J., Sun, L., Yang, F., … Wang, F.-S. (2021). Effect Of Human Umbilical Cord-Derived Mesenchymal Stem Cells On Lung Damage In Severe COVID-19 Patients: A Randomized, Double-Blind, Placebo-Controlled Phase 2 Trial. *Signal Transduction and Targeted Therapy*, *6*(1), 58. https://doi.org/10.1038/s41392-021-00488-5

Shi, L., Wang, L., Xu, R., Zhang, C., Xie, Y., Liu, K., Li, T., Hu, W., Zhen, C., & Wang, F.-S. (2021). Mesenchymal Stem Cell Therapy For Severe COVID-19. *Signal Transduction and Targeted Therapy*, *6*(1), 339. https://doi.org/10.1038/s41392-021-00754-6

Shi, X., Leng, L., Wang, T., Wang, W., Du, X., Li, J., McDonald, C., Chen, Z., Murphy, J. W., Lolis, E., Noble, P., Knudson, W., & Bucala, R. (2006). CD44 Is the Signaling Component of the Macrophage Migration Inhibitory Factor-CD74 Receptor Complex. *Immunity*, *25*(4), 595–606. https://doi.org/10.1016/j.immuni.2006.08.020

Shimizu, T., Abe, R., Ohkawara, A., & Nishihira, J. (1999). Increased Production Of Macrophage Migration Inhibitory Factor By Pbmcs Of Atopic Dermatitis. *Journal of Allergy and Clinical Immunology*, *104*(3), 659–664. https://doi.org/10.1016/S0091-6749(99)70339-8

Shin, J. J., Fan, W., Par-Young, J., Piecychna, M., Leng, L., Israni-Winger, K., Qing, H., Gu, J., Zhao, H., Schulz, W. L., Unlu, S., Kuster, J., Young, G., Liu, J., Ko, A. I., Baeza Garcia, A., Sauler, M., Wisnewski, A. V, Young, L., Bucala, R. (2022). MIF Is A Common Genetic Determinant Of COVID-19 Symptomatic Infection And Severity. *QJM: An International Journal of Medicine*. https://doi.org/10.1093/qjmed/hcac234

Shin, J. W., Ryu, S., Ham, J., Jung, K., Lee, S., Chung, D. H., Kang, H.-R., & Kim, H. Y. (2021). Mesenchymal Stem Cells Suppress Severe Asthma by Directly Regulating Th2 Cells and Type 2 Innate Lymphoid Cells. *Molecules and Cells*, *44*(8), 580–590. https://doi.org/10.14348/molcells.2021.0101

Shinomiya, S., Naraba, H., Ueno, A., Utsunomiya, I., Maruyama, T., Ohuchida, S., Ushikubi, F., Yuki, K., Narumiya, S., Sugimoto, Y., Ichikawa, A., & Oh-ishi, S. (2001). Regulation Of Tnfα And

Interleukin-10 Production By Prostaglandins I2 And E2: Studies With Prostaglandin Receptor-Deficient Mice And Prostaglandin E-Receptor Subtype-Selective Synthetic Agonists. *Biochemical Pharmacology*, *61*(9), 1153–1160. https://doi.org/10.1016/S0006-2952(01)00586-X

Silva, L. H. A., Antunes, M. A., Dos Santos, C. C., Weiss, D. J., Cruz, F. F., & Rocco, P. R. M. (2018). Strategies To Improve The Therapeutic Effects Of Mesenchymal Stromal Cells In Respiratory Diseases. *Stem Cell Research & Therapy*, *9*(1), 45. https://doi.org/10.1186/s13287-018-0802-8

Silverberg, J. I., Guttman-Yassky, E., Thaçi, D., Irvine, A. D., Stein Gold, L., Blauvelt, A., Simpson, E. L., Chu, C.-Y., Liu, Z., Gontijo Lima, R., Pillai, S. G., & Seneschal, J. (2023). Two Phase 3 Trials of Lebrikizumab for Moderate-to-Severe Atopic Dermatitis. *New England Journal of Medicine*, *388*(12), 1080–1091. https://doi.org/10.1056/NEJMoa2206714

Simms-Williams, N., Nagakumar, P., Thayakaran, R., Adderley, N., Hotham, R., Mansur, A., Nirantharakumar, K., & Haroon, S. (2022). Preventing Unscheduled Hospitalisations From Asthma: A Retrospective Cohort Study Using Routine Primary And Secondary Care Data In The UK (The PUSH-Asthma Study)—Protocol Paper. *BMJ Open*, *12*(8), e058356. https://doi.org/10.1136/bmjopen-2021-058356

Sinitski, D., Kontos, C., Krammer, C., Asare, Y., Kapurniotu, A., & Bernhagen, J. (2019). Macrophage Migration Inhibitory Factor (MIF)-Based Therapeutic Concepts in Atherosclerosis and Inflammation. *Thrombosis and Haemostasis*, *119*(04), 553–566. https://doi.org/10.1055/s-0039-1677803

Smith, C. A., Tyrell, D. J., Kulkarni, U. A., Wood, S., Leng, L., Zemans, R. L., Bucala, R., & Goldstein, D. R. (2019). Macrophage Migration Inhibitory Factor Enhances Influenza-Associated Mortality In Mice. *JCI Insight*, *4*(13). https://doi.org/10.1172/jci.insight.128034

Soh, W. T., Zhang, J., Hollenberg, M. D., Vliagoftis, H., Rothenberg, M. E., Sokol, C. L., Robinson, C., & Jacquet, A. (2023). Protease Allergens As Initiators–Regulators Of Allergic Inflammation. *Allergy*, *78*(5), 1148–1168. https://doi.org/10.1111/all.15678

Sorg, C., Michels, E., Malorny, U., & Neumann, C. (1984). Migration Inhibitory Factors And Macrophage Differentiation. *Springer Seminars in Immunopathology*, *7*(4). https://doi.org/10.1007/BF00201964

Stevens, H. Y., Bowles, A. C., Yeago, C., & Roy, K. (2020). Molecular Crosstalk Between Macrophages and Mesenchymal Stromal Cells. *Frontiers in Cell and Developmental Biology*, *8*. https://doi.org/10.3389/fcell.2020.600160

Stewart, G. A., Boyd, S. M., Bird, C. H., Krska, K. D., Kollinger, M. R., & Thompson, P. J. (1994). Immunobiology Of The Serine Protease Allergens From House Dust Mites. *American Journal of Industrial Medicine*, *25*(1), 105–107. https://doi.org/10.1002/ajim.4700250128

Stockmann, C., Kerdiles, Y., Nomaksteinsky, M., Weidemann, A., Takeda, N., Doedens, A., Torres-Collado, A. X., Iruela-Arispe, L., Nizet, V., & Johnson, R. S. (2010). Loss Of Myeloid Cell-Derived Vascular Endothelial Growth Factor Accelerates Fibrosis. *Proceedings of the National Academy of Sciences*, *107*(9), 4329–4334. https://doi.org/10.1073/pnas.0912766107

Stosic-Grujicic, S., Stojanovic, I., Maksimovic-Ivanic, D., Momcilovic, M., Popadic, D., Harhaji, L., Miljkovic, D., Metz, C., Mangano, K., Papaccio, G., Al-Abed, Y., & Nicoletti, F. (2008). Macrophage Migration Inhibitory Factor (MIF) Is Necessary For Progression Of Autoimmune Diabetes Mellitus. *Journal of Cellular Physiology*, *215*(3), 665–675. https://doi.org/10.1002/jcp.21346

Su, V. Y.-F., Lin, C.-S., Hung, S.-C., & Yang, K.-Y. (2019). Mesenchymal Stem Cell-Conditioned Medium Induces Neutrophil Apoptosis Associated with Inhibition of the NF-κB Pathway in Endotoxin-Induced Acute Lung Injury. *International Journal of Molecular Sciences*, *20*(9), 2208. https://doi.org/10.3390/ijms20092208

Su, Y., Xu, C., Cheng, W., Zhao, Y., Sui, L., & Zhao, Y. (2023). Pretreated Mesenchymal Stem Cells and Their Secretome: Enhanced Immunotherapeutic Strategies. *International Journal of Molecular Sciences*, *24*(2), 1277. https://doi.org/10.3390/ijms24021277

Sugimoto, H., Suzuki, M., Nakagawa, A., Tanaka, I., & Nishihira, J. (1996). Crystal Structure Of Macrophage Migration Inhibitory Factor From Human Å Lymphocyte At 2.1 Å Resolution. *FEBS Letters*, *389*(2), 145–148. https://doi.org/10.1016/0014-5793(96)00553-4

Sugimoto, H., Taniguchi, M., Nakagawa, A., Tanaka, I., Suzuki, M., & Nishihira, J. (1997). Crystallization and Preliminary X-Ray Analysis of Humand-Dopachrome Tautomerase. *Journal of Structural Biology*, *120*(1), 105–108. https://doi.org/10.1006/jsbi.1997.3904

Sugimoto, H., Taniguchi, M., Nakagawa, A., Tanaka, I., Suzuki, M., & Nishihira, J. (1999). Crystal Structure of Human <scp>d</scp> -Dopachrome Tautomerase, a Homologue of Macrophage Migration Inhibitory Factor, at 1.54 Å Resolution ·. *Biochemistry*, *38*(11), 3268–3279. https://doi.org/10.1021/bi982184o

Sun, H. W., Bernhagen, J., Bucala, R., & Lolis, E. (1996). Crystal Structure At 2.6-A Resolution Of Human Macrophage Migration Inhibitory Factor. *Proceedings of the National Academy of Sciences*, *93*(11), 5191–5196. https://doi.org/10.1073/pnas.93.11.5191

Sun, J., Bai, S., Zhao, J., Li, D., Ma, X., Ma, L., & Su, X. (2023). Mapping Knowledge Structure And Research Of The Biologic Treatment Of Asthma: A Bibliometric Study. *Frontiers in Immunology*, *14*. https://doi.org/10.3389/fimmu.2023.1034755

Synek, M., Beasley, R., Frew, A. J., Goulding, D., Holloway, L., Lampe, F. C., Roche, W. R., & Holgate, S. T. (1996). Cellular Infiltration Of The Airways In Asthma Of Varying Severity. *American Journal of Respiratory and Critical Care Medicine*, *154*(1), 224–230. https://doi.org/10.1164/ajrccm.154.1.8680684

Szabłowska-Gadomska, I., Rudziński, S., & Dymowska, M. (2023). Secretome of Mesenchymal Stromal Cells as a Possible Innovative Therapeutic Tool in Facial Nerve Injury Treatment. *BioMed Research International*, *2023*, 1–7. https://doi.org/10.1155/2023/8427200

Szefler, S. J., Roberts, G., Rubin, A. S., Zielen, S., Kuna, P., Alpan, O., Anzures-Cabrera, J., Chen, Q., Holweg, C. T. J., Kaminski, J., Putnam, W. S., Matthews, J. G., & Kamath, N. (2022). Efficacy, Safety, And Tolerability Of Lebrikizumab In Adolescent Patients With Uncontrolled Asthma (ACOUSTICS). *Clinical and Translational Allergy*, *12*(7). https://doi.org/10.1002/clt2.12176

Tang, H., Luo, H., Zhang, Z., & Yang, D. (2022). Mesenchymal Stem Cell-Derived Apoptotic Bodies: Biological Functions and Therapeutic Potential. *Cells*, *11*(23), 3879. https://doi.org/10.3390/cells11233879

Tang, K., Rossiter, H. B., Wagner, P. D., & Breen, E. C. (2004). Lung-Targeted VEGF Inactivation Leads To An Emphysema Phenotype In Mice. *Journal of Applied Physiology*, *97*(4), 1559–1566. https://doi.org/10.1152/japplphysiol.00221.2004

Tanino, Y., Makita, H., Miyamoto, K., Betsuyaku, T., Ohtsuka, Y., Nishihira, J., & Nishimura, M. (2002). Role Of Macrophage Migration Inhibitory Factor In Bleomycin-Induced Lung Injury And Fibrosis In Mice. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *283*(1), L156–L162. https://doi.org/10.1152/ajplung.00155.2001

Tatler, A. L. (2023). Asthmatic Airway Remodeling: Long Overlooked But Too Important To Ignore. *Annals of Translational Medicine*, *11*(2), 29–29. https://doi.org/10.21037/atm-22-5733

The Global Asthma Report 2022. (2022). *The International Journal of Tuberculosis and Lung Disease*, *26*(1), 1–104. https://doi.org/10.5588/ijtld.22.1010

Thelen, J. C., van Zelst, C. M., van Brummelen, S. E., Rauh, S., in 't Veen, J. C. C. M., Kappen, J. H., & Braunstahl, G.-J. (2023). Efficacy And Safety Of Dupilumab As Add-On Therapy For Patients With Severe Asthma: A Real-World Dutch Cohort Study. *Respiratory Medicine*, *206*, 107058. https://doi.org/10.1016/j.rmed.2022.107058

Thiele, M., Donnelly, S. C., & Mitchell, R. A. (2022). Oxmif: A Druggable Isoform Of Macrophage Migration Inhibitory Factor In Cancer And Inflammatory Diseases. *Journal for ImmunoTherapy of Cancer*, *10*(9), e005475. https://doi.org/10.1136/jitc-2022-005475

Thiriou, D., Morianos, I., Xanthou, G., & Samitas, K. (2017). Innate Immunity As The Orchestrator Of Allergic Airway Inflammation And Resolution In Asthma. *International Immunopharmacology*, *48*, 43–54. https://doi.org/10.1016/j.intimp.2017.04.027

Tilstam, P. V., Schulte, W., Holowka, T., Kim, B.-S., Nouws, J., Sauler, M., Piecychna, M., Pantouris, G., Lolis, E., Leng, L., Bernhagen, J., Fingerle-Rowson, G., & Bucala, R. (2021). MIF But Not MIF-2 Recruits Inflammatory Macrophages In An Experimental Polymicrobial Sepsis Model. *Journal of Clinical Investigation*, *131*(23). https://doi.org/10.1172/JCI127171

Tobin, L. M., Healy, M. E., English, K., & Mahon, B. P. (2013). Human Mesenchymal Stem Cells Suppress Donor CD4+ T Cell Proliferation And Reduce Pathology In A Humanized Mouse Model

Of Acute Graft- *Versus* -Host Disease. *Clinical and Experimental Immunology*, *172*(2), 333–348. https://doi.org/10.1111/cei.12056

Toldi, J., Nemeth, D., Hegyi, P., Molnar, Z., Solymar, M., Farkas, N., Alizadeh, H., Rumbus, Z., Pakai, E., & Garami, A. (2021). Macrophage Migration Inhibitory Factor As A Diagnostic And Predictive Biomarker In Sepsis: Meta-Analysis Of Clinical Trials. *Scientific Reports*, *11*(1), 8051. https://doi.org/10.1038/s41598-021-87613-0

Tomita, K., Tanigawa, T., Yajima, H., Fukutani, K., Matsumoto, Y., Tanaka, Y., & Sasaki, T. (1995). Identification And Characterization Of Monocyte Subpopulations From Patients With Bronchial Asthma. *Journal of Allergy and Clinical Immunology*, *96*(2), 230–238. https://doi.org/10.1016/S0091-6749(95)70012-9

Tran, J., Rak, J., Sheehan, C., Saibil, S. D., LaCasse, E., Korneluk, R. G., & Kerbel, R. S. (1999). Marked Induction of the IAP Family Antiapoptotic Proteins Survivin and XIAP by VEGF in Vascular Endothelial Cells. *Biochemical and Biophysical Research Communications*, *264*(3), 781–788. https://doi.org/10.1006/bbrc.1999.1589

Tsitoura, D. C., Blumenthal, R. L., Berry, G., DeKruyff, R. H., & Umetsu, D. T. (2000). Mechanisms Preventing Allergen-Induced Airways Hyperreactivity: Role Of Tolerance And Immune Deviation. *Journal of Allergy and Clinical Immunology*, *106*(2), 239–246. https://doi.org/10.1067/mai.2000.108429

Tu, Y., Guo, R., Li, J., Wang, S., Leng, L., Deng, J., Bucala, R., & Lu, L. (2019). MiRNA Regulation of MIF in SLE and Attenuation of Murine Lupus Nephritis With miR-654. *Frontiers in Immunology*, *10*. https://doi.org/10.3389/fimmu.2019.02229

Tuazon, J. A., Kilburg-Basnyat, B., Oldfield, L. M., Wiscovitch-Russo, R., Dunigan-Russell, K., Fedulov, A. V., Oestreich, K. J., & Gowdy, K. M. (2022). Emerging Insights into the Impact of Air Pollution on Immune-Mediated Asthma Pathogenesis. *Current Allergy and Asthma Reports*, *22*(7), 77–92. https://doi.org/10.1007/s11882-022-01034-1

Tuder, R., & Yun, J. (2008). Vascular endothelial growth factor of the lung: friend or foe. *Current Opinion in Pharmacology*, *8*(3), 255–260. https://doi.org/10.1016/j.coph.2008.03.003

Tynan, A., Mawhinney, L., Armstrong, M. E., O'Reilly, C., Kennedy, S., Caraher, E., Jülicher, K., O'Dwyer, D., Maher, L., Schaffer, K., Fabre, A., McKone, E. F., Leng, L., Bucala, R., Bernhagen, J., Cooke, G., & Donnelly, S. C. (2017). Macrophage Migration Inhibitory Factor Enhances *Pseudomonas Aeruginosa* Biofilm Formation, Potentially Contributing To Cystic Fibrosis Pathogenesis. *The FASEB Journal*, *31*(11), 5102–5110. https://doi.org/10.1096/fj.201700463R

Ullah, M., Liu, D. D., & Thakor, A. S. (2019). Mesenchymal Stromal Cell Homing: Mechanisms and Strategies for Improvement. *IScience*, *15*, 421–438. https://doi.org/10.1016/j.isci.2019.05.004

Valerio, C., Murray, P., Arlian, L., & Slater, J. (2005). Bacterial 16S Ribosomal DNA In House Dust Mite Cultures. *Journal of Allergy and Clinical Immunology*, *116*(6), 1296–1300. https://doi.org/10.1016/j.jaci.2005.09.046

Valero, A., Molina, J., Nuevo, J., Simon, S., Capel, M., Sicras-Mainar, A., Sicras-Navarro, A., & Plaza, V. (2023). Economic Consequences of the Overuse of Short-Acting ß-Adrenergic Agonists in the Treatment of Asthma in Spain. *Journal of Investigational Allergy and Clinical Immunology*, *33*(2), 109–118. https://doi.org/10.18176/jiaci.0767

van Dijk, A., Anten, J., Bakker, A., Evers, N., Hoekstra, A. T., Chang, J.-C., Scheenstra, M. R., Veldhuizen, E. J. A., Netea, M. G., Berkers, C. R., & Haagsman, H. P. (2022). Innate Immune Training of Human Macrophages by Cathelicidin Analogs. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.777530

Varricchi, G., Ferri, S., Pepys, J., Poto, R., Spadaro, G., Nappi, E., Paoletti, G., Virchow, J. C., Heffler, E., & Canonica, W. G. (2022). Biologics And Airway Remodeling In Severe Asthma. *Allergy*, *77*(12), 3538–3552. https://doi.org/10.1111/all.15473

Vasandan, A. B., Jahnavi, S., Shashank, C., Prasad, P., Kumar, A., & Prasanna, S. J. (2016). Human Mesenchymal Stem Cells Program Macrophage Plasticity By Altering Their Metabolic Status Via A PGE2-Dependent Mechanism. *Scientific Reports*, *6*(1), 38308. https://doi.org/10.1038/srep38308

Vieira-de-Abreu, A., Calheiros, A. S., Mesquita-Santos, F. P., Magalhães, E. S., Mourão-Sá, D., Castro-Faria-Neto, H. C., Bozza, M. T., Bandeira-Melo, C., & Bozza, P. T. (2011). Cross-Talk between Macrophage Migration Inhibitory Factor and Eotaxin in Allergic Eosinophil Activation Forms

Leukotriene C $_4$ –Synthesizing Lipid Bodies. *American Journal of Respiratory Cell and Molecular Biology*, *44*(4), 509–516. https://doi.org/10.1165/rcmb.2010-0004OC

Vincent, F. B., Lin, E., Sahhar, J., Ngian, G., Kandane-Rathnayake, R., Mende, R., Hoi, A. Y., Morand, E. F., Lang, T., & Harris, J. (2018). Analysis Of Serum Macrophage Migration Inhibitory Factor And D-Dopachrome Tautomerase In Systemic Sclerosis. *Clinical & Translational Immunology*, *7*(12), e1042. https://doi.org/10.1002/cti2.1042

von Mutius, E. (2009). Gene-Environment Interactions In Asthma. *Journal of Allergy and Clinical Immunology*, *123*(1), 3–11. https://doi.org/10.1016/j.jaci.2008.10.046

Walsh, L. J., Casey, D., Vairamani, P., Arnott, F., Plant, B. J., & Murphy, D. M. (2023). Real-World Clinical Outcomes Of Asthma Patients Switched From Reslizumab To Mepolizumab Or Benralizumab. *Frontiers in Allergy*, *3*. https://doi.org/10.3389/falgy.2022.1052339

Wan, H., Winton, H. L., Soeller, C., Taylor, G. W., Gruenert, D. C., Thompson, P. J., Cannell, M. B., Stewart, G. A., Garrod, D. R., & Robinson, C. (2001). The Transmembrane Protein Occludin Of Epithelial Tight Junctions Is A Functional Target For Serine Peptidases From Faecal Pellets Of *Dermatophagoides Pteronyssinus*. *Clinical & Experimental Allergy*, *31*(2), 279–294. https://doi.org/10.1046/j.1365-2222.2001.00970.x

Wan, H., Winton, H. L., Soeller, C., Tovey, E. R., Gruenert, D. C., Thompson, P. J., Stewart, G. A., Taylor, G. W., Garrod, D. R., Cannell, M. B., & Robinson, C. (1999). Der P 1 Facilitates Transepithelial Allergen Delivery By Disruption Of Tight Junctions. *Journal of Clinical Investigation*, *104*(1), 123–133. https://doi.org/10.1172/JCI5844

Wang, F., Li, Y., Wang, B., Li, J., & Peng, Z. (2023). The Safety And Efficacy Of Mesenchymal Stromal Cells In ARDS: A Meta-Analysis Of Randomized Controlled Trials. *Critical Care*, *27*(1), 31. https://doi.org/10.1186/s13054-022-04287-4

Wang, X., Bove, A. M., Simone, G., & Ma, B. (2020). Molecular Bases of VEGFR-2-Mediated Physiological Function and Pathological Role. *Frontiers in Cell and Developmental Biology*, *8*. https://doi.org/10.3389/fcell.2020.599281

Waters, M., McKinnell, J. A., Kalil, A. C., Martin, G. S., Buchman, T. G., Theess, W., Yang, X., Lekkerkerker, A. N., Staton, T., Rosenberger, C. M., Pappu, R., Wang, Y., Zhang, W., Brooks, L., Cheung, D., Galanter, J., Chen, H., Mohan, D., & Peck, M. C. (2023). Astegolimab or Efmarodocokin Alfa in Patients With Severe COVID-19 Pneumonia: A Randomized, Phase 2 Trial*. *Critical Care Medicine*, *51*(1), 103–116. https://doi.org/10.1097/CCM.0000000000005716

Webb, D. C., McKenzie, A. N. J., & Foster, P. S. (2001). Expression of the Ym2 Lectin-binding Protein Is Dependent on Interleukin (IL)-4 and IL-13 Signal Transduction. *Journal of Biological Chemistry*, *276*(45), 41969–41976. https://doi.org/10.1074/jbc.M106223200

Weiser, W. Y., Temple, P. A., Witek-Giannotti, J. S., Remold, H. G., Clark, S. C., & David, J. R. (1989). Molecular Cloning Of A Cdna Encoding A Human Macrophage Migration Inhibitory Factor. *Proceedings of the National Academy of Sciences*, *86*(19), 7522–7526. https://doi.org/10.1073/pnas.86.19.7522

Weiss, D. J., English, K., Krasnodembskaya, A., Isaza-Correa, J. M., Hawthorne, I. J., & Mahon, B. P. (2019). The Necrobiology of Mesenchymal Stromal Cells Affects Therapeutic Efficacy. *Frontiers in Immunology*, *10*. https://doi.org/10.3389/fimmu.2019.01228

Weiss, D. J., Filiano, A., Galipeau, J., Khoury, M., Krampera, M., Lalu, M., Blanc, K. Le, Nolta, J., Phinney, D. G., Rocco, P. R. M., Shi, Y., Tarte, K., Viswanathan, S., & Martin, I. (2022). An International Society For Cell And Gene Therapy Mesenchymal Stromal Cells Committee Editorial On Overcoming Limitations In Clinical Trials Of Mesenchymal Stromal Cell Therapy For Coronavirus Disease-19: Time For A Global Registry. *Cytotherapy*, *24*(11), 1071–1073. https://doi.org/10.1016/j.jcyt.2022.07.010

Weiss, D. J., Segal, K., Casaburi, R., Hayes, J., & Tashkin, D. (2021). Effect Of Mesenchymal Stromal Cell Infusions On Lung Function In COPD Patients With High CRP Levels. *Respiratory Research*, *22*(1), 142. https://doi.org/10.1186/s12931-021-01734-8

White, M. J. V., Briquez, P. S., White, D. A. V., & Hubbell, J. A. (2021). VEGF-A, PDGF-BB And HB-EGF Engineered For Promiscuous Super Affinity To The Extracellular Matrix Improve Wound Healing In A Model Of Type 1 Diabetes. *Npj Regenerative Medicine*, *6*(1), 76. https://doi.org/10.1038/s41536-021-00189-1

Wick, K. D., Leligdowicz, A., Zhuo, H., Ware, L. B., & Matthay, M. A. (2021). Mesenchymal Stromal Cells Reduce Evidence Of Lung Injury In Patients With ARDS. *JCI Insight*, *6*(12). https://doi.org/10.1172/jci.insight.148983

Wiese, D. M., Wood, C. A., & Braid, L. R. (2022). From Vial to Vein: Crucial Gaps in Mesenchymal Stromal Cell Clinical Trial Reporting. *Frontiers in Cell and Developmental Biology*, *10*. https://doi.org/10.3389/fcell.2022.867426

Wilson, A. J., Rand, E., Webster, A. J., & Genever, P. G. (2021). Characterisation Of Mesenchymal Stromal Cells In Clinical Trial Reports: Analysis Of Published Descriptors. *Stem Cell Research & Therapy*, *12*(1), 360. https://doi.org/10.1186/s13287-021-02435-1

Wolterink, R. G. J. K., KleinJan, A., van Nimwegen, M., Bergen, I., de Bruijn, M., Levani, Y., & Hendriks, R. W. (2012). Pulmonary Innate Lymphoid Cells Are Major Producers Of IL-5 And IL-13 In Murine Models Of Allergic Asthma. *European Journal of Immunology*, *42*(5), 1106–1116. https://doi.org/10.1002/eji.201142018

Wright, A., Arthaud-Day, M. L., & Weiss, M. L. (2021). Therapeutic Use of Mesenchymal Stromal Cells: The Need for Inclusive Characterization Guidelines to Accommodate All Tissue Sources and Species. *Frontiers in Cell and Developmental Biology*, *9*. https://doi.org/10.3389/fcell.2021.632717

Xia, J., Minamino, S., Kuwabara, K., & Arai, S. (2019). Stem Cell Secretome As A New Booster For Regenerative Medicine. *BioScience Trends*, *13*(4), 299–307. https://doi.org/10.5582/bst.2019.01226

Xia, W., & Hou, M. (2017). Macrophage Migration Inhibitory Factor Rescues Mesenchymal Stem Cells From Doxorubicin-Induced Senescence Though The PI3K-Akt Signaling Pathway. *International Journal of Molecular Medicine*. https://doi.org/10.3892/ijmm.2017.3282

Xia, W., Xie, C., Jiang, M., & Hou, M. (2015). Improved Survival Of Mesenchymal Stem Cells By Macrophage Migration Inhibitory Factor. *Molecular and Cellular Biochemistry*, *404*(1–2), 11–24. https://doi.org/10.1007/s11010-015-2361-y

Xia, W., Zhang, F., Xie, C., Jiang, M., & Hou, M. (2015). Macrophage Migration Inhibitory Factor Confers Resistance To Senescence Through CD74-Dependent AMPK-FOXO3a Signaling In Mesenchymal Stem Cells. *Stem Cell Research & Therapy*, *6*(1), 82. https://doi.org/10.1186/s13287-015-0076-3

Xia, W., Zhuang, L., & Hou, M. (2018). Role Of Lincrna-P21 In The Protective Effect Of Macrophage Inhibition Factor Against Hypoxia/Serum Deprivation-Induced Apoptosis In Mesenchymal Stem Cells. *International Journal of Molecular Medicine*. https://doi.org/10.3892/ijmm.2018.3767

Xie, Z., Hao, H., Tong, C., Cheng, Y., Liu, J., Pang, Y., Si, Y., Guo, Y., Zang, L., Mu, Y., & Han, W. (2016). Human Umbilical Cord-Derived Mesenchymal Stem Cells Elicit Macrophages Into An Anti-Inflammatory Phenotype To Alleviate Insulin Resistance In Type 2 Diabetic Rats. *Stem Cells*, *34*(3), 627–639. https://doi.org/10.1002/stem.2238

Xu, R., Feng, Z., & Wang, F.-S. (2022). Mesenchymal stem cell treatment for COVID-19. *EBioMedicine*, *77*, 103920. https://doi.org/10.1016/j.ebiom.2022.103920

Xu, X., Mann, M., Qiao, D., Li, Y., Zhou, J., & Brasier, A. R. (2021). Bromodomain Containing Protein 4 (BRD4) Regulates Expression of its Interacting Coactivators in the Innate Response to Respiratory Syncytial Virus. *Frontiers in Molecular Biosciences*, *8*. https://doi.org/10.3389/fmolb.2021.728661

Xu, Y., Zhang, Z., He, J., & Chen, Z. (2022). Immune Effects of Macrophages in Rheumatoid Arthritis: A Bibliometric Analysis From 2000 to 2021. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.903771

Yamaguchi, Nishihira, Shimizu, Takahashi, Kitashiro, Hizawa, Kamishima, & Kawakami. (2000). Macrophage Migration Inhibitory Factor (MIF) In Bronchial Asthma. *Clinical & Experimental Allergy*, *30*(9), 1244–1249. https://doi.org/10.1046/j.1365-2222.2000.00888.x

Yaneva, M., & Darlenski, R. (2021). The Link Between Atopic Dermatitis And Asthma- Immunological Imbalance And Beyond. *Asthma Research and Practice*, *7*(1), 16. https://doi.org/10.1186/s40733-021-00082-0

Yang, S., Liu, P., Jiang, Y., Wang, Z., Dai, H., & Wang, C. (2021). Therapeutic Applications of Mesenchymal Stem Cells in Idiopathic Pulmonary Fibrosis. *Frontiers in Cell and Developmental Biology*, *9*. https://doi.org/10.3389/fcell.2021.639657

Yao, W., Shi, L., Zhang, Y., Dong, H., & Zhang, Y. (2022). Mesenchymal Stem/Stromal Cell Therapy For COVID-19 Pneumonia: Potential Mechanisms, Current Clinical Evidence, And Future Perspectives. *Stem Cell Research & Therapy*, *13*(1), 124. https://doi.org/10.1186/s13287-022-02810-6

Yao, X., Jin, G., Liu, D., Zhang, X., Yang, Y., chen, Y., Duan, Z., Bi, Y., Yan, F., Yang, Y., Zhang, H., Dong, G., Li, S., Cheng, S., Tang, H., Hong, F., & Si, C. (2022). Inducible Nitric Oxide Synthase Regulates Macrophage Polarization Via The MAPK Signals In Concanavalin A-Induced Hepatitis. *Immunity, Inflammation and Disease*, *10*(7). https://doi.org/10.1002/iid3.643

Yao, Y., Fan, X.-L., Jiang, D., Zhang, Y., Li, X., Xu, Z.-B., Fang, S.-B., Chiu, S., Tse, H.-F., Lian, Q., & Fu, Q.-L. (2018). Connexin 43-Mediated Mitochondrial Transfer of iPSC-MSCs Alleviates Asthma Inflammation. *Stem Cell Reports*, *11*(5), 1120–1135. https://doi.org/10.1016/j.stemcr.2018.09.012

Yin, K., Wang, S., & Zhao, R. C. (2019). Exosomes From Mesenchymal Stem/Stromal Cells: A New Therapeutic Paradigm. *Biomarker Research*, *7*(1), 8. https://doi.org/10.1186/s40364-019-0159-x

Yoo, S.-A., Leng, L., Kim, B.-J., Du, X., Tilstam, P. V., Kim, K. H., Kong, J.-S., Yoon, H.-J., Liu, A., Wang, T., Song, Y., Sauler, M., Bernhagen, J., Ritchlin, C. T., Lee, P., Cho, C.-S., Kim, W.-U., & Bucala, R. (2016). *MIF* Allele-Dependent Regulation Of The MIF Coreceptor CD44 And Role In Rheumatoid Arthritis. *Proceedings of the National Academy of Sciences*, *113*(49). https://doi.org/10.1073/pnas.1612717113

Yousuf, A. J., Mohammed, S., Carr, L., Yavari Ramsheh, M., Micieli, C., Mistry, V., Haldar, K., Wright, A., Novotny, P., Parker, S., Glover, S., Finch, J., Quann, N., Brookes, C. L., Hobson, R., Ibrahim, W., Russell, R. J., John, C., Grimbaldeston, M. A., … Brightling, C. E. (2022). Astegolimab, An Anti-ST2, In Chronic Obstructive Pulmonary Disease (COPD-ST2OP): A Phase 2a, Placebo-Controlled Trial. *The Lancet Respiratory Medicine*, *10*(5), 469–477. https://doi.org/10.1016/S2213-2600(21)00556-7

Yu, X., Yu, L., Guo, B., Chen, R., & Qiu, C. (2020). A Narrative Review Of Research Advances In Mesenchymal Stem Cell Therapy For Asthma. *Annals of Translational Medicine*, *8*(21), 1461–1461. https://doi.org/10.21037/atm-20-6389

Yuan, Y., Yuan, L., Li, L., Liu, F., Liu, J., Chen, Y., Cheng, J., & Lu, Y. (2021). Mitochondrial Transfer from Mesenchymal Stem Cells to Macrophages Restricts Inflammation and Alleviates Kidney Injury in Diabetic Nephropathy Mice via PGC-1α Activation. *Stem Cells*, *39*(7), 913–928. https://doi.org/10.1002/stem.3375

Zahalka, S., Starkl, P., Watzenboeck, M. L., Farhat, A., Radhouani, M., Deckert, F., Hladik, A., Lakovits, K., Oberndorfer, F., Lassnig, C., Strobl, B., Klavins, K., Matsushita, M., Sanin, D. E., Grzes, K. M., Pearce, E. J., Gorki, A.-D., & Knapp, S. (2022). Trained Immunity Of Alveolar Macrophages Requires Metabolic Rewiring And Type 1 Interferon Signaling. *Mucosal Immunology*, *15*(5), 896–907. https://doi.org/10.1038/s41385-022-00528-5

Zeng, Z., Huang, H., Zhang, J., Liu, Y., Zhong, W., Chen, W., Lu, Y., Qiao, Y., Zhao, H., Meng, X., Zou, F., Cai, S., & Dong, H. (2022). HDM Induce Airway Epithelial Cell Ferroptosis And Promote Inflammation By Activating Ferritinophagy In Asthma. *The FASEB Journal*, *36*(6). https://doi.org/10.1096/fj.202101977RR

Zhang, C., Ramsey, C., Berical, A., Yu, L., Leng, L., McGinnis, K. A., Song, Y., Michael, H., McCormack, M. C., Allore, H., Morris, A., Crothers, K., Bucala, R., Lee, P. J., & Sauler, M. (2019). A Functional Macrophage Migration Inhibitory Factor Promoter Polymorphism Is Associated With Reduced Diffusing Capacity. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *316*(2), L400–L405. https://doi.org/10.1152/ajplung.00439.2018

Zhang, X., Wei, X., Deng, Y., Yuan, X., Shi, J., Huang, W., Huang, J., Chen, X., Zheng, S., Chen, J., Chen, K., Xu, R., Wang, H., Li, W., Li, S., Yi, H., & Xiang, A. P. (2022). Mesenchymal Stromal Cells Alleviate Acute Respiratory Distress Syndrome Through The Cholinergic Anti-Inflammatory Pathway. *Signal Transduction and Targeted Therapy*, *7*(1), 307. https://doi.org/10.1038/s41392-022-01124-6

Zhang, Y., Xu, L., Zhang, Y., Pan, J., Wang, P. qing, Tian, S., Li, H. ting, Gao, B. wen, Hou, T. jun, Zhen, X. chu, & Zheng, L. T. (2022). Discovery Of Novel MIF Inhibitors That Attenuate Microglial Inflammatory Activation By Structures-Based Virtual Screening And In Vitro

Bioassays. *Acta Pharmacologica Sinica*, *43*(6), 1508–1520. https://doi.org/10.1038/s41401-021-00753-x

Zhang, Y., Zhou, Y., Chen, S., Hu, Y., Zhu, Z., Wang, Y., Du, N., Song, T., Yang, Y., Guo, A., & Wang, Y. (2019). Macrophage Migration Inhibitory Factor Facilitates Prostaglandin E2 Production Of Astrocytes To Tune Inflammatory Milieu Following Spinal Cord Injury. *Journal of Neuroinflammation*, *16*(1), 85. https://doi.org/10.1186/s12974-019-1468-6

Zhang, Y., Zhu, W., He, H., Fan, B., Deng, R., Hong, Y., Liang, X., Zhao, H., Li, X., & Zhang, F. (2019). Macrophage Migration Inhibitory Factor Rejuvenates Aged Human Mesenchymal Stem Cells And Improves Myocardial Repair. *Aging*, *11*(24), 12641–12660. https://doi.org/10.18632/aging.102592

Zhang, Z., Zhou, X., Guo, J., Zhang, F., Qian, Y., Wang, G., Duan, M., Wang, Y., Zhao, H., Yang, Z., Liu, Z., & Jiang, X. (2022). TA-Mscs, TA-Mscs-Evs, MIF: Their Crosstalk In Immunosuppressive Tumor Microenvironment. *Journal of Translational Medicine*, *20*(1), 320. https://doi.org/10.1186/s12967-022-03528-y

Zhao, J., Lv, Z., Wang, F., Wei, J., Zhang, Q., Li, S., Yang, F., Zeng, X., Wu, X., & Wu, Z. (2013). Ym1, An Eosinophilic Chemotactic Factor, Participates In The Brain Inflammation Induced By Angiostrongylus Cantonensis In Mice. *Parasitology Research*, *112*(7), 2689–2695. https://doi.org/10.1007/s00436-013-3436-x

Zhao, J., Zhu, H., Wong, C. H., Leung, K. Y., & Wong, W. S. F. (2005). Increased Lungkine And Chitinase Levels In Allergic Airway Inflammation: A Proteomics Approach. *PROTEOMICS*, *5*(11), 2799–2807. https://doi.org/10.1002/pmic.200401169

Zhou, B.-R., Xu, Y., Guo, S.-L., Xu, Y., Wang, Y., Zhu, F., Permatasari, F., Wu, D., Yin, Z.-Q., & Luo, D. (2013). The Effect Of Conditioned Media Of Adipose-Derived Stem Cells On Wound Healing After Ablative Fractional Carbon Dioxide Laser Resurfacing. *BioMed Research International*, *2013*, 1–9. https://doi.org/10.1155/2013/519126

Zhou, Y., Zhu, X., Cui, H., Shi, J., Yuan, G., Shi, S., & Hu, Y. (2021). The Role of the VEGF Family in Coronary Heart Disease. *Frontiers in Cardiovascular Medicine*, *8*. https://doi.org/10.3389/fcvm.2021.738325

Zhou, Z., Hua, Y., Ding, Y., Hou, Y., Yu, T., Cui, Y., & Nie, H. (2021). Conditioned Medium of Bone Marrow Mesenchymal Stem Cells Involved in Acute Lung Injury by Regulating Epithelial Sodium Channels via miR-34c. *Frontiers in Bioengineering and Biotechnology*, *9*. https://doi.org/10.3389/fbioe.2021.640116

Zhu, W., Sun, L., Zhao, P., Liu, Y., Zhang, J., Zhang, Y., Hong, Y., Zhu, Y., Lu, Y., Zhao, W., Chen, X., & Zhang, F. (2021). Macrophage Migration Inhibitory Factor Facilitates The Therapeutic Efficacy Of Mesenchymal Stem Cells Derived Exosomes In Acute Myocardial Infarction Through Upregulating Mir-133a-3p. *Journal of Nanobiotechnology*, *19*(1), 61. https://doi.org/10.1186/s12951-021-00808-5

Zhuang, L., Xia, W., Chen, D., Ye, Y., Hu, T., Li, S., & Hou, M. (2020). Exosomal Lncrna–NEAT1 Derived From MIF-Treated Mesenchymal Stem Cells Protected Against Doxorubicin-Induced Cardiac Senescence Through Sponging Mir-221-3p. *Journal of Nanobiotechnology*, *18*(1), 157. https://doi.org/10.1186/s12951-020-00716-0

Zhuang, L., Xia, W., & Hou, M. (2018). Role Of Lincrna-P21 In The Protective Effect Of Macrophage Inhibition Factor Against Hypoxia/Serum Deprivation-Induced Apoptosis In Mesenchymal Stem Cells. *International Journal of Molecular Medicine*. https://doi.org/10.3892/ijmm.2018.3767

Chapter 7:

Appendices

cells

MDPI

Review

The Inflammatory Lung Microenvironment; a Key Mediator in MSC Licensing

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Abstract: Recent clinical trials of mesenchymal stromal cell (MSC) therapy for various inflammatory conditions have highlighted the significant benefit to patients who respond to MSC administration. Thus, there is strong interest in investigating MSC therapy in acute inflammatory lung conditions, such as acute respiratory distress syndrome (ARDS). Unfortunately, not all patients respond, and evidence now suggests that the differential disease microenvironment present across patients and sub-phenotypes of disease or across disease severities influences MSC licensing, function and therapeutic efficacy. Here, we discuss the importance of licensing MSCs and the need to better understand how the disease microenvironment influences MSC activation and therapeutic actions, in addition to the need for a patient-stratification approach.

Keywords: MSC; licensing; lung; microenvironment; inflammatory; cytokines; immunomodulatory

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1. Introduction

The morbidity and mortality associated with acute respiratory disease have never been more prominent than during the COVID-19 pandemic. In particular, the lack of therapeutics for treating lung inflammatory conditions including acute respiratory distress syndrome (ARDS) have highlighted the urgent unmet need for new therapeutic approaches. Mesenchymal stromal cells (MSCs) derived from both mouse and human bone marrow (BM), human umbilical cord (UC), adipose tissue (AT) and amniotic (A) tissue have shown positive outcomes in a broad spectrum of lung diseases [1] in preclinical studies, including asthma [2,3], idiopathic pulmonary fibrosis (IPF) [4–6], chronic obstructive pulmonary disease (COPD) [7,8], acute lung injury (ALI) [9–11], and acute respiratory distress syndrome (ARDS) [12–14]. Clinical trials have investigated MSC therapy (BM and UC) in IPF [15], COPD [16–18], ARDS [19,20] and in COVID-19 associated ARDS [21] showing safety but have not yet shown efficacy. While MSCs (BM or AT) have been approved in some countries for use in treating acute graft versus host disease (aGvHD) [22–24] and for Crohn's fistula [25,26], MSC therapy has not yet been approved for lung inflammatory disorders [27]. However, MSCs have a proven safety profile in clinical trials [27] for inflammatory lung disorders and there are currently 151 clinical trials investigating MSCs as a lung intervention (https://ClinicalTrials.gov/, accessed on 5 October 2021), many of these for COVID-19-associated ARDS. While significant progress has been made in understanding the mechanisms by which MSC mediate their anti-inflammatory and

pro-reparative effects in vitro, there are gaps in our understanding of how MSCs mediate their therapeutic effects in vivo [28,29]. A significant body of work has clearly identified the requirement for MSCs to be activated or licensed by signals such as pro-inflammatory cytokines to mediate MSC therapeutic effects [28,30]. It is becoming increasingly apparent that the disease inflammatory environment into which MSCs are administered is of critical importance for MSC capacity to suppress inflammation and promote repair [27,28]. The success of clinical application of MSC administration is limited by unclear understanding of the role of the microenvironment on MSC capacity to suppress inflammation and modulate immune responses. In the context of inflammatory lung disorders such as ARDS, there is now a general consensus that hyper- and hypo- inflammatory traits can be identified in ARDS patients [31]. Similarly, there are significant differences between the lung microenvironments of mild, moderate and severe asthmatics [32]. Thus, a better understanding of how the disease microenvironment may influence MSC therapeutic efficacy would help to identify the patients in which MSC therapy may be of most benefit. Herein, we provide an overview of our current understanding of how the disease microenvironment impacts MSC therapeutic efficacy and potential strategies to license or activate the anti-inflammatory and pro-reparative functions of MSCs. Where possible, we will focus on studies in inflammatory lung disease as well as evidence from other inflammatory conditions that might help us to better understand the role of the disease microenvironment and the potential for licensing to impact on MSC protective effects and therapeutic efficacy.

2. The Progress in the Clinical Translation of MSCs for Inflammatory Lung Disease

While earlier studies have not provided clear evidence of efficacy in phase 1/2 randomised controlled trials of MSCs in COPD [17] and ARDS [19,20] there are some reports of positive effects. A phase 2 trial enrolling 62 randomised patients with COPD, deemed the systemic administration of BM-MSC safe, although there were no differences in pulmonary function testing or with the 6-min walking test. However, a decrease in C-reactive protein was observed in comparison to elevated C-reactive protein (CRP) levels upon study entry (NCT00683722) [17]. In the context of the growing body of research suggesting the importance of the inflammatory lung profile of patients and its role in activating or licensing MSCs, a post-hoc analysis of the trial data was performed with stratification of COPD patients based on baseline levels of circulating inflammatory marker CRP. Interestingly, the data demonstrated that Remestemcel-L (BM-MSC) provided significant improvements in forced expiratory volume in one second, forced vital capacity and six-minute walk distance at 120 days post-infusion in patients with a higher baseline CRP [18]. In the context of ARDS, the findings from MSC clinical trials have not been clear-cut with respect to efficacy but studies have had favourable outcomes. Many of the current clinical trials investigating MSCs in lung inflammatory conditions are in COVID-19 ARDS and there is now a growing body of literature supportive of MSC efficacy in both non COVID-19 ARDS and in COVID-19 ARDS. A double-blinded randomised phase 2a safety trial investigating the use of allogeneic bone marrow derived-MSCs (BM-MSC) in severe ARDS patients showed there was no infusion-related haemodynamic or respiratory adverse events, proving their safety, although, the data could not support a claim for MSC efficacy in this trial and that may have been associated with reduced viability of the cell therapy product (NCT02097641). However, patients infused with BM-MSCs (with higher viability post-thaw) had lower concentrations of angiopoietin 2 (Ang-2) in their plasma after 6 h [19]. Interestingly, a nested cohort study within a phase 2a trial investigating BM-MSCs for moderate-to-severe ARDS, demonstrated that MSC treatment significantly reduced airspace total protein, Ang-2, IL-6 and soluble tumour necrosis factor (TNF) receptor-1 concentrations within a 48 h window following administration (NCT02097641) [33]. In addition, a phase 1 study of UC-MSCs in moderate-to-severe ARDS showed safety and reduction of circulating inflammatory biomarkers (ISRCTN52319075) [34].

Positive findings from the completed Athersys MUST-ARDS phase 1/2 randomised, double blind, placebo-controlled exploratory clinical study of MultiStem® (BM derived

human MSC-like cells) therapy in ARDS have been reported in a published conference abstract [35]. Interestingly, MultiStem®therapy enhanced ventilator-free days and ICU-free days and reduced mortality [35]. Ricordi and colleagues have also published the findings from their double-blind randomised control phase 1/2a trial of UC-MSCs in COVID-19 ARDS reporting significantly improved patient survival and significant decreases in pro-inflammatory cytokines in UC-MSC treated subjects at day 6 (NCT04355728) [21]. Similarly, a randomised controlled clinical trial investigating UC-MSCs reported improved survival rate, reduced length of stay and ventilator use as well as a decrease in IL-6 in patients who received UC-MSCs in COVID-19 ARDS (NCT04457609) [36]. Moreover, Mesoblast have reported positive initial findings from their phase 3 randomised, double-blind, placebo-controlled trial investigating Remestemcel-L in COVID-19 ARDS at international conferences (NCT04371393) [37]. Importantly, the work from Calfee and colleagues and others supports the idea of identifying phenotypes of ARDS [38,39] or treatable traits [40] and using that information to facilitate a personalised medicine approach. Together these studies suggest that patient stratification to identify disease phenotypes that might best respond to MSC therapy may increase the chance for MSC therapeutic efficacy.

3. The Importance of the Inflammatory Disease Microenvironment on MSC Therapeutic Efficacy

Pre-clinical studies have shown that the microenvironment present at the time of MSC administration influences MSCs' actions and thus potential therapeutic efficacy (Figure 1). For example, if MSCs are administered too early before disease onset (such as in GvHD) [41–43], or if signals required for their immunomodulation (such as NFκB, TNF-α receptor, or IFN-γ) [41,44] are blocked, MSCs lose their protective effects. Activation or licensing of MSC immunomodulation often involves activation of NF-κB and NF-κB regulated genes. In situations, where pro-inflammatory cytokines are limited or NF-κB is inhibited, MSC efficacy is impaired [42,43,45]. Moreover, in microenvironments which prevent MSC immunomodulation or promote MSC death, the presence of allogeneic MSCs may even promote harm [29,44,46]. For example, MSCs promoted fibrotic changes and inhibited re-epithelialization in an acid-induced ALI model with high levels of oxidative stress, whereby the conditions present in this model either negatively impacted the MSCs or prevented their ability to immunomodulate and promote repair [47].

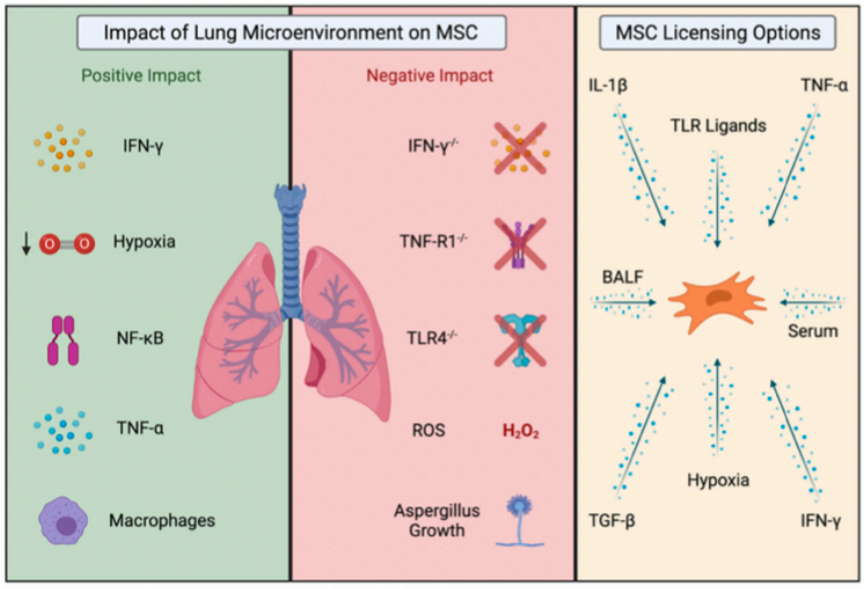

Figure 1. The Lung Microenvironment can positively or negatively influence MSC licensing and therapeutic effects. The presence of pro-inflammatory cytokines IFN-γ and TNF-α license MSCs and

promote MSC immunomodulation. A hypoxic environment can license MSCs and enhance their survival. Activation of NFκB signalling has been identified as an important factor in MSC licensing. The crosstalk or interaction between MSCs and macrophages following intravenous administration has been shown to play a key role in shaping MSC therapeutic effects. The absence of TLR4, TNF-R1 or IFN-γ on MSC or in the disease microenvironment has been shown to negatively impact MSC function. The presence of Aspergillus growth or reactive oxygen species (ROS) negatively impacts MSC survival. There are several licensing options whereby prior licensing of MSCs can be achieved, in vitro, prior to administration. Licensing options include proinflammatory cytokines, the anti-inflammatory cytokine TGF-β, via TLR ligand activation or by exposure of MSCs to disease microenvironments in the form of patient derived serum or bronchoalveolar lavage fluid (BALF). Image created using Biorender.com.

Notably, expression of PPARδ in mouse MSCs has been shown to impair MSC efficacy in a mouse model of arthritis, while knockdown or antagonism of PPARδ-enhanced mouse MSC efficacy via increased nitric oxide (NO) production [48]. In a humanised mouse model of acute GvHD, agonism of PPARδ in human BM-derived MSCs significantly impaired MSC therapeutic efficacy [49]. With respect to pre-clinical lung injury and sepsis models, upregulation of PPARδ signalling has been shown to play an important role in LPS-induced ALI and in caecal ligation puncture-induced sepsis [50,51]. Notably, angiopoietin-like protein 4 (Angptl4), a known target gene of PPARδ is upregulated in LPS-induced ALI [52] and has recently been reported as a clinical biomarker for ARDS [53]. Together, this evidence suggests that PPARδ ligands (agonists) are present in LPS-induced ALI which may contribute to microenvironmental impact on MSC therapeutic efficacy.

4. Licensing of MSCs Enhances Their Therapeutic Efficacy

IFN-γ, TNF-α and IL-1β have been identified as key mediators that facilitate the activation of MSC immunomodulation, in vitro [54–57]. A number of pre-clinical studies have demonstrated the capacity to enhance MSC therapeutic effects using a licensing approach in lung diseases including ALI, asthma and IPF (Table 1, Figure 1). Moreover, there is much we can learn about the mechanistic effects of licensed MSCs in other disease models, particularly where licensed MSCs have been administered systemically via the tail vein (Table 2). The licensing of mouse MSCs [41] or human BM-MSCs with IFN-γ before administration, enhanced MSC therapeutic efficacy in a mouse model [41] and a humanised mouse model of GvHD [43,49,54]. IFN-γ licensing of human BM-MSCs also enhanced therapeutic efficacy in pre-clinical models of TNBS-induced colitis and DSS-induced colitis [58]. Importantly, MSCs require stimulation with pro-inflammatory cytokines to produce immunomodulatory secreted factors like prostaglandin-E2 (PGE-2) [55,59], indolamine-2,3-dioxygenase (IDO) [60] and TNFα-stimulated gene 6 (TSG-6) [61], responsible for MSC therapeutic efficacy. TNF-α can act as an adjuvant for IFN-γ, where the two pro-inflammatory cytokines can work synergistically [62]. Licensing MSCs with TNF-α alone, enhances the production or secretion of factors such as PGE2, IDO, HGF and TSG-6 [55,63–65] and has been shown to enhance MSC efficacy in a number of disease models (reviewed in [66]).

Table 1. Pre-clinical models of lung disease that received licensed MSCs.

Murine Model	MSC Source	Route of Administration	Licensing Method	Licensing Location	Outcome	Ref
IPF (bleomycin)	Mouse BM-MSC	I.T.	Hypoxia (1.5% O_2)	in vitro	↑ HIF1α, HGF, VEGF ↓ IL-6, pro-IL-1β	[67]
ALI	Rat BM-MSC	In perfusate	Hypoxia (1% O_2)	in vitro	↑ IL-10, PGE2 ↓ Lung injury score ↓ TNF-α, IL-1β, MIP-2	[68]

Table 1. *Cont.*

Murine Model	MSC Source	Route of Administration	Licensing Method	Licensing Location	Outcome	Ref
ALI	Human UC-MSC	I.V.	TGF-β1	in vitro	↑ MSC survival ↑ Expression of RhoA ↓ LPS-induced injury	[69]
ALI	Human UC-MSC (EVs)	I.V.	IFN-γ	in vitro	↑ Animal survival ↑ eNOS ↓ Lung injury score ↓ TNF-α	[70]
ALI	Rat lung MSCs	I.V.	Culture on lung ECM-cyclic stretch	in vitro	↑ Lung elastance ↓ TNF-α, CXCL2 + neutrophils	[71]
ALI	Human UC-MSC	I.N.	Heatshock (42 °C for 1 h)	in vitro	↑ HSP70 expression ↑ IL-10 + PGE2 ↓ NLRP3 inflammasome formation ↓ IL-1β secretion by macrophages	[72]
ALI	Human BM-MSC	I.V.	Co-culture w/ serum from ARDS patients	ex vivo	↑ IL-10 + IL-1RN ↓ IL-6, IL-1α, IL-8, IL-1β, IFN-γ, TGFβ3, TGFβ2 + TNFAIP6	[73]
ALI	Human BM-MSC	AM treated with MSC-EVs-I.V.	Co-culture w/ BALF from ARDS patients	ex vivo	↑ M2 macrophage marker expression ↑ Phagocytic capacity of human MDMs ↓ Cytokine production	[74]
ALI	Human BM-MSC	I.T.	Cco-culture w/ plasma from ARDS patients	ex vivo	↑ IL-6 production ↑ N-cadherin expression at mRNA + protein levels ↓ CD105 + CD90 marker expression at day 5	[47]
ALI	Mouse BM-MSC	I.V.	Co-culture w/ serum from ALI mice	ex vivo	↑ Expression of anti-inflammatory mediators (TGF-β + IL-10) in AM in vitro ↓ iNOS + IL-6	[75]
Asthma	Mouse BM-MSC	I.T.	Co-culture w/ serum or BALF from asthmatic mice	ex vivo	↑ TGF-β, IFN-γ, IL-10,TSG-6, IDO-1, IL-1RN, iNOS, TNF-α, IL-1β + arginase-2 ↑ caspase-3, bax ↓ bcl-2Serum:↓ IL-4, IL-13 + eotaxin	[2]

Footnote ↑ = Increased, ↓ = Decreased. Idiopathic Pulmonary Fibrosis (IPF), Acute Lung Injury (ALI), Acute Respiratory Distress Syndrome (ARDS), Bone-Marrow Mesenchymal Stromal Cells (BM-MSC), Umbilical-Cord Mesenchymal Stromal Cells (UC-MSC), Intratracheal (I.T.), Intranasal (I.N.), Intravenous (I.V.), Alveolar Macrophages (AM), Extracellular Vesicles (EV), Extracellular Matrix (ECM), Bronchoalveolar Lavage Fluid (BALF).

Table 2. In vivo studies utilising MSC licensing.

Murine Model	MSC Source	Route of Administration	Licensing Method	Licensing Location	Outcome	Ref
GvHD	Human BM-MSC	I.V.	IFN-γ	in vitro	↑ Animal survival ↓ Cellular infiltration ↓ Pathology severity in small intestine + liver	[43]
GvHD	Human MAPC	I.V.	IFN-γ / PPARδ antagonist	in vitro	↑ Efficacy on day 0 ↑ Retention in spleen + liver	[49]
GvHD	Human BM-MSC	I.V.	Cyclosporine A + IFN-γ	in vitro	↑ IDO production + activity ↑ Animal survival	[54]
GvHD	Human BM-MSC	I.V.	N/A	N/A	↑ Immunosuppression ↑ IDO production in recipient phagocytes	[76]
Corneal allograft	Mouse BM-MSC	I.V.	TGF-β1	in vitro	↑ CD73 expression ↑ Treg expansion ↑ Immunosuppression ↑ Allograft survival ↓ Syngeneic T cell proliferation	[77]
Corneal allograft	Rat BM-MSC	I.V.	IFN-γ + TNF-α + IL-1β	in vitro	↑ Nitric oxide production ↑ Suppression of syngeneic lymphocytes ↑ Allograft survival ↑ Myeloid cells in lung ↑ FoxP3$^+$ Treg population in lung + spleen	[78]
Sepsis	BM-MSC	I.V.	N/A	N/A	↑ Animal survival ↑ Organ function ↓ TNF-α + IL-6	[79]
Arthritis	Human BM-MSC	I.V.	PPARβ/δ antagonist orIFN-γ + TNF-α	in vitro	↑ Immunosuppression ↑ NF-kB activity ↑ p65 binding on iNOS promoter ↓ Severity of disease	[48]
Myocardial Infarction	Human BM-MSC	I.V.	TNF-α	in vitro	↑ TSG-6 mRNA in lung ↓ Infarct size ↓ Pro-inflammatory proteases ↓ MMP9	[64]

Footnote: ↑ = Increased, ↓ = Decreased. Graft versus Host Disease (GvHD), Bone-Marrow Mesenchymal Stromal Cells (BM-MSC), Multipotent Adult Progenitor Cells (MAPC), Intravenous (I.V.).

Trophic factors, including those contained within extracellular vesicles (EVs) produced by MSCs, are thought to play a key role in mediating MSC therapeutics effects. EVs can transfer therapeutic cargo, such as mRNA, miRNA and even organelles like mitochondria, to ameliorate lung injury [70,80–82]. EVs are of particular interest, as they have been identified as a main effector of MSC paracrine function, playing a pivotal role for intracellular communication and boasting a therapeutic effect equivalent to that of their parent cells, MSCs [81]. Along with boosting MSC therapeutic potential, licensing with inflammatory cytokines can also boost EV efficacy [70,83,84]. The use of human and mouse BM- and human UC-MSC-derived EVs has been documented in pre-clinical models of lung disease, such as neonatal chronic lung disease [85], pneumonia [70,86,87], allergic asthma [3,88] and ALI [74,89]. EVs secreted from UC-MSCs and licensed with IFN-γ were more effective in attenuating *E. coli*-induced injury compared with EV from unlicensed UC-MSC in a rat

model of *E. coli* pneumonia [70]. Furthermore, IFN-γ licensed UC-MSC EVs, but not naïve UC-MSC EVs, had the ability to reduce lung protein permeability, alveolar inflammation and alveolar-arterial oxygen gradient in injured lungs compared with controls [70].

Other cytokine licensing approaches include the use of oncostatin M and TGF-β. Licensing of BM-MSCs with oncostatin M enhanced MSC therapeutic efficacy in a bleomycin-induced fibrosis model [90], while TGF-β licensing of MSC enhanced MSC survival in a rat ALI model [69]. TGF-β-licensed mouse BM-MSCs have also been utilised in a corneal allograft mouse model, where they modulated the immune response by suppressing the effector T cell population and induced Tregs within the lung following intravenous administration [77].

'Multi-cytokine licensing', or 'composite priming' involving a cocktail of cytokines, including combinations of IFN-γ, TNF-α and IL-1β for MSC licensing, has also been investigated [43,55]. In this context, TNF-α and IL-1β increased the MSC expression of the IFN-γ receptor, enhancing the MSC immunoregulatory effects; thus, IL-1β can optimise the therapeutic effects initiated by MSC licensing with IFN-γ/TNF-α [91]. In line with this, monocyte-derived IL-1β activation of multipotent adult progenitor cells (MAPCs) was required for MAPC suppression of IL-7-induced CD4 T-cell proliferation [92]. In vivo, rat BM-MSCs, pre-licensed with TNF-α and IL-1β, promoted corneal allograft survival via myeloid cell-mediated induction of regulatory T cells in the lung [78].

In addition to cytokines, pre-conditioning, or licensing MSCs with toll-like receptor (TLR) ligands can enhance therapeutic efficacy in a wide array of inflammatory diseases. MSCs express a number of TLRs [93]. MSCs licensed with the TLR3 ligand Poly(I:C) provided enhanced therapeutic effects in pre-clinical models of TNBS-induced colitis [94], in cardiomyopathic hamsters [95] and in atopic dermatitis [96]. Licensed MSC-derived EVs via the TLR3 ligand Poly I:C, also exhibited beneficial effects with enhanced antimicrobial activity in pre-clinical mouse and ex-vivo-perfused human lung injured with severe E. coli pneumonia [87,97]. TLR4 priming of MSCs via LPS enhanced MSC efficacy in an experimental autoimmune encephalitis (EAE) model [98], while LPS-primed MSC-derived exosomes provided accelerated wound healing in a diabetic cutaneous wound model [99]. Using an alternative approach, Yu and Chiang utilised the TLR2 agonist Pam3CSK4 to license mouse BM-MSCs and showed that TLR2 activated mouse MSCs further decreased eosinophil infiltration in the lung and IL-4/IL-5 secretion in the bronchoalveolar lavage fluid (BALF) and had a greater impact on lung function compared to MSCs in an OVA-induced allergic airway model [100]. Early work from Waterman et al. highlighted the role of differential TLR ligation in driving pro- or anti-inflammatory activation of human MSCs [93]. Pre-conditioning with differential TLR agonists can modulate the MSC secretome, where TLR4 activation with LPS enhances secretion of pro-inflammatory mediators and TLR3 activation with Poly(I:C) increases secretion of immunosuppressive factors such as IDO and PGE2 [101,102]. Ligation of TLR2, but not TLR4, inhibited the chemotaxis of murine BM-MSC and reduced their ability to expand Treg populations in vitro [103]. Moreover, murine BM-MSCs licensed with TLR4 and IFN-γ alleviated liver fibrosis in mice infected with S. japonicum cercariae, compared with BM-MSCs licensed with TLR2 and IFN-γ, which exacerbated the immunopathology in vivo [104]. In a mouse model of experimental autoimmune encephalomyelitis (EAE), BM-MSCs licensed with Poly(I:C) reduced the proliferation of $CD3^+$ T cells, compared with BM-MSCs licensed with LPS, which increased $CD3^+$ T-cell proliferation. Following i.p. administration, Poly(I:C)-licensed BM-MSCs alleviated EAE severity in contrast to LPS-licensed BM-MSCs, where their immunosuppressive effects were reversed [105]. The differential effects of various TLR ligands on MSCs may be associated with the downstream activation of pro- or anti-inflammatory mediators by MSCs. It is also likely that differential disease microenvironments will alter TLR activation of MSCs in vivo.

The oxygen concentration in ex-vivo culture can have a significant impact on MSC function, particularly when there is a large difference between the oxygen concentration used during ex-vivo culture (usually normoxic) and the concentration available at the site

of in vivo administration in inflammatory disease (usually hypoxic). Pre-conditioning in a hypoxic environment can enhance MSC survival [106–110] and MSCs' secretion of trophic factors associated with their therapeutic efficacy [110–113]. Hypoxic licensing is of relevance, as a disruption in oxygen homeostasis results in a hypoxic environment in many inflammatory lung diseases [114], such as ARDS, wherein gas exchange is impaired [115,116]. Hypoxic pre-conditioning of MSCs enhanced MSC survival and therapeutic efficacy in bleomycin-induced lung fibrosis [67]. This enhanced survival was found to be partially linked to an upregulation in hepatocyte growth factor (HGF) [4]. The importance of HGF's role in MSC cytoprotection has also been demonstrated using shRNA knockdown of HGF in human MSCs in a bleomycin-induced IPF model [4] and in an elastase-induced COPD model [8]. Differentially, MSCs exposed to hyperoxia (95% oxygen) can have an enhanced paracrine effect when administered to rats with oxygen-induced neonatal lung injury, due to an increase in stannocalcin-1 (STC-1) [117]. STC-1 has been described as having an important role in anti-apoptotic effects when secreted by MSCs [118]. UC-MSC derived microvesicles had enhanced angiogenesis potential following licensing with hypoxia, both in vitro and in vivo [119]. Alternatively, MSCs can also be pre-conditioned by culturing in anoxia, where ischemic MSC derived-exosomes have been shown to have enhanced protection in endotoxin-induced ALI in mice [120].

5. Exogenous Licensing of MSCs

There is growing evidence that different lung inflammatory environments, illustrated by utilising serum and BALF collected from various different inflammatory lung conditions as a surrogate, leads to altered MSC behaviours [2,47,121,122]. For example, ex-vivo exposure of murine MSCs to BALF or serum from mice with house dust mite (HDM)-induced allergic airway inflammation promoted increased expression of anti-inflammatory mediators (IDO, IL1RN, TSG-6, IL-10, TGF-β) and enhanced MSC therapeutic efficacy in HDM-mediated allergic airway inflammation [2]. Pre-conditioning of human MSCs with ARDS patient (moderately severe ARDS secondary to bacterial pneumonia) serum led to enhanced production of IL-10 and IL-1RN and decreased production of IL-6, IL-1 and IL-8 [73]. In contrast, BALF from patients with cystic fibrosis had toxic effects on human BM-MSCs, mediated by Aspergillus species-induced mitochondrial dysfunction and MSC death [123]. In the context of BALF from ARDS patients, a range of pro and anti-inflammatory mediators are induced in human MSCs following exposure to ARDS BALF (taken from ARDS patients without sepsis). Of particular interest, IL-1β present in these ARDS BALF samples was predictive of the induction of IL-6, IL-8 and FAS by human BM-MSCs following ex-vivo exposure to the BALF [124]. MSCs exposed to ARDS patient BALF samples were less effective at driving an anti-inflammatory macrophage phenotype compared to MSCs exposed to BALF from other lung conditions (including acute exacerbations of CF) [121]. A different study used pooled ARDS BALF and following exposure to these samples, human BM-MSCs promoted an anti-inflammatory and phagocytic macrophage phenotype, in vitro [74]. The limitations of these studies are that the ARDS patient etiologic profiles were not well described, and the use of different experimental conditions make it difficult to compare the findings from these studies. However, these studies highlight that the disease microenvironment present in the lung has the potential to have a significant positive or indeed negative effect on MSC therapeutic effects. As such, this underscores the need to better understand the disease microenvironment, how it influences MSC efficacy, and the potential benefits associated with a patient stratification approach to identify the patients who are most likely to respond to MSC therapy.

6. Endogenous Licensing of MSCs

Thus far, we have discussed the evidence supporting the fact that MSCs can influence their microenvironment once administered in vivo and the positive impact that exogenous licensing can have on MSC therapeutic effects. The previous paragraph alluded to the fact that MSCs can also be influenced by their microenvironment, for example, ex vivo,

following exposure to BALF or serum from inflammatory conditions. Based on the status of the microenvironment, MSCs can either have a beneficial or detrimental effect in the case of acute lung injury [47]. One of the earliest studies focusing on the microenvironmental effects on MSC efficacy showed that MSCs failed to modulate the immune response in GvHD driven by IFN-γ knock out T cells, demonstrating the importance of IFN-γ for activation of MSC immunomodulatory function in vivo [41]. In alignment with this, protective effects of BM-MSC administration in a mouse model of allergic airway inflammation were lost in IFN-γ receptor knockout mice [125]. While systemic administration of mouse BM-MSCs protected against ventilator-induced lung injury in mice, the same MSCs exacerbated injury in an acid-induced ALI mouse model [47]. Exacerbated injury was associated with higher BALF concentrations of IL-6, fibronectin, and lower levels of total antioxidant capacity (TAC) [47], highlighting conditions which negatively impact MSC functions. The proteomic profile of more than half of a cohort of ARDS (severe pneumonia without sepsis) patients serum samples (n = 33) shared this profile of high plasma fibronectin and low levels of TAC, suggesting that the microenvironment present in these patients may not be optimal for MSC administration [47]. This study from Islam et al. was an important study in highlighting that there may be lung inflammatory conditions that may not be compatible with MSC therapeutic efficacy. Notably, a recent clinical trial of BM-MSC in ARDS (inclusion criteria: sepsis with/out pneumonia, pneumonia without sepsis, aspiration only) showed a non-significant trend of higher 28-day mortality in patients after treatment with MSCs compared to that of placebo (NCT02097641) [19]. This may be associated with baseline imbalances in the severity of illness and low viability of the MSCs utilized in this trial, however, it is also possible that the microenvironment present in some of these patients was sub-optimal for MSC therapeutic efficacy. These investigators are now conducting another trial in a select population of patients with ARDS resulting from trauma as opposed to sepsis or pneumonia to better evaluate this issue. Importantly, there are also many pre-clinical studies that have identified ways in which the lung inflammatory environment facilitates the licensing or activation of MSCs. In addition to the positive effects associated with endogenous production or presence of NF-κB, TNF-α receptor, or IFN-γ [41,44,45], endogenous TNF-α has also been shown to play a key role in licensing MSCs, in vivo, through the induction of TSG-6. Human MSCs expressing high levels of TSG-6 improved survival and preserved body weight in a murine bleomycin model, compared with the control. Similarly, human MSCs attenuated LPS-induced inflammation in the lung via secretion of TSG-6, as knock down of TSG-6 expression abrogated the human BM-MSCs' anti-inflammatory effects in this murine model of ALI [126]. Moreover, entrapment of BM-MSCs in the lung leads to their activation and production of TSG-6, which has been shown to play a role in protection against myocardial infarction in mice [64]. Endogenous TNF-α or TNF receptors present in the disease microenvironment have been shown to play a key role in MSC efficacy in pre-clinical models of sepsis [127] and cardiomyopathy [128]. BM-MSCs from TNF-α or TNF-R1 knockout mice did not protect against caecal ligation and puncture-induced sepsis following intravenous administration [79]. An elegant study identified an important role for induced pluripotent stem cells (iPSC)-MSCs sensitivity to endogenous TNF-α in protection mediated via mitochondrial transfer in anthracycline-induced cardiomyopathy [128]. Specifically, transplantation of iPSC-MSCs but not TNFαIP2 (TNF-α induced protein that regulates tunnelling nanotube (TNT) formation) knockdown iPSC-MSCs protected against cardiomyopathy [128].

In addition to pro-inflammatory cytokines, toll-like receptors (TLR) and their ligands also influence MSC efficacy and function in vivo. TLR4 knockout mouse BM-MSCs failed to protect against *E. coli*-induced pneumonia [10] and were not efficacious in pre-clinical models of EAE [98] in comparison with wildtype MSCs. On the other hand, the microenvironment in the failing heart or myocardial infarct promoted a pro-inflammatory phenotype in both resident and transplanted mouse MSCs via TLR4 activation [129]. Interestingly, TLR4 knockout MSCs maintained their expression of CD47 (a "don't eat me" signal), in-

creasing their survival and facilitating their protective effects in a pre-clinical model of myocardial infarction [129].

Some of the most interesting studies focused on understanding how MSCs mediate their effect, have identified an important role for host macrophages present at the site of administration. A growing body of recent literature suggests that MSC–macrophage crosstalk plays a key role [130,131] in shaping MSC anti-inflammatory effects. An increase in anti-inflammatory or non-classical monocytes or macrophages has been reported in a range of disease models following MSC administration [79,82,92,132–136]. With respect to mechanisms, the transfer of mitochondria from MSCs to macrophages has been shown to enhance their bioenergetics [84] and promote an anti-inflammatory phenotype [11,82]. Use of clodronate liposomes to delete macrophages in vivo has demonstrated that the presence of macrophages are essential for MSC-mediated anti-inflammatory effects in preclinical models of ARDS, corneal allo-transplantation, liver injury and DSS-induced colitis [11,133,137,138]. Phagocytosis of MSCs [139,140], MSC-EVs [141], MSC cytoplasm [142] or MSC mitochondrial transfer via tunnelling nanotubes [11] can drive anti-inflammatory macrophage phenotypes. Building complexity upon those findings, one study showed that macrophage phagocytosis of MSCs that have been killed by cytotoxic T cells plays a key role in human BM-MSC protection against GvHD following i.v. administration [76]. Importantly, i.v. administration of apoptotic MSCs did not have the same level of protection as live MSCs [76]. Moreover, while live MSCs were effective in combination with immunosuppressive drugs, heat-killed MSCs were not efficacious in pre-clinical allogeneic heart transplantation [143]. Many of these findings highlighting the importance of the MSC-macrophage crosstalk in driving MSC activation and therapeutic effects are in studies using systemic administration of MSCs, whereby MSCs become trapped in the lung and mediate their effects even in inflammatory conditions distal to the lung. Thus, if we are to better understand exactly how the disease microenvironment influences MSC licensing and therapeutic efficacy and to identify the mechanism used by MSCs in mediating their effects, then we need to include in-depth studies of the lung environments present before and after MSC administration when MSCs are administered intravenously.

7. Patient Stratification to Identify Responders to MSC-Based Therapy

MSCs are usually detected for only a short time (72 h) in the lung or any other organ following systemic administration [29,140,144], however, their longevity can be enhanced in an injured lung or in licensed MSCs. Interestingly, exposure to healthy control BALF promotes human BM-MSC expression of HLA-DR, arguably increasing recognition and clearance of the MSCs [124]. This doesn't occur with ARDS BALF exposure, suggesting that the ARDS inflammatory environment may be protective of MSC survival [124]. We have also demonstrated that IFN-γ licensing of human BM-MSCs enhances their longevity in the short-term, in vivo, while pre-exposure to a PPARδ agonist significantly reduces MSC longevity in vivo in a humanised mouse model of aGvHD [49]. Despite the short time-frame, MSCs can mediate significant protective effects when administered to conditions where there is an acute inflammatory insult such as in ARDS. Data from chronic lung patients where MSCs have been investigated within clinical trials but have not demonstrated efficacy in COPD [17,145–147], or IPF [15,148]. Furthermore, preclinical evidence suggests that MSCs cannot promote the regeneration of fibrotic tissue when administered during established bleomycin-induced IPF [4,149,150] and delayed administration of MSC in an elastase-induced COPD model reduced MSC efficacy. Given that patients with IPF and COPD are likely to receive MSCs at a time when the disease is fully established, this data suggests that MSCs may not be efficacious. In the context of the acute nature of ARDS [151], and the growing body of literature supporting the potential for MSC efficacy in ARDS [19,21,35,36], it seems sensible that MSCs may be most suitable in acute inflammatory conditions. Moreover, the data discussed in this review also supports the idea that differential disease microenvironments present in some diseases or sub-phenotypes of disease may be better suited to facilitate MSC activation and lead to optimal MSC

therapeutic effects. For example, stratification of ARDS patients based on the hyper and hypo-inflammatory phenotypes may lead to the identification of responders to MSC therapy. Similarly, COPD patient stratification based on CRP baseline levels may enhance MSC efficacy, suggesting that even in some chronic diseases, the inflammatory environment may dictate potential MSC efficacy. Moreover, the potential to enhance MSC efficacy via pre-conditioning or licensing before administration to patients may provide a solution to try to enhance MSC therapy in heterogeneous patients where MSCs may not receive activation signals, negatively influencing their efficacy. Interestingly, Horwitz and colleagues have registered a phase I clinical trial investigating IFN-γ-licensed MSCs as a prophylaxis against aGvHD (NCT04328714). The findings from this study are eagerly awaited as the first study evaluating licensed MSCs in clinical trials.

8. Conclusions

In patients who respond to MSC therapy, these cells can have significant effects on the morbidity and in some cases mortality of patients who are very unwell and who have limited options. Although it may seem that limited progress has been made in the translation of MSC therapy to patients with inflammatory conditions (particularly in the lung), the field has learned much about how these cells respond to the inflammatory/disease microenvironment in which they find themselves following administration. Considering this, there is also a significant volume of research to be done in order for us to fully appreciate and understand how best to utilise these cells, so that we can identify the mechanisms of action and critical quality attributes required by the regulatory agencies. In our opinion, significant efforts should be made at the pre-clinical model stage and in patients following MSC administration to better identify how the disease microenvironment influences MSC licensing, function and efficacy.

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References

1. Cruz, F.F.; Rocco, P.R.M. The potential of mesenchymal stem cell therapy for chronic lung disease. *Expert Rev. Respir. Med.* **2019**, *14*, 31–39. [CrossRef] [PubMed]
2. Abreu, S.C.; Xisto, D.G.; De Oliveira, T.B.; Blanco, N.G.; De Castro, L.L.; Kitoko, J.; Olsen, P.; Lopes-Pacheco, M.; Morales, M.M.; Weiss, D.J.; et al. Serum from Asthmatic Mice Potentiates the Therapeutic Effects of Mesenchymal Stromal Cells in Experimental Allergic Asthma. *Stem Cells Transl. Med.* **2018**, *8*, 301–312. [CrossRef] [PubMed]
3. Cruz, F.F.; Borg, Z.D.; Goodwin, M.; Sokocevic, D.; Wagner, D.E.; Coffey, A.; Antunes, M.; Robinson, K.L.; Mitsialis, S.A.; Kourembanas, S.; et al. Systemic Administration of Human Bone Marrow-Derived Mesenchymal Stromal Cell Extracellular Vesicles AmelioratesAspergillusHyphal Extract-Induced Allergic Airway Inflammation in Immunocompetent Mice. *Stem Cells Transl. Med.* **2015**, *4*, 1302–1316. [CrossRef] [PubMed]
4. Cahill, E.F.; Kennelly, H.; Carty, F.; Mahon, B.P.; English, K. Hepatocyte Growth Factor Is Required for Mesenchymal Stromal Cell Protection Against Bleomycin-Induced Pulmonary Fibrosis. *Stem Cells Transl. Med.* **2016**, *5*, 1307–1318. [CrossRef] [PubMed]
5. Cargnoni, A.; Romele, P.; Signoroni, P.B.; Farigu, S.; Magatti, M.; Vertua, E.; Toschi, I.; Cesari, V.; Silini, A.R.; Stefani, F.R.; et al. Amniotic MSCs reduce pulmonary fibrosis by hampering lung B-cell recruitment, retention, and maturation. *Stem Cells Transl. Med.* **2020**, *9*, 1023–1035. [CrossRef] [PubMed]

6. Moroncini, G.; Paolini, C.; Orlando, F.; Capelli, C.; Grieco, A.; Tonnini, C.; Agarbati, S.; Mondini, E.; Saccomanno, S.; Goteri, G.; et al. Mesenchymal stromal cells from human umbilical cord prevent the development of lung fibrosis in immunocompetent mice. *PLoS ONE* **2018**, *13*, e0196048. [CrossRef]
7. Antunes, M.A.; Abreu, S.C.; Cruz, F.F.; Teixeira, A.C.; Lopes-Pacheco, M.; Bandeira, E.; Olsen, P.; Diaz, B.L.; Takyia, C.M.; Freitas, I.P.R.G.; et al. Effects of different mesenchymal stromal cell sources and delivery routes in experimental emphysema. *Respir. Res.* **2014**, *15*, 1–14. [CrossRef] [PubMed]
8. Kennelly, H.; Mahon, B.; English, K. Human mesenchymal stromal cells exert HGF dependent cytoprotective effects in a human relevant pre-clinical model of COPD. *Sci. Rep.* **2016**, *6*, 38207. [CrossRef]
9. Devaney, J.; Horie, S.; Masterson, C.; Elliman, S.; Barry, F.; O'Brien, T.; Curley, G.; O'Toole, D.; Laffey, J. Human mesenchymal stromal cells decrease the severity of acute lung injury induced by *E. coli* in the rat. *Thorax* **2015**, *70*, 625–635. [CrossRef] [PubMed]
10. Gupta, N.; Sinha, R.; Krasnodembskaya, A.; Xu, X.; Nizet, V.; Matthay, M.A.; Griffin, J.H. The TLR4-PAR1 Axis Regulates Bone Marrow Mesenchymal Stromal Cell Survival and Therapeutic Capacity in Experimental Bacterial Pneumonia. *Stem Cells* **2018**, *36*, 796–806. [CrossRef] [PubMed]
11. Jackson, M.V.; Morrison, T.J.; Doherty, D.F.; McAuley, D.; Matthay, M.A.; Kissenpfennig, A.; O'Kane, C.; Krasnodembskaya, A.D. Mitochondrial Transfer via Tunneling Nanotubes is an Important Mechanism by Which Mesenchymal Stem Cells Enhance Macrophage Phagocytosis in the In Vitro and In Vivo Models of ARDS. *Stem Cells* **2016**, *34*, 2210–2223. [CrossRef] [PubMed]
12. Curley, G.; Jerkic, M.; Dixon, S.; Hogan, G.; Masterson, C.; O'Toole, D.; Devaney, J.; Laffey, J. Cryopreserved, Xeno-Free Human Umbilical Cord Mesenchymal Stromal Cells Reduce Lung Injury Severity and Bacterial Burden in Rodent Escherichia coli–Induced Acute Respiratory Distress Syndrome. *Crit. Care Med.* **2017**, *45*, e202–e212. [CrossRef] [PubMed]
13. Horie, S.; Gonzalez, H.E.; Laffey, J.G.; Masterson, C.H. Cell therapy in acute respiratory distress syndrome. *J. Thorac. Dis.* **2018**, *10*, 5607–5620. [CrossRef] [PubMed]
14. Masterson, C.; Devaney, J.; Horie, S.; O'Flynn, L.; Deedigan, L.; Elliman, S.; Barry, F.; O'Brien, T.; O'Toole, D.; Laffey, J.G. Syndecan-2–positive, Bone Marrow–derived Human Mesenchymal Stromal Cells Attenuate Bacterial-induced Acute Lung Injury and Enhance Resolution of Ventilator-induced Lung Injury in Rats. *Anesthesiology* **2018**, *129*, 502–516. [CrossRef] [PubMed]
15. Glassberg, M.K.; Minkiewicz, J.; Toonkel, R.L.; Simonet, E.S.; Rubio, G.A.; DiFede, D.; Shafazand, S.; Khan, A.; Pujol, M.V.; LaRussa, V.F.; et al. Allogeneic Human Mesenchymal Stem Cells in Patients With Idiopathic Pulmonary Fibrosis via Intravenous Delivery (AETHER). *Chest* **2017**, *151*, 971–981. [CrossRef] [PubMed]
16. Armitage, J.D.; Tan, D.B.; Sturm, M.; Moodley, Y.P. Transcriptional profiling of circulating mononuclear cells from patients with chronic obstructive pulmonary disease receiving mesenchymal stromal cell infusions. *Stem Cells Transl. Med.* **2021**, *10*, 1470–1481. [CrossRef]
17. Weiss, D.J.; Casaburi, R.; Flannery, R.; LeRoux-Williams, M.; Tashkin, D.P. A Placebo-Controlled, Randomized Trial of Mesenchymal Stem Cells in COPD. *Chest* **2013**, *143*, 1590–1598. [CrossRef]
18. Weiss, D.J.; Segal, K.; Casaburi, R.; Hayes, J.; Tashkin, D. Effect of mesenchymal stromal cell infusions on lung function in COPD patients with high CRP levels. *Respir. Res.* **2021**, *22*, 1–11. [CrossRef]
19. Matthay, M.A.; Calfee, C.S.; Zhuo, H.; Thompson, B.T.; Wilson, J.G.; Levitt, J.E.; Rogers, A.J.; Gotts, J.E.; Wiener-Kronish, J.P.; Bajwa, E.K.; et al. Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): A randomised phase 2a safety trial. *Lancet Respir. Med.* **2018**, *7*, 154–162. [CrossRef]
20. Wilson, J.G.; Liu, K.D.; Zhuo, H.; Caballero, L.; McMillan, M.; Fang, X.; Cosgrove, K.; Vojnik, R.; Calfee, C.S.; Lee, J.-W.; et al. Mesenchymal stem (stromal) cells for treatment of ARDS: A phase 1 clinical trial. *Lancet Respir. Med.* **2014**, *3*, 24–32. [CrossRef]
21. Lanzoni, G.; Linetsky, E.; Correa, D.; Cayetano, S.M.; Alvarez, R.A.; Kouroupis, D.; Gil, A.A.; Poggioli, R.; Ruiz, P.; Martos, A.C.; et al. Umbilical cord mesenchymal stem cells for COVID-19 acute respiratory distress syndrome: A double-blind, phase 1/2a, randomized controlled trial. *Stem Cells Transl. Med.* **2021**, *10*, 660–673. [CrossRef] [PubMed]
22. Galipeau, J. Mesenchymal Stromal Cells for Graft-versus-Host Disease: A Trilogy. *Biol. Blood Marrow Transplant.* **2020**, *26*, e89–e91. [CrossRef] [PubMed]
23. Galipeau, J.; Sensébé, L. Mesenchymal Stromal Cells: Clinical Challenges and Therapeutic Opportunities. *Cell Stem Cell* **2018**, *22*, 824–833. [CrossRef] [PubMed]
24. Pittenger, M.F.; Discher, D.E.; Péault, B.M.; Phinney, D.G.; Hare, J.M.; Caplan, A.I. Mesenchymal stem cell perspective: Cell biology to clinical progress. *NPJ Regen. Med.* **2019**, *4*, 1–15. [CrossRef] [PubMed]
25. Meng, Z.W.; Baumgart, D.C. Darvadstrocel for the treatment of perianal fistulas in Crohn's disease. *Expert Rev. Gastroenterol. Hepatol.* **2020**, *14*, 405–410. [CrossRef] [PubMed]
26. Panés, J.; García-Olmo, D.; Van Assche, G.; Colombel, J.F.; Reinisch, W.; Baumgart, D.C.; Dignass, A.; Nachury, M.; Ferrante, M.; Kazemi-Shirazi, L.; et al. Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: A phase 3 randomised, double-blind controlled trial. *Lancet* **2016**, *388*, 1281–1290. [CrossRef]
27. Enes, S.R.; Krasnodembskaya, A.D.; English, K.; Dos Santos, C.C.; Weiss, D.J. Research Progress on Strategies that can Enhance the Therapeutic Benefits of Mesenchymal Stromal Cells in Respiratory Diseases With a Specific Focus on Acute Respiratory Distress Syndrome and Other Inflammatory Lung Diseases. *Front. Pharmacol.* **2021**, *12*, 811. [CrossRef]
28. English, K. Mechanisms of mesenchymal stromal cell immunomodulation. *Immunol. Cell Biol.* **2012**, *91*, 19–26. [CrossRef] [PubMed]

29. Weiss, D.J.; English, K.; Krasnodembskaya, A.; Isaza-Correa, J.M.; Hawthorne, I.J.; Mahon, B.P. The Necrobiology of Mesenchymal Stromal Cells Affects Therapeutic Efficacy. *Front. Immunol.* **2019**, *10*, 1228. [CrossRef]
30. English, K.; French, A.; Wood, K.J. Mesenchymal Stromal Cells: Facilitators of Successful Transplantation? *Cell Stem Cell* **2010**, *7*, 431–442. [CrossRef] [PubMed]
31. Sinha, P.; Calfee, C.S. Phenotypes in acute respiratory distress syndrome. *Curr. Opin. Crit. Care* **2019**, *25*, 12–20. [CrossRef] [PubMed]
32. Desai, D.; Brightling, C. Cytokine and anti-cytokine therapy in asthma: Ready for the clinic? *Clin. Exp. Immunol.* **2009**, *158*, 10–19. [CrossRef]
33. Wick, K.D.; Leligdowicz, A.; Zhuo, H.; Ware, L.B.; Matthay, M.A. Mesenchymal stromal cells reduce evidence of lung injury in patients with ARDS. *JCI Insight* **2021**, *6*. [CrossRef]
34. Yip, H.-K.; Fang, W.-F.; Li, Y.-C.; Lee, F.-Y.; Lee, C.-H.; Pei, S.-N.; Ma, M.-C.; Chen, K.-H.; Sung, P.-H.; Lee, M.S. Human Umbilical Cord-Derived Mesenchymal Stem Cells for Acute Respiratory Distress Syndrome. *Crit. Care Med.* **2020**, *48*, e391–e399. [CrossRef]
35. Bellingan, G.; Jacono, F.; Bannard-Smith, J.; Brealey, D.; Meyer, N.; Thickett, D.; Young, D.; Bentley, A.; McVerry, B.; Wunderink, R.; et al. Primary Analysis of a Phase 1/2 Study to Assess MultiStem® Cell Therapy, a Regenerative Advanced Therapy Medicinal Product (ATMP), in Acute Respiratory Distress Syndrome (MUST-ARDS). *Am. J. Respir. Crit. Care Med.* **2019**, *199*, A7353. [CrossRef]
36. Dilogo, I.H.; Aditianingsih, D.; Sugiarto, A.; Burhan, E.; Damayanti, T.; Sitompul, P.A.; Mariana, N.; Antarianto, R.D.; Liem, I.K.; Kispa, T.; et al. Umbilical cord mesenchymal stromal cells as critical COVID-19 adjuvant therapy: A randomized controlled trial. *Stem Cells Transl. Med.* **2021**, *10*, 1279–1287. [CrossRef] [PubMed]
37. Ilic, D.; Liovic, M. Industry updates from the field of stem cell research and regenerative medicine in November 2020. *Regen. Med.* **2021**, *16*, 323–329. [CrossRef] [PubMed]
38. Matthay, M.A.; Arabi, Y.M.; Siegel, E.R.; Ware, L.B.; Bos, L.D.J.; Sinha, P.; Beitler, J.R.; Wick, K.D.; Curley, M.A.Q.; Constantin, J.-M.; et al. Phenotypes and personalized medicine in the acute respiratory distress syndrome. *Intensiv. Care Med.* **2020**, *46*, 2136–2152. [CrossRef]
39. Vasquez, C.R.; Gupta, S.; Miano, T.A.; Roche, M.; Hsu, J.; Yang, W.; Holena, D.N.; Reilly, J.P.; Schrauben, S.J.; Leaf, D.E.; et al. Identification of Distinct Clinical Subphenotypes in Critically Ill Patients With COVID-19. *Chest* **2021**, *160*, 929–943. [CrossRef] [PubMed]
40. Reddy, K.; Calfee, C.S.; McAuley, D.F. Acute Respiratory Distress Syndrome Subphenotypes beyond the Syndrome: A Step toward Treatable Traits? *Am. J. Respir. Crit. Care Med.* **2021**, *203*, 1449–1451. [CrossRef]
41. Polchert, D.; Sobinsky, J.; Douglas, G.W.; Kidd, M.; Moadsiri, A.; Reina, E.; Genrich, K.; Mehrotra, S.; Setty, S.; Smith, B.J.; et al. IFN-γ activation of mesenchymal stem cells for treatment and prevention of graft versus host disease. *Eur. J. Immunol.* **2008**, *38*, 1745–1755. [CrossRef] [PubMed]
42. Sudres, M.; Norol, F.; Trenado, A.; Grégoire, S.; Charlotte, F.; Levacher, B.; Lataillade, J.-J.; Bourin, P.; Holy, X.; Vernant, J.-P.; et al. Bone Marrow Mesenchymal Stem Cells Suppress Lymphocyte Proliferation In Vitro but Fail to Prevent Graft-versus-Host Disease in Mice. *J. Immunol.* **2006**, *176*, 7761–7767. [CrossRef] [PubMed]
43. Tobin, L.M.; Healy, M.E.; English, K.; Mahon, B.P. Human mesenchymal stem cells suppress donor CD4+T cell proliferation and reduce pathology in a humanized mouse model of acute graft-versus-host disease. *Clin. Exp. Immunol.* **2012**, *172*, 333–348. [CrossRef] [PubMed]
44. Ankrum, J.; Ong, J.F.; Karp, J.M. Mesenchymal stem cells: Immune evasive, not immune privileged. *Nat. Biotechnol.* **2014**, *32*, 252–260. [CrossRef] [PubMed]
45. Dorronsoro, A.; Ferrin, I.; Salcedo, J.M.; Jakobsson, E.; Fernández-Rueda, J.; Lang, V.; Sepulveda, P.; Fechter, K.; Pennington, D.; Trigueros, C. Human mesenchymal stromal cells modulate T-cell responses through TNF-α-mediated activation of NF-κB. *Eur. J. Immunol.* **2013**, *44*, 480–488. [CrossRef]
46. Zhang, H.; Li, Y.; Slutsky, A.S. Precision medicine for cell therapy in acute respiratory distress syndrome. *Lancet Respir. Med.* **2019**, *7*, e13. [CrossRef]
47. Islam, D.; Huang, Y.; Fanelli, V.; Delsedime, L.; Wu, S.; Khang, J.; Han, B.; Grassi, A.; Li, M.; Xu, Y.; et al. Identification and Modulation of Microenvironment Is Crucial for Effective Mesenchymal Stromal Cell Therapy in Acute Lung Injury. *Am. J. Respir. Crit. Care Med.* **2019**, *199*, 1214–1224. [CrossRef] [PubMed]
48. Luz-Crawford, P.; Ipseiz, N.; Espinosa-Carrasco, G.; Caicedo, A.; Tejedor, G.; Toupet, K.; Loriau, J.; Scholtysek, C.; Stoll, C.; Khoury, M.; et al. PPARβ/δ directs the therapeutic potential of mesenchymal stem cells in arthritis. *Ann. Rheum. Dis.* **2016**, *75*, 2166–2174. [CrossRef] [PubMed]
49. Carty, F.; Dunbar, H.; Hawthorne, I.J.; Ting, A.E.; Stubblefield, S.R.; Hof, W.V.; English, K. IFN-γ and PPARδ influence the efficacy and retention of multipotent adult progenitor cells in graft vs host disease. *Stem Cells Transl. Med.* **2021**, *10*, 1561–1574. [CrossRef]
50. Haskova, Z.; Hoang, B.; Luo, G.; Morgan, L.A.; Billin, A.; Barone, F.C.; Shearer, B.G.; Barton, M.E.; Kilgore, K.S. Modulation of LPS-induced pulmonary neutrophil infiltration and cytokine production by the selective PPARβ/δ ligand GW0742. *Inflamm. Res.* **2008**, *57*, 314–321. [CrossRef]
51. Kapoor, A.; Shintani, Y.; Collino, M.; Osuchowski, M.F.; Busch, D.; Patel, N.S.A.; Sepodes, B.; Castiglia, S.; Fantozzi, R.; Bishop-Bailey, D.; et al. Protective Role of Peroxisome Proliferator–activated Receptor-β/δ in Septic Shock. *Am. J. Respir. Crit. Care Med.* **2010**, *182*, 1506–1515. [CrossRef] [PubMed]

52. Guo, L.; Li, S.; Zhao, Y.; Qian, P.; Ji, F.; Qian, L.; Wu, X.; Qian, G. Silencing Angiopoietin-Like Protein 4 (ANGPTL4) Protects Against Lipopolysaccharide-Induced Acute Lung Injury Via Regulating SIRT1 /NF-kB Pathway. *J. Cell. Physiol.* **2015**, *230*, 2390–2402. [CrossRef] [PubMed]
53. Wang, T.; Lan, Y.; Wang, H.; Zeng, N.; Wen, F. Angiopoietin like 4 as a clinical biomarker for acute respiratory distress syndrome. *Eur. Respir. J.* **2019**, *54*. [CrossRef]
54. Corbett, J.M.; Hawthorne, I.; Dunbar, H.; Coulter, I.; Ni Chonghaile, M.; Flynn, C.M.; English, K. Cyclosporine A and IFNγ licencing enhances human mesenchymal stromal cell potency in a humanised mouse model of acute graft versus host disease. *Stem Cell Res. Ther.* **2021**, *12*, 1–10. [CrossRef] [PubMed]
55. English, K.; Barry, F.P.; Field-Corbett, C.P.; Mahon, B. IFN-γ and TNF-α differentially regulate immunomodulation by murine mesenchymal stem cells. *Immunol. Lett.* **2007**, *110*, 91–100. [CrossRef] [PubMed]
56. Krampera, M.; Cosmi, L.; Angeli, R.; Pasini, A.; Liotta, F.; Andreini, A.; Santarlasci, V.; Mazzinghi, B.; Pizzolo, G.; Vinante, F.; et al. Role for Interferon-γ in the Immunomodulatory Activity of Human Bone Marrow Mesenchymal Stem Cells. *Stem Cells* **2006**, *24*, 386–398. [CrossRef] [PubMed]
57. Ren, G.; Su, J.; Zhang, L.; Zhao, X.; Ling, W.; L'Huillie, A.; Zhang, J.; Lu, Y.; Roberts, A.I.; Ji, W.; et al. Species Variation in the Mechanisms of Mesenchymal Stem Cell-Mediated Immunosuppression. *Stem Cells* **2009**, *27*, 1954–1962. [CrossRef] [PubMed]
58. Duijvestein, M.; Wildenberg, M.E.; Welling, M.M.; Hennink, S.; Molendijk, I.; van Zuylen, V.L.; Bosse, T.; Vos, A.C.W.; de Jonge-Muller, E.S.M.; Roelofs, H.; et al. Pretreatment with Interferon-γ Enhances the Therapeutic Activity of Mesenchymal Stromal Cells in Animal Models of Colitis. *Stem Cells* **2011**, *29*, 1549–1558. [CrossRef]
59. Rabani, R.; Volchuk, A.; Jerkic, M.; Ormesher, L.; Garces-Ramirez, L.; Canton, J.; Masterson, C.; Gagnon, S.; Tatham, K.C.; Marshall, J.; et al. Mesenchymal stem cells enhance NOX2-dependent reactive oxygen species production and bacterial killing in macrophages during sepsis. *Eur. Respir. J.* **2018**, *51*, 1702021. [CrossRef]
60. Boyt, D.T.; Boland, L.K.; Burand, A.J.; Brown, A.J.; Ankrum, J.A. Dose and duration of interferon γ pre-licensing interact with donor characteristics to influence the expression and function of indoleamine-2,3-dioxygenase in mesenchymal stromal cells. *J. R. Soc. Interface.* **2020**, *17*, 20190815. [CrossRef]
61. Wang, G.; Cao, K.; Liu, K.; Xue, Y.; Roberts, A.I.; Li, F.; Han, Y.; Rabson, A.B.; Wang, Y.; Shi, Y. Kynurenic acid, an IDO metabolite, controls TSG-6-mediated immunosuppression of human mesenchymal stem cells. *Cell Death Differ.* **2017**, *25*, 1209–1223. [CrossRef] [PubMed]
62. Carvalho, A.S.; Sousa, M.R.R.; Alencar-Silva, T.; Carvalho, J.L.; Saldanha-Araujo, F. Mesenchymal stem cells immunomodulation: The road to IFN-γ licensing and the path ahead. *Cytokine Growth Factor Rev.* **2019**, *47*, 32–42. [CrossRef]
63. De Witte, S.F.H.; Franquesa, M.; Baan, C.; Hoogduijn, M.J. Toward Development of iMesenchymal Stem Cells for Immunomodulatory Therapy. *Front. Immunol.* **2016**, *6*, 648. [CrossRef] [PubMed]
64. Lee, R.H.; Pulin, A.A.; Seo, M.J.; Kota, D.J.; Ylostalo, J.; Larson, B.L.; Semprun-Prieto, L.; Delafontaine, P.; Prockop, D.J. Intravenous hMSCs Improve Myocardial Infarction in Mice because Cells Embolized in Lung Are Activated to Secrete the Anti-inflammatory Protein TSG-6. *Cell Stem Cell* **2009**, *5*, 54–63. [CrossRef]
65. Prasanna, S.J.; Gopalakrishnan, D.; Shankar, S.R.; Vasandan, A.B. Pro-Inflammatory Cytokines, IFNγ and TNFα, Influence Immune Properties of Human Bone Marrow and Wharton Jelly Mesenchymal Stem Cells Differentially. *PLoS ONE* **2010**, *5*, e9016. [CrossRef] [PubMed]
66. Yan, L.; Zheng, D.; Xu, R.-H. Critical Role of Tumor Necrosis Factor Signaling in Mesenchymal Stem Cell-Based Therapy for Autoimmune and Inflammatory Diseases. *Front. Immunol.* **2018**, *9*, 1658. [CrossRef] [PubMed]
67. Lan, Y.-W.; Choo, K.-B.; Chen, C.-M.; Chung-Hsing, H.; Chen, Y.-B.; Hsieh, C.-H.; Kuo, H.-P.; Chong, K.-Y. Hypoxia-preconditioned mesenchymal stem cells attenuate bleomycin-induced pulmonary fibrosis. *Stem Cell Res. Ther.* **2015**, *6*, 1–17. [CrossRef] [PubMed]
68. Liu, Y.-Y.; Chiang, C.-H.; Hung, S.-C.; Chian, C.-F.; Tsai, C.-L.; Chen, W.-C.; Zhang, H. Hypoxia-preconditioned mesenchymal stem cells ameliorate ischemia/reperfusion-induced lung injury. *PLoS ONE* **2017**, *12*, e0187637. [CrossRef] [PubMed]
69. Li, D.; Liu, Q.; Qi, L.; Dai, X.; Liu, H.; Wang, Y. Low levels of TGF-β1 enhance human umbilical cord-derived mesenchymal stem cell fibronectin production and extend survival time in a rat model of lipopolysaccharide-induced acute lung injury. *Mol. Med. Rep.* **2016**, *14*, 1681–1692. [CrossRef]
70. Varkouhi, A.K.; Jerkic, M.; Ormesher, L.; Gagnon, S.; Goyal, S.; Rabani, R.; Masterson, C.; Spring, C.; Chen, P.Z.; Gu, F.X.; et al. Extracellular Vesicles from Interferon-γ–primed Human Umbilical Cord Mesenchymal Stromal Cells Reduce *Escherichia coli*-induced Acute Lung Injury in Rats. *Anesthesiology* **2019**, *130*, 778–790. [CrossRef] [PubMed]
71. Nonaka, P.N.; Falcones, B.; Farre, R.; Artigas, A.; Almendros, I.; Navajas, D. Biophysically Preconditioning Mesenchymal Stem Cells Improves Treatment of Ventilator-Induced Lung Injury. *Arch. Bronconeumol.* **2019**, *56*, 179–181. [CrossRef] [PubMed]
72. Lv, H.; Yuan, X.; Zhang, J.; Lu, T.; Yao, J.; Zheng, J.; Cai, J.; Xiao, J.; Chen, H.; Xie, S.; et al. Heat shock preconditioning mesenchymal stem cells attenuate acute lung injury via reducing NLRP3 inflammasome activation in macrophages. *Stem Cell Res. Ther.* **2021**, *12*, 1–16. [CrossRef]
73. Bustos, M.L.; Huleihel, L.; Meyer, E.M.; Donnenberg, A.D.; Donnenberg, V.S.; Sciurba, J.D.; Mroz, L.; McVerry, B.J.; Ellis, B.M.; Kaminski, N.; et al. Activation of Human Mesenchymal Stem Cells Impacts Their Therapeutic Abilities in Lung Injury by Increasing Interleukin (IL)-10 and IL-1RN Levels. *Stem Cells Transl. Med.* **2013**, *2*, 884–895. [CrossRef]

74. Morrison, T.J.; Jackson, M.V.; Cunningham, E.K.; Kissenpfennig, A.; McAuley, D.; O'Kane, C.; Krasnodembskaya, A.D. Mesenchymal Stromal Cells Modulate Macrophages in Clinically Relevant Lung Injury Models by Extracellular Vesicle Mitochondrial Transfer. *Am. J. Respir. Crit. Care Med.* **2017**, *196*, 1275–1286. [CrossRef] [PubMed]
75. Silva, J.D.; De Castro, L.L.; Braga, C.L.; Oliveira, G.P.; Trivelin, S.A.; Barbosa-Junior, C.M.; Morales, M.M.; Dos Santos, C.C.; Weiss, D.J.; Lopes-Pacheco, M.; et al. Mesenchymal Stromal Cells Are More Effective Than Their Extracellular Vesicles at Reducing Lung Injury Regardless of Acute Respiratory Distress Syndrome Etiology. *Stem Cells Int.* **2019**, *2019*, 1–15. [CrossRef] [PubMed]
76. Galleu, A.; Riffo-Vasquez, Y.; Trento, C.; Lomas, C.; Dolcetti, L.; Cheung, T.S.; von Bonin, M.; Barbieri, L.; Halai, K.; Ward, S.; et al. Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation. *Sci. Transl. Med.* **2017**, *9*, 158–175. [CrossRef] [PubMed]
77. Lynch, K.; Treacy, O.; Chen, X.; Murphy, N.; Lohan, P.; Islam, N.; Donohoe, E.; Griffin, M.D.; Watson, L.; McLoughlin, S.; et al. TGF-β1-Licensed Murine MSCs Show Superior Therapeutic Efficacy in Modulating Corneal Allograft Immune Rejection In Vivo. *Mol. Ther.* **2020**, *28*, 2023–2043. [CrossRef] [PubMed]
78. Murphy, N.; Treacy, O.; Lynch, K.; Morcos, M.; Lohan, P.; Howard, L.; Fahy, G.; Griffin, M.D.; Ryan, A.E.; Ritter, T. TNF-α/IL-1β—Licensed mesenchymal stromal cells promote corneal allograft survival via myeloid cell-mediated induction of Foxp3 + regulatory T cells in the lung. *FASEB J.* **2019**, *33*, 9404–9421. [CrossRef] [PubMed]
79. Németh, K.; Leelahavanichkul, A.; Yuen, P.; Mayer, B.; Parmelee, A.; Doi, K.; Robey, P.; Leelahavanichkul, K.; Koller, B.H.; Brown, J.M.; et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E2–dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat. Med.* **2008**, *15*, 42–49. [CrossRef] [PubMed]
80. Abreu, S.C.; Weiss, D.J.; Rocco, P.R.M. Extracellular vesicles derived from mesenchymal stromal cells: A therapeutic option in respiratory diseases? *Stem Cell Res. Ther.* **2016**, *7*, 1–10. [CrossRef] [PubMed]
81. Lee, M.J.W.; Matthay, M.M.A. Is a Part Better than the Whole for Cell-based Therapy for Acute Respiratory Distress Syndrome? *Anesthesiology* **2019**, *130*, 683–685. [CrossRef]
82. Phinney, D.; Di Giuseppe, M.; Njah, J.; Sala-Llinas, E.; Shiva, S.; Croix, C.M.S.; Stolz, D.B.; Watkins, S.; Di, Y.P.; Leikauf, G.; et al. Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. *Nat. Commun.* **2015**, *6*, 8472. [CrossRef] [PubMed]
83. Harting, M.T.; Srivastava, A.; Zhaorigetu, S.; Bair, H.; Prabhakara, K.S.; Furman, N.E.T.; Vykoukal, J.V.; Ruppert, K.A.; Cox, C.S.; Olson, S.D. Inflammation-Stimulated Mesenchymal Stromal Cell-Derived Extracellular Vesicles Attenuate Inflammation. *Stem Cells* **2017**, *36*, 79–90. [CrossRef] [PubMed]
84. Zhang, Q.; Fu, L.; Liang, Y.; Guo, Z.; Wang, L.; Ma, C.; Wang, H. Exosomes originating from MSCs stimulated with TGF-β and IFN-γ promote Treg differentiation. *J. Cell. Physiol.* **2018**, *233*, 6832–6840. [CrossRef] [PubMed]
85. Aslam, M.; Baveja, R.; Liang, O.D.; Fernandez-Gonzalez, A.; Lee, C.; Mitsialis, S.A.; Kourembanas, S. Bone Marrow Stromal Cells Attenuate Lung Injury in a Murine Model of Neonatal Chronic Lung Disease. *Am. J. Respir. Crit. Care Med.* **2009**, *180*, 1122–1130. [CrossRef] [PubMed]
86. Hao, Q.; Gudapati, V.; Monsel, A.; Park, J.H.; Hu, S.; Kato, H.; Lee, J.H.; Zhou, L.; He, H. Mesenchymal Stem Cell–Derived Extracellular Vesicles Decrease Lung Injury in Mice. *J. Immunol.* **2019**, *203*, 1961–1972. [CrossRef] [PubMed]
87. Monsel, A.; Zhu, Y.-G.; Gennai, S.; Hao, Q.; Hu, S.; Rouby, J.-J.; Rosenzwajg, M.; Matthay, M.A.; Lee, J.W. Therapeutic Effects of Human Mesenchymal Stem Cell–derived Microvesicles in Severe Pneumonia in Mice. *Am. J. Respir. Crit. Care Med.* **2015**, *192*, 324–336. [CrossRef]
88. De Castro, L.L.; Xisto, D.G.; Kitoko, J.Z.; Cruz, F.F.; Olsen, P.C.; Redondo, P.A.G.; Ferreira, T.P.T.; Weiss, D.J.; Martins, M.A.; Morales, M.M.; et al. Human adipose tissue mesenchymal stromal cells and their extracellular vesicles act differentially on lung mechanics and inflammation in experimental allergic asthma. *Stem Cell Res. Ther.* **2017**, *8*, 151. [CrossRef] [PubMed]
89. Silva, J.D.; Su, Y.; Calfee, C.S.; Delucchi, K.L.; Weiss, D.; McAuley, D.F.; O'Kane, C.; Krasnodembskaya, A.D. Mesenchymal stromal cell extracellular vesicles rescue mitochondrial dysfunction and improve barrier integrity in clinically relevant models of ARDS. *Eur. Respir. J.* **2020**, *58*, 2002978. [CrossRef] [PubMed]
90. Lan, Y.-W.; Theng, S.-M.; Huang, T.-T.; Choo, K.-B.; Chen, C.-M.; Kuo, H.-P.; Chong, K.Y. Oncostatin M-Preconditioned Mesenchymal Stem Cells Alleviate Bleomycin-Induced Pulmonary Fibrosis Through Paracrine Effects of the Hepatocyte Growth Factor. *Stem Cells Transl. Med.* **2016**, *6*, 1006–1017. [CrossRef]
91. Hackel, A.; Aksamit, A.; Bruderek, K.; Lang, S.; Brandau, S. TNF-α and IL-1β sensitize human MSC for IFN-γ signaling and enhance neutrophil recruitment. *Eur. J. Immunol.* **2020**, *51*, 319–330. [CrossRef] [PubMed]
92. Reading, J.L.; Vaes, B.; Hull, C.; Sabbah, S.; Hayday, T.; Wang, N.S.; DiPiero, A.; A Lehman, N.; Taggart, J.M.; Carty, F.; et al. Suppression of IL-7-dependent Effector T-cell Expansion by Multipotent Adult Progenitor Cells and PGE2. *Mol. Ther.* **2015**, *23*, 1783–1793. [CrossRef] [PubMed]
93. Waterman, R.S.; Tomchuck, S.L.; Henkle, S.L.; Betancourt, A.M. A New Mesenchymal Stem Cell (MSC) Paradigm: Polarization into a Pro-Inflammatory MSC1 or an Immunosuppressive MSC2 Phenotype. *PLoS ONE* **2010**, *5*, e10088. [CrossRef]
94. Qiu, Y.; Guo, J.; Mao, R.; Chao, K.; Chen, B.-L.; He, Y.; Zeng, Z.-R.; Zhang, S.-H.; Chen, M.-H. TLR3 preconditioning enhances the therapeutic efficacy of umbilical cord mesenchymal stem cells in TNBS-induced colitis via the TLR3-Jagged-1-Notch-1 pathway. *Mucosal Immunol.* **2016**, *10*, 727–742. [CrossRef] [PubMed]

95. Mastri, M.; Shah, Z.; McLaughlin, T.; Greene, C.J.; Baum, L.; Suzuki, G.; Lee, T. Activation of Toll-like receptor 3 amplifies mesenchymal stem cell trophic factors and enhances therapeutic potency. *Am. J. Physiol. Physiol.* **2012**, *303*, C1021–C1033. [CrossRef] [PubMed]
96. Park, A.; Park, H.; Yoon, J.; Kang, D.; Kang, M.-H.; Park, Y.-Y.; Suh, N.; Yu, J. Priming with Toll-like receptor 3 agonist or interferon-gamma enhances the therapeutic effects of human mesenchymal stem cells in a murine model of atopic dermatitis. *Stem Cell Res. Ther.* **2019**, *10*, 1–11. [CrossRef]
97. Park, J.; Kim, S.; Lim, H.; Liu, A.; Hu, S.; Lee, J.; Zhuo, H.; Hao, Q.; Matthay, M.A.; Lee, J.-W. Therapeutic effects of human mesenchymal stem cell microvesicles in an ex vivo perfused human lung injured with severe *E. coli* pneumonia. *Thorax* **2018**, *74*, 43–50. [CrossRef] [PubMed]
98. Kurte, M.; Vega-Letter, A.M.; Luz-Crawford, P.; Djouad, F.; Noël, D.; Khoury, M.; Carrión, F. Time-dependent LPS exposure commands MSC immunoplasticity through TLR4 activation leading to opposite therapeutic outcome in EAE. *Stem Cell Res. Ther.* **2020**, *11*, 1–14. [CrossRef]
99. Ti, D.; Hao, H.; Tong, C.; Liu, J.; Dong, L.; Zheng, J.; Zhao, Y.; Liu, H.; Fu, X.; Han, W. LPS-preconditioned mesenchymal stromal cells modify macrophage polarization for resolution of chronic inflammation via exosome-shuttled let-7b. *J. Transl. Med.* **2015**, *13*, 1–14. [CrossRef] [PubMed]
100. Yu, H.-C.; Chiang, B.-L. Toll-like receptor 2 ligation of mesenchymal stem cells alleviates asthmatic airway inflammation. *J. Allergy Clin. Immunol.* **2018**, *142*, 284–287.e5. [CrossRef]
101. Kota, D.J.; Dicarlo, B.; Hetz, R.A.; Smith, P.; Cox, C.S.; Olson, S.D. Differential MSC activation leads to distinct mononuclear leukocyte binding mechanisms. *Sci. Rep.* **2014**, *4*, 4565. [CrossRef]
102. Najar, M.; Krayem, M.; Meuleman, N.; Bron, D.; Lagneaux, L. Mesenchymal Stromal Cells and Toll-Like Receptor Priming: A Critical Review. *Immune Netw.* **2017**, *17*, 89–102. [CrossRef]
103. Lei, J.; Wang, Z.; Hui, D.; Yu, W.; Zhou, D.; Xia, W.; Chen, C.; Zhang, Q.; Wang, Z.; Zhang, Q.; et al. Ligation of TLR2 and TLR4 on murine bone marrow-derived mesenchymal stem cells triggers differential effects on their immunosuppressive activity. *Cell. Immunol.* **2011**, *271*, 147–156. [CrossRef]
104. Liu, C.; Zhang, Y.-S.; Chen, F.; Wu, X.-Y.; Zhang, B.-B.; Wu, Z.-D.; Lei, J.-X. Immunopathology in schistosomiasis is regulated by TLR2,4- and IFN-γ-activated MSC through modulating Th1/Th2 responses. *Stem Cell Res. Ther.* **2020**, *11*, 217. [CrossRef] [PubMed]
105. Vega-Letter, A.M.; Kurte, M.; Fernández-O'Ryan, C.; Gauthier-Abeliuk, M.; Fuenzalida, P.; Moya-Uribe, I.; Altamirano, C.; Figueroa, F.; Irarrázabal, C.; Carrión, F. Differential TLR activation of murine mesenchymal stem cells generates distinct immunomodulatory effects in EAE. *Stem Cell Res. Ther.* **2016**, *7*, 1–12. [CrossRef] [PubMed]
106. Amiri, F.; Jahanian-Najafabadi, A.; Roudkenar, M.H. In vitro augmentation of mesenchymal stem cells viability in stressful microenvironments. *Cell Stress Chaperon-* **2014**, *20*, 237–251. [CrossRef] [PubMed]
107. Boregowda, S.V.; Krishnappa, V.; Chambers, J.; Lograsso, P.V.; Lai, W.-T.; Ortiz, L.; Phinney, D.G. Atmospheric Oxygen Inhibits Growth and Differentiation of Marrow-Derived Mouse Mesenchymal Stem Cells via a p53-Dependent Mechanism: Implications for Long-Term Culture Expansion. *Stem Cells* **2012**, *30*, 975–987. [CrossRef]
108. Hu, X.; Yu, S.P.; Fraser, J.L.; Lu, Z.; Ogle, M.E.; Wang, J.-A.; Wei, L. Transplantation of hypoxia-preconditioned mesenchymal stem cells improves infarcted heart function via enhanced survival of implanted cells and angiogenesis. *J. Thorac. Cardiovasc. Surg.* **2008**, *135*, 799–808. [CrossRef]
109. Levy, O.; Kuai, R.; Siren, E.M.J.; Bhere, D.; Milton, Y.; Nissar, N.; De Biasio, M.; Heinelt, M.; Reeve, B.; Abdi, R.; et al. Shattering barriers toward clinically meaningful MSC therapies. *Sci. Adv.* **2020**, *6*, eaba6884. [CrossRef] [PubMed]
110. Bader, A.M.; Klose, K.; Bieback, K.; Korinth, D.; Schneider, M.; Seifert, M.; Choi, Y.-H.; Kurtz, A.; Falk, V.; Stamm, C. Hypoxic Preconditioning Increases Survival and Pro-Angiogenic Capacity of Human Cord Blood Mesenchymal Stromal Cells In Vitro. *PLoS ONE* **2015**, *10*, e0138477. [CrossRef]
111. Lee, J.H.; Yoon, Y.M.; Lee, S.H. Hypoxic Preconditioning Promotes the Bioactivities of Mesenchymal Stem Cells via the HIF-1α-GRP78-Akt Axis. *Int. J. Mol. Sci.* **2017**, *18*, 1320. [CrossRef] [PubMed]
112. Sun, J.; Wei, Z.Z.; Gu, X.; Zhang, J.Y.; Zhang, Y.; Li, J.; Wei, L. Intranasal delivery of hypoxia-preconditioned bone marrow-derived mesenchymal stem cells enhanced regenerative effects after intracerebral hemorrhagic stroke in mice. *Exp. Neurol.* **2015**, *272*, 78–87. [CrossRef] [PubMed]
113. Yu, J.; Yin, S.; Zhang, W.; Gao, F.; Liu, Y.; Chen, Z.; Zhang, M.; He, J.; Zheng, S. Hypoxia preconditioned bone marrow mesenchymal stem cells promote liver regeneration in a rat massive hepatectomy model. *Stem Cell Res. Ther.* **2013**, *4*, 1–9. [CrossRef]
114. Brahimi-Horn, M.C.; Pouysségur, J. Oxygen, a source of life and stress. *FEBS Lett.* **2007**, *581*, 3582–3591. [CrossRef] [PubMed]
115. Noronha, N.C.; Mizukami, A.; Caliári-Oliveira, C.; Cominal, J.G.; Rocha, J.L.M.; Covas, D.T.; Swiech, K.; Malmegrim, K.C.R. Priming approaches to improve the efficacy of mesenchymal stromal cell-based therapies. *Stem Cell Res. Ther.* **2019**, *10*, 1–21.
116. Da Silva, L.H.A.; Antunes, M.A.; Dos Santos, C.C.; Weiss, D.J.; Cruz, F.F.; Rocco, P.R.M. Strategies to improve the therapeutic effects of mesenchymal stromal cells in respiratory diseases. *Stem Cell Res. Ther.* **2018**, *9*, 1–9. [CrossRef]
117. Waszak, P.; Alphonse, R.; Vadivel, A.; Ionescu, L.; Eaton, F.; Thébaud, B. Preconditioning Enhances the Paracrine Effect of Mesenchymal Stem Cells in Preventing Oxygen-Induced Neonatal Lung Injury in Rats. *Stem Cells Dev.* **2012**, *21*, 2789–2797. [CrossRef]

118. Block, G.J.; Ohkouchi, S.; Fung, F.; Frenkel, J.; Gregory, C.; Pochampally, R.; DiMattia, G.; Sullivan, D.E.; Prockop, D.J. Multipotent stromal cells are activated to reduce apoptosis in part by upregulation and secretion of stanniocalcin-1. *Stem Cells* **2009**, *27*, 670–681. [CrossRef]
119. Zhang, H.; Liu, X.-B.; Huang, S.; Bi, X.-Y.; Wang, H.-X.; Xie, L.-X.; Wang, Y.-Q.; Cao, X.-F.; Lv, J.; Xiao, F.-J.; et al. Microvesicles Derived from Human Umbilical Cord Mesenchymal Stem Cells Stimulated by Hypoxia Promote Angiogenesis Both In Vitro and In Vivo. *Stem Cells Dev.* **2012**, *21*, 3289–3297. [CrossRef]
120. Li, L.; Jin, S.; Zhang, Y. Ischemic preconditioning potentiates the protective effect of mesenchymal stem cells on endotoxin-induced acute lung injury in mice through secretion of exosome. *Int. J. Clin. Exp. Med.* **2015**, *8*, 3825–3832. [PubMed]
121. Abreu, S.C.; Enes, S.R.; Dearborn, J.; Goodwin, M.; Coffey, A.; Borg, Z.D.; Dos Santos, C.C.; Wargo, M.J.; Cruz, F.F.; Loi, R.; et al. Lung inflammatory environments differentially alter mesenchymal stromal cell behavior. *Am. J. Physiol. Cell. Mol. Physiol.* **2019**, *317*, L823–L831. [CrossRef] [PubMed]
122. Xu, A.; Ii, L.A.R.; Walker, K.P.; Mohammadipoor, A.; Kamucheka, R.M.; Cancio, L.C.; Batchinsky, A.I.; Antebi, B. Mesenchymal Stem Cells Reconditioned in Their Own Serum Exhibit Augmented Therapeutic Properties in the Setting of Acute Respiratory Distress Syndrome. *Stem Cells Transl. Med.* **2019**, *8*, 1092–1106. [CrossRef]
123. Abreu, S.C.; Hampton, T.H.; Hoffman, E.; Dearborn, J.; Ashare, A.; Singh Sidhu, K.; Matthews, D.E.; McKenna, D.H.; Amiel, E.; Barua, J.; et al. Differential effects of the cystic fibrosis lung inflammatory environment on mesenchymal stromal cells. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2020**, *319*, 908–925. [CrossRef] [PubMed]
124. Enes, S.R.; Hampton, T.H.; Barua, J.; McKenna, D.H.; dos Santos, C.C.; Amiel, E.; Ashare, A.; Liu, K.D.; Krasnodembskaya, A.D.; English, K.; et al. Healthy versus inflamed lung environments differentially affect mesenchymal stromal cells. *Eur. Respir. J.* **2021**, *58*, 2004149. [CrossRef]
125. Goodwin, M.; Sueblinvong, V.; Eisenhauer, P.; Ziats, N.P.; LeClair, L.; Poynter, M.E.; Steele, C.; Rincon, M.; Weiss, D.J. Bone marrow-derived mesenchymal stromal cells inhibit Th2-mediated allergic airways inflammation in mice. *Stem Cells* **2011**, *29*, 1137–1148. [CrossRef] [PubMed]
126. Danchuk, S.; Ylostalo, J.H.; Hossain, F.; Sorge, R.; Ramsey, A.; Bonvillain, R.W.; Lasky, J.A.; Bunnell, B.A.; Welsh, D.A.; Prockop, D.J.; et al. Human multipotent stromal cells attenuate lipopolysaccharide-induced acute lung injury in mice via secretion of tumor necrosis factor-α-induced protein 6. *Stem Cell Res. Ther.* **2011**, *2*, 27. [CrossRef]
127. Jahromi, S.H.; Li, Y.; Davies, J.E.; Jahromi, M.S.H.; Li, M.Y. Effect of Tumor Necrosis Factor Alpha Dose and Exposure Time on Tumor Necrosis Factor-Induced Gene-6 Activation by Neonatal and Adult Mesenchymal Stromal Cells. *Stem Cells Dev.* **2018**, *27*, 44–54. [CrossRef]
128. Zhang, Y.; Yu, Z.; Jiang, D.; Liang, X.; Liao, S.; Zhang, Z.; Yue, W.; Li, X.; Chiu, S.-M.; Chai, Y.-H.; et al. iPSC-MSCs with High Intrinsic MIRO1 and Sensitivity to TNF-α Yield Efficacious Mitochondrial Transfer to Rescue Anthracycline-Induced Cardiomyopathy. *Stem Cell Rep.* **2016**, *7*, 749–763. [CrossRef]
129. Naftali-Shani, N.; Levin-Kotler, L.-P.; Palevski, D.; Amit, U.; Kain, D.; Landa, N.; Hochhauser, E.; Leor, J. Left Ventricular Dysfunction Switches Mesenchymal Stromal Cells Toward an Inflammatory Phenotype and Impairs Their Reparative Properties Via Toll-Like Receptor-4. *Circulation* **2017**, *135*, 2271–2287. [CrossRef] [PubMed]
130. Carty, F.; Mahon, B.P.; English, K. The influence of macrophages on mesenchymal stromal cell therapy: Passive or aggressive agents? *Clin. Exp. Immunol.* **2017**, *188*, 1–11. [CrossRef]
131. Stevens, H.Y.; Bowles, A.C.; Yeago, C.; Roy, K. Molecular Crosstalk Between Macrophages and Mesenchymal Stromal Cells. *Front. Cell Dev. Biol.* **2020**, *8*, 1436. [CrossRef] [PubMed]
132. Chiossone, L.; Conte, R.; Spaggiari, G.M.; Serra, M.; Romei, C.; Bellora, F.; Becchetti, F.; Andaloro, A.; Moretta, L.; Bottino, C. Mesenchymal Stromal Cells Induce Peculiar Alternatively Activated Macrophages Capable of Dampening Both Innate and Adaptive Immune Responses. *Stem Cells* **2016**, *34*, 1909–1921. [CrossRef] [PubMed]
133. Ko, J.H.; Lee, H.J.; Jeong, H.J.; Kim, M.K.; Wee, W.R.; Yoon, S.-O.; Choi, H.; Prockop, D.J.; Oh, J.Y. Mesenchymal stem/stromal cells precondition lung monocytes/macrophages to produce tolerance against allo- and autoimmunity in the eye. *Proc. Natl. Acad. Sci. USA* **2015**, *113*, 158–163. [CrossRef]
134. Liu, Y.; Zhang, R.; Yan, K.; Chen, F.; Huang, W.; Lv, B.; Sun, C.; Xu, L.; Li, F.; Jiang, X. Mesenchymal stem cells inhibit lipopolysaccharide-induced inflammatory responses of BV2 microglial cells through TSG-6. *J. Neuroinflammation* **2014**, *11*, 1–12. [CrossRef] [PubMed]
135. Luz-Crawford, P.; Djouad, F.; Toupet, K.; Bony, C.; Franquesa, M.; Hoogduijn, M.J.; Jorgensen, C.; Noël, D. Mesenchymal Stem Cell-Derived Interleukin 1 Receptor Antagonist Promotes Macrophage Polarization and Inhibits B Cell Differentiation. *Stem Cells* **2015**, *34*, 483–492. [CrossRef] [PubMed]
136. Ortiz, L.A.; DuTreil, M.; Fattman, C.; Pandey, A.C.; Torres, G.; Go, K.; Phinney, D.G. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 11002–11007. [CrossRef]
137. Ghanem, L.Y.; Mansour, I.M.; Abulata, N.; Akl, M.M.; Demerdash, Z.A.; El Baz, H.G.; Mahmoud, S.S.; Mohamed, S.H.; Mahmoud, F.S.; Hassan, A.S.M. Liver Macrophage Depletion Ameliorates The Effect of Mesenchymal Stem Cell Transplantation in a Murine Model of Injured Liver. *Sci. Rep.* **2019**, *9*, 35. [CrossRef] [PubMed]
138. Giri, J.; Das, R.; Nylen, E.; Chinnadurai, R.; Galipeau, J. CCL2 and CXCL12 Derived from Mesenchymal Stromal Cells Cooperatively Polarize IL-10+ Tissue Macrophages to Mitigate Gut Injury. *Cell Rep.* **2020**, *30*, 1923–1934.e4. [CrossRef]

139. Braza, F.; Dirou, S.; Forest, V.; Sauzeau, V.; Hassoun, D.; Chesné, J.; Cheminant-Muller, M.-A.; Sagan, C.; Magnan, A.; Lemarchand, P. Mesenchymal Stem Cells Induce Suppressive Macrophages Through Phagocytosis in a Mouse Model of Asthma. *Stem Cells* **2016**, *34*, 1836–1845. [CrossRef] [PubMed]
140. De Witte, S.F.; Luk, F.; Sierra-Parraga, J.M.; Gargesha, M.; Merino, A.; Korevaar, S.S.; Shankar, A.S.; O'Flynn, L.; Elliman, S.J.; Roy, D.; et al. Immunomodulation By Therapeutic Mesenchymal Stromal Cells (MSC) Is Triggered Through Phagocytosis of MSC By Monocytic Cells. *Stem Cells* **2018**, *36*, 602–615. [CrossRef] [PubMed]
141. Ko, J.H.; Kim, H.J.; Jeong, H.J.; Lee, H.J.; Oh, J.Y. Mesenchymal Stem and Stromal Cells Harness Macrophage-Derived Amphiregulin to Maintain Tissue Homeostasis. *Cell Rep.* **2020**, *30*, 3806–3820. [CrossRef] [PubMed]
142. Min, H.; Xu, L.; Parrott, R.; Overall, C.C.; Lillich, M.; Rabjohns, E.M.; Rampersad, R.R.; Tarrant, T.K.; Meadows, N.; Fernandez-Castaneda, A.; et al. Mesenchymal stromal cells reprogram monocytes and macrophages with processing bodies. *Stem Cells* **2021**, *39*, 115–128. [PubMed]
143. Weiss, A.R.R.; Lee, O.; Eggenhofer, E.; Geissler, E.; Korevaar, S.S.; Soeder, Y.; Schlitt, H.J.; Geissler, E.K.; Hoogduijn, M.J.; Dahlke, M.H.; et al. Differential effects of heat-inactivated, secretome-deficient MSC and metabolically active MSC in sepsis and allogenic heart transplantation. *Stem Cells* **2020**, *38*, 797–807. [CrossRef]
144. Carty, F.; Corbett, J.M.; da Cunha, J.P.M.C.M.; Reading, J.L.; Tree, T.; Ting, A.; Stubblefield, S.R.; English, K. Multipotent Adult Progenitor Cells Suppress T Cell Activation in In Vivo Models of Homeostatic Proliferation in a Prostaglandin E2-Dependent Manner. *Front. Immunol.* **2018**, *9*, 645. [CrossRef] [PubMed]
145. Armitage, J.; Tan, D.B.; Troedson, R.; Young, P.; Lam, K.-V.; Shaw, K.; Sturm, M.; Weiss, D.J.; Moodley, Y.P. Mesenchymal stromal cell infusion modulates systemic immunological responses in stable COPD patients: A phase I pilot study. *Eur. Respir. J.* **2018**, *51*, 1702369. [CrossRef] [PubMed]
146. De Oliveira, H.G.; Cruz, F.; Antunes, M.A.; Neto, A.V.D.M.; Oliveira, G.A.; Svartman, F.M.; Borgonovo, T.; Rebelatto, C.L.K.; Weiss, D.J.; Brofman, P.R.S.; et al. Combined Bone Marrow-Derived Mesenchymal Stromal Cell Therapy and One-Way Endobronchial Valve Placement in Patients with Pulmonary Emphysema: A Phase I Clinical Trial. *Stem Cells Transl. Med.* **2016**, *6*, 962–969. [CrossRef] [PubMed]
147. Stolk, J.; Broekman, W.; Mauad, T.; Zwaginga, J.; Roelofs, H.; Fibbe, W.; Oostendorp, J.; Bajema, I.; Versteegh, M.; Taube, C.; et al. A phase I study for intravenous autologous mesenchymal stromal cell administration to patients with severe emphysema. *Qjm: Int. J. Med.* **2016**, *109*, 331–336. [CrossRef] [PubMed]
148. Chambers, D.C.; Enever, D.; Ilic, N.; Sparks, L.; Whitelaw, K.; Ayres, J.; Yerkovich, S.; Khalil, D.; Atkinson, K.M.; Hopkins, P.M. A phase 1b study of placenta-derived mesenchymal stromal cells in patients with idiopathic pulmonary fibrosis. *Respirology* **2014**, *19*, 1013–1018. [CrossRef]
149. Ortiz, L.A.; Gambelli, F.; McBride, C.; Gaupp, D.; Baddoo, M.; Kaminski, N.; Phinney, D. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 8407–8411. [CrossRef]
150. Sabry, M.M.; Elkalawy, S.A.-E.; Abo-Elnour, R.K.E.-D.; Abd-El-Maksod, D.F. Histolgical AND Immunohistochemical Study on the Effect of Stem Cell Therapy on Bleomycin Induced Pulmonary Fibrosis in Albino Rat. *Int. J. Stem Cells* **2014**, *7*, 33–42. [CrossRef] [PubMed]
151. Laffey, J.G.; Matthay, M.A. FiftyYearsofResearchinARDS.Cell-based Therapy for Acute Respiratory Distress Syndrome. Biology and Potential Therapeutic Value. *Am. J. Respir. Crit. Care Med.* **2017**, *196*, 266–273. [CrossRef] [PubMed]

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RESEARCH ARTICLE

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Blockade of MIF biological activity ameliorates house dust mite-induced allergic airway inflammation in humanized MIF mice

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Abstract

Macrophage migration inhibitory factor (MIF) expression is controlled by a functional promoter polymorphism, where the number of tetranucleotide repeats ($CATT_n$) corresponds to the level of MIF expression. To examine the role of this polymorphism in a pre-clinical model of allergic asthma, novel humanized MIF mice with increasing CATT repeats ($CATT_5$ and $CATT_7$) were used to generate a physiologically relevant scale of airway inflammation following house dust mite (HDM) challenge. $CATT_7$ mice expressing high levels of human MIF developed an aggressive asthma phenotype following HDM challenge with significantly elevated levels of immune cell infiltration, production of inflammatory mediators, goblet cell hyperplasia, subepithelial collagen deposition, and airway resistance compared to wild-type controls. Importantly the potent MIF inhibitor SCD-19 significantly mitigated the pathophysiology observed in $CATT_7$ mice after HDM challenge, demonstrating the fundamental role of endogenous human MIF expression in the severity of airway inflammation in vivo. Up to now, there are limited reproducible in vivo models of asthma airway remodeling. Current asthma medications are focused on reducing the acute inflammatory response but have limited effects on airway remodeling. Here, we present a reproducible pre-clinical model that capitulates asthma airway remodeling and suggests that in addition to having pro-inflammatory effects MIF may play a role in driving airway remodeling.

Abbreviations: AHR, airway hyperresponsiveness; ANOVA, analysis of variance; BALF, bronchoalveolar lavage fluid; BMDM, bone marrow derived macrophage; CATT, tetranucleotide repeat sequence; CD74, cluster of differentiation 74; G, tissue damping; H, tissue elasticity; H&E, haematoxylin & eosin; HDM, house dust mite; hMIF, human macrophage migration inhibitory factor; IgE, Immunoglobulin E; IL, interleukin; I.N., intranasal; I.P., intraperitoneal; ISO-1, (S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester; Mch, methacholine; MIF, macrophage migration inhibitory factor; OVA, ovalbumin; PAS, periodic acid-schiff; PBS, phosphate buffered saline; R_N, airway resistance; RNA, ribonucleic acid; SCD-19, 3-(2-methylphenyl)-1H-isochromen-1-one; SEM, standard error mean; Th2, T helper type 2; WT, wildtype.

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KEYWORDS
airway inflammation, airway remodeling, allergic asthma, house dust mite, macrophage migration inhibitory factor, MIF, MIF inhibitors, severe asthma

1 | INTRODUCTION

Asthma is a complex multifactorial disease affecting over 300 million people worldwide.[1] Allergic asthma is characterized by sensitization to specific and/or non-specific stimuli resulting in airway hyperresponsiveness (AHR), airway inflammation, and goblet cell hyperplasia.[2,3] Common environmental stimuli include house dust mite (HDM), mold, cigarette smoke, and pet dander.[4,5] HDM, a trigger in up to 85% of asthmatic patients,[6] has proteolytic activity to cleave epithelial tight junctions after inhalation to permit uptake by submucosal antigen-presenting cells surrounding the upper airways.[7] Lung inflammation is orchestrated by the release of prototypical Th2 cytokines, IL-4, IL-5, and IL-13 which drive the release of inflammatory mediators into the surrounding microenvironment.[8] Preclinical models of allergic asthma have provided significant contributions for the understanding of allergic airway inflammation; however, we have limited access to reproducible models of asthma airway remodeling.[9]

Macrophage migration inhibitory factor (MIF) is detected at high levels in the bronchoalveolar lavage fluid (BALF) and serum of asthmatic patients.[10] The level of MIF expression can vary in humans due to a functional repeat polymorphism implicating a tetranucleotide sequence 'CATT', found at position -794 in the promoter region of the MIF gene.[11] Four types of allelic variations were found, classified as 5-CATT, 6-CATT, 7-CATT, and 8-CATT, with the 5-CATT repeat allele presenting the lowest promoter activity.[12] Interestingly low MIF 5-CATT allele correlates with lower levels of inflammation and thus milder forms of asthma. Studies have established a role for MIF in asthma, with the use of MIF deficient mice ($MIF^{-/-}$), anti-MIF antibodies, and small molecular weight inhibitors. In a mouse model of ovalbumin (OVA)-induced allergic airway inflammation, $MIF^{-/-}$ mice had lower levels of pulmonary inflammation, Th2 cytokines, and airway hyperresponsiveness (AHR) compared to wild-type controls.[10] Administration of a MIF neutralizing antibody mitigated the MIF-related induction of AHR in an OVA model, but notably had no effect on the production of Th2 cytokines or IgE.[13] The prototypical MIF antagonist ISO-1 could abrogate AHR and airway inflammation in mice challenged with HDM, along with illustrating MIF's role in epithelial barrier dysfunction in vitro.[14] A polyclonal anti-MIF antibody decreased cellular infiltration in BALF from OVA-induced allergic mice but failed to decrease Th2 cytokines or IgE.[15] The link between MIF and airway remodeling has also been investigated in OVA-challenged mice. ISO-1 decreased autophagy in smooth muscle cells, thus reducing the incidence of airway remodeling after OVA sensitization.[16]

Current treatments for asthma focus on the management of symptoms and consist of utilization of bronchodilators and glucocorticoid steroids to control the intensity and number of allergic exacerbations. The more recently developed biologics target Th2-driven inflammation; however, these medications have limited effects on airway remodeling.[9] MIF is known to suppress the action of glucocorticoids[17,18] and therefore the levels of MIF expressed by asthma patients may have a major impact on their responsiveness to therapeutic strategies.

To better understand the potential impact of high allele human MIF expression on the severity of allergic airway inflammation and remodeling, we have generated novel humanized mice expressing high (CATT7) or low (CATT5) levels of human MIF. Using a specific MIF inhibitor, we have examined the specificity of human MIF expression in driving HDM-induced allergic airway inflammation.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

All procedures involving the use of animals were carried out by licensed personnel. Ethical approval for all work was granted by the ethics committee of Maynooth University (BRESC-2018-13). Project Authorization was received from the HPRA (AE19124/P022), whereby the terms of the animal experiments within this project were outlined and adhered to.

2.2 | Humanized MIF mice

Two mouse strains expressing the human high- or low-expression *MIF* alleles (e.g., MIF^{CATT7} [C57BL/6NTac-Miftm3884.1(MIF)Tac-Tg(CAG-Flpe)2Arte] and MIF^{CATT5} [C57BL/6NTac-Miftm3883.1(MIF)Tac-Tg(CAG-Flpe)2Arte] mice) were created using vector-based recombinant replacement of murine *Mif* by Taconic Biosciences (Rensselaer, NY, US). Validation of the expression of human and not murine *MIF* mRNA was verified by qPCR, and -794 CATT-length dependent stimulated MIF production was confirmed in vivo.[19]

2.3 | Mouse model of house dust mite-induced acute allergic airway inflammation

WT, CATT5 and CATT7 mice (6–18 weeks old) were challenged with 25 μg of house dust mite (HDM) allergen, *Dermatophagoides pteronyssinus* (Greer Labs, Lenoir, NC, US) or PBS control intranasally (I.N.) 3 days weekly for 3 weeks under light isoflurane anesthesia.

2.4 | MIF inhibitors

SCD-19 (3-(2-methylphenyl)-1H-isochromen-1-one) (Specs.net), or ISO-1 ((S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester) (Tocris) small molecular weight inhibitors of macrophage migration inhibitory factor (MIF) enzymatic activity was used in a house dust mite model of acute allergic airway inflammation. 35 mg/kg of SCD-19 was administered intraperitoneally (I.P.) twice weekly for three weeks; day 0, 4, 7, 11, 14, and 18.

2.5 | Histology

On day 21 of the model, lungs were harvested. Tissue was fixed in 10% (v/v) neutral buffered formalin (Sigma-Aldrich) for 24 h. Tissue was then processed using an automated processor (Shandon Pathcentre, Runcorn, UK) and embedded in paraffin wax using a Shannon Histocentre 2 (Shandon). Once sectioned with a Shandon Finesse 325 microtome (Thermo-Shandon, Waltham, MA, USA), tissue sections (5 μm) were stained for Masson's Trichrome (Sigma-Aldrich), Periodic Acid Schiff (Abcam) and Haemotoxylin and Eosin-Y (Richard Allan Scientific). Samples were air dried and a coverslip was mounted with DPX mounting media (BDH). 4× and 20× images were taken using an Olympus BX51 light microscope.

2.6 | Histological scoring

Following staining, slides were coded without reference to prior treatment and examined in a blind manner. For H&E, images were scored using a composite scale from 1 to 9; comprising of infiltration or aggregation of inflammatory cells in air space or vessel wall [1 = only wall, 2 = few cells (1–5 cells) in air space, 3 = intermediate, 4 = severe (air space congested)]; interstitial congestion and hyaline membrane [formation: 1 = normal lung, 2 = moderate (<25% of lung section), 3 = intermediate (25%–50% of lung section), 4 = severe (>50% of lung section)]; hemorrhage: (0 = absent, 1 = present).[20] For periodic acid-schiff (PAS), images were scored by counting the number of PAS-positive (magenta) mucin-producing goblet cells present within the airway, relative to the diameter to the airway. Collagen deposition was calculated by analyzing the % of positive staining following Masson's Trichrome staining using the trainable Weka segmentation plugin on Fiji open-source software.

2.7 | Bronchoalveolar lavage fluid (BALF) Retrieval

Mice were sacrificed by lethal overdose of sodium pentobarbital via I.P. injection on day 18 of the model, 4 hr after last challenge. A tracheostomy and cannulation was performed, where a 27 gauge cannula was secured in place with sutures. 1 mL of cold endotoxin-free PBS was infused into the lungs through the cannula using a 1 mL syringe for 3 gentle instillations. BALF was placed into an eppendorf and kept on ice before being centrifuged at 300*g* for 5 min at 4°C. The supernatant was collected, aliquoted and 10× protease inhibitor solution (Roche) was added to prevent protein degradation.

2.8 | BALF cell analysis

Cells were isolated and resuspended in 100 μL of endotoxin-free PBS for counting. Cyto-spin funnels were pre-wet by spinning with 300 μL of PBS onto glass slides at 600 rpm for 5 min. 1×10^5 cells in a volume of 300 μL of PBS were spun onto fresh labeled glass slides at 600 rpm for 10 min using a RotoFix 32 cytocentrifuge (Hettich Zentrifugen). Slides were airdried before being stained with Kwik-Diff™ Stain (Shandon, ThermoScientific); 25 s in fixative, 15 s in solution I and 15 s in solution II. Slides were imaged on an Olympus BX51 light microscope until 300 cells could be counted. Cells were identified as being neutrophils, eosinophils, macrophages, or lymphocytes.

2.9 | Cytokine analysis

BALF supernatants were analyzed for Th2 cytokines IL-4 (Biolegend), IL-5 (Biolegend), IL-13 (eBioscience), and human MIF (R&D Systems) by ELISA following the manufacturer's instructions.

2.10 | FlexiVent® lung function

Mice were anesthetized with 150 mg/kg ketamine and 2 mg/kg medetomidine via subcutaneous injection and

the surgical plane of anesthesia was reached. A tail vein catheter was inserted. Tracheostomy and cannulation was carried out. The mouse was placed close to the FlexiVent FX system (SCIREQ, Emka Technologies, Paris, France) and mechanical ventilation was initiated by selecting a predefined ventilation. Every 6 min, alfaxan and 0.5 mg/kg Tracium, a neuromuscular blocking agent (NMBA), was administered through the tail vail catheter. The measurement of lung function was initiated and approximately 100 µL of PBS or increasing concentrations of the bronchoconstrictor methacholine (MCh) (3.125, 12.5, and 25 mg/mL) was loaded into the nebulizer. Upon completion of lung function measurements at baseline and following increasing aerosolized methacholine challenges, the ventilator was stopped and the mouse was euthanized using either I.P. injection of sodium pentobarbital or via cervical dislocation.

2.11 | Statistical methods

All data are presented as mean ± SEM. Results of two or more groups were compared by analysis of variance (ANOVA) followed by the post-hoc Tukey's multiple comparison test. were analyzed using a statistical software package (GraphPad Prism, San Diego, CA). Response to different concentrations of methacholine was analyzed by 2-way ANOVA followed by the *post-hoc* Tukey's multiple comparison test. GraphPad Prism (GraphPad Software Inc, San Diego, CA, USA) was used for all statistical analyses.

3 | RESULTS

3.1 | Functional –794CATT polymorphic mouse tissues express different levels of hMIF under basal and disease conditions

Novel humanized MIF mice were generated to capitulate the varying levels of MIF expression under the functional promoter polymorphism within the human population. C57BL/6 mice were humanized by replacing the murine MIF gene with the human counterpart. Within this human MIF gene, 794 downstream of the promoter region, where the number of tetranucleotide repeats correlates with MIF allele expression,[11] 5 repeats of this tetranucleotide sequence 'CATT' generated $CATT_5$ mice, containing the low expressing MIF allele. 7 repeats of this tetranucleotide sequence 'CATT' generated $CATT_7$ mice, containing the high expressing MIF allele (Figure 1A). To characterize the effect of the CATT microsatellite repeat we analyzed hMIF production under basal and disease conditions. Bronchoalveolar lavage fluid (BALF) (Figure 1B), bone marrow-derived macrophages (BMDMs) (Figure 1C), and splenocytes (Figure 1D) isolated from $CATT_7$ mice secrete significantly higher levels of hMIF than $CATT_5$ or wildtype (WT) mice (Figure 1B–D).

To investigate the role of the human MIF polymorphism in a disease setting, a model of acute allergic airway inflammation was generated. The clinically relevant house dust mite (HDM) allergen was administered intranasally three times a week for three weeks to induce airway inflammation (Figure 1E). BALF was obtained, and lungs were snap frozen for RNA isolation and qPCR analysis on day 21. Significantly higher levels of hMIF were detected in $CATT_7$ BALF compared to BALF from $CATT_5$ or WT mice (Figure 1F). Similarly, the relative expression of human *Mif* was significantly increased in the $CATT_7$ compared to the $CATT_5$ mouse lung tissue (Figure 1G).

These data comprehensively show that the CATT polymorphism is responsible for differential production of hMIF under basal and disease conditions. This model allows us to investigate the role of high versus low hMIF in the pathophysiology of acute airway inflammation in a relevant pre-clinical model.

3.2 | Human $CATT_7$ allele significantly increases the Th2 signature in an HDM model of allergic airway inflammation

Absence of MIF in models of allergic inflammation has been shown to reduce the levels of Th2 cytokines in the BALF.[10,21] To study the effect of different levels of endogenous MIF on asthma severity, $CATT_7$, $CATT_5$, and WT C57BL/6 mice were challenged with HDM intranasally 3 times a week for 3 consecutive weeks. 4 hr post final challenge the mice were sacrificed, and the BALF was obtained. The BALF total leukocyte counts show that $CATT_7$ mice challenged with HDM have significantly higher numbers of immune cells in the bronchoalveolar space compared to WT mice (Figure 2A). $CATT_7$ mice also exhibit a marked increase in cell number compared to $CATT_5$ although not significant (Figure 2A). Differential cell counts demonstrated that the predominant cell type in the BALF are eosinophils (Figure 2B,C).

To further characterize the influence of hMIF on asthma pathophysiology we explored its effects on the prototypical Th2 signature. IL-4, IL-5, and IL-13 have been shown to be critical in the development of airway hyperresponsiveness, eosinophilic responses, and goblet cell hyperplasia, and responsible for the overall asthma phenotype. Th2 cytokines were detected in the BALF of $CATT_5$ and WT HDM mice (Figure 2D–F). However, we observed significantly elevated levels of IL-4, IL-5,

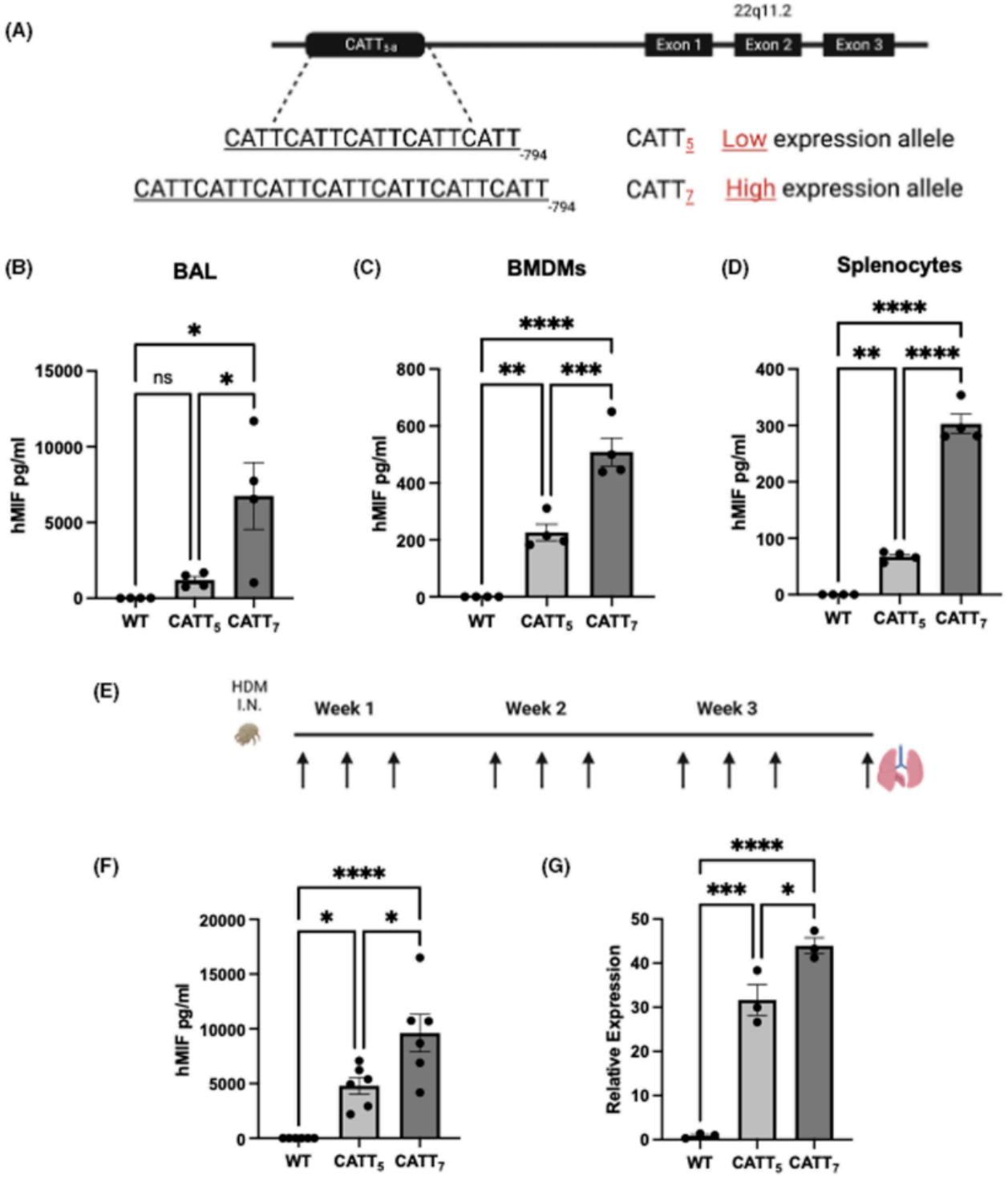

FIGURE 1 Functional −794CATT polymorphisms lead to different expression levels of hMIF under basal and disease conditions. (A) Human MIF gene containing a functional polymorphism in position 794 downstream of the promoter region, where the number of tetranucleotide repeats correlates with promoter activity and MIF allele expression; 5 repeats = $CATT_5$ mice, 7 repeats = $CATT_7$ mice. (B) hMIF levels in BAL fluid of naïve WT, $CATT_5$ and $CATT_7$ mice. (C) hMIF production from BMDMs of WT, $CATT_5$, and $CATT_7$ mice. (D) hMIF production from splenocytes of WT, $CATT_5$, and $CATT_7$ mice. (E) Timeline for HDM exposure. Mice were intranasally challenged with HDM 3 times a week for 3 consecutive weeks. (F) Levels of hMIF detected in the BAL fluid of WT, $CATT_5$, and $CATT_7$ mice challenged with HDM. (G) Relative expression of hMIF detected in lung homogenates of WT, $CATT_5$, and $CATT_7$ mice challenged with HDM detected by real-time PCR. Data are presented as mean ± SEM; $N = 3–6$ per group. $^*p < .05$; $^{**}p < .01$; $^{****}p < .0001$.

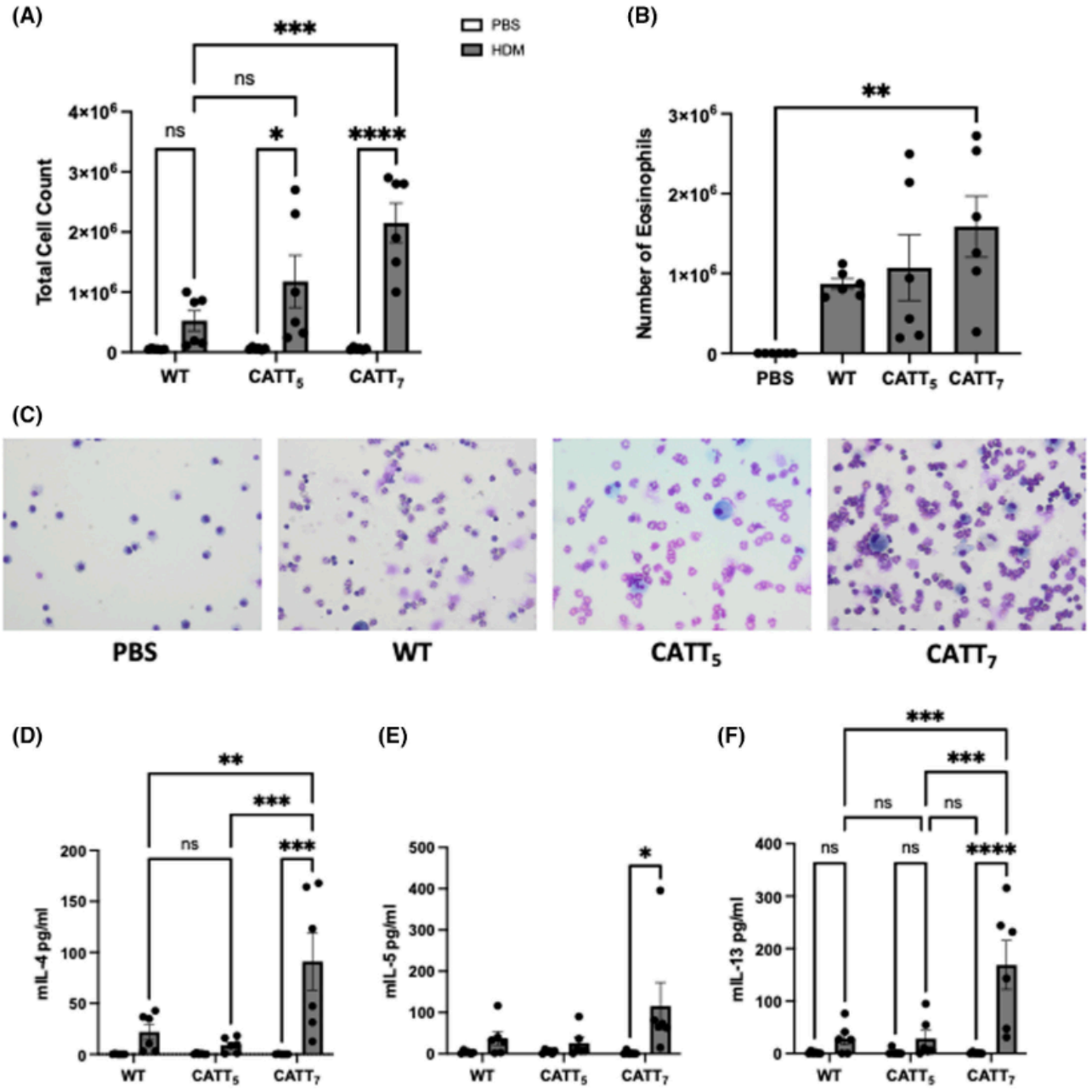

FIGURE 2 Human $CATT_7$ allele significantly increases the Th2 cytokine signature in a HDM model of allergic airway inflammation. (A) Total cell count recovered from the BAL. (B) BAL fluid eosinophil count determined by differential staining of cytospins. (C) Representative imgaes of cytosins. Cytokine levels of (D) IL-4, (E) IL-5, and (F) IL-13 in the BAL fluid determined by ELISA. Data are presented as mean ± SEM; $N = 6$ per group. $^*p < .05$; $^{**}p < .01$; $^{***}p < .001$; $^{****}p < .0001$.

ad IL-13 in $CATT_7$ HDM compared to $CATT_7$ PBS group (Figure 2D–F). Furthermore, significantly higher levels of IL-4 and IL-13 were detected in $CATT_7$ HDM compared to $CATT_5$ and WT HDM (Figure 2D,F). A marked increase in IL-5 was also detected although not significant (Figure 2E). These data show that the $CATT_7$ polymorphism generates a prominent Th2 cytokine profile which may contribute to a more severe asthma phenotype.

3.3 | The human $CATT_7$ allele exacerbates hallmarks of asthma pathophysiology

Excessive production of mucus and the associated pathophysiological changes are hallmarks in a range of respiratory diseases including asthma.[22] We investigated the effect of the CATT polymorphism on goblet cell hyperplasia by staining lung tissue with PAS (Figure 3A). PBS control groups exhibited very low levels of PAS-positive

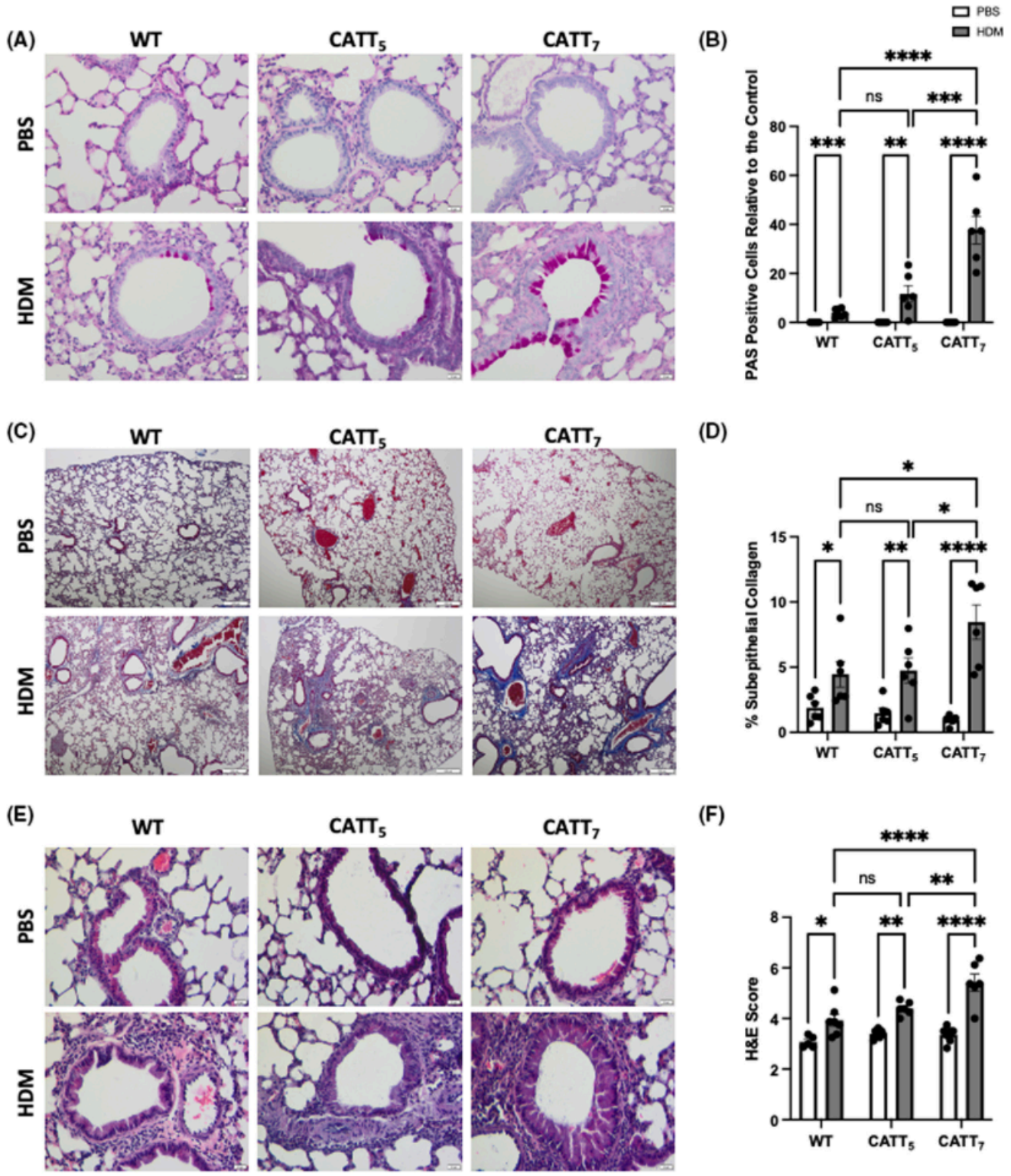

FIGURE 3 Human $CATT_7$ allele exacerbates airway inflammation in a house dust mite model of allergic asthma. (A) Representative images of lung tissue stained with periodic acid Schiff at 20× magnification, scale bar = 20 μm. (B) Goblet cell hyperplasia was investigated through the quantitation of PAS-positive cells. (C) Representative images of lung tissue stained with Masson's trichrome at 4× magnification, scale bar = 200 μm. (D) Quantitation of % subepithelial collagen. (E) Representative images of lung tissue stained with H&E from WT, 5CATT, and 7CATT mice challenged with HDM or PBS control at 20× magnification, scale bar = 20 μm. (F) Quantitation of airway inflammation in H&E-stained lung tissue. Data are presented as mean ± SEM; $N = 6$ per group. *$p < .05$; **$p < .01$; ***$p < .001$; ****$p < .0001$.

staining, whilst $CATT_5$ HDM exhibit slightly higher numbers of PAS-positive cells relative to the control compared to the WT (Figure 3A,B). Strikingly, $CATT_7$ mice have significantly higher levels of goblet cell hyperplasia compared to $CATT_5$ and WT mice following the HDM challenge (Figure 3B).

In addition to investigating goblet cell hyperplasia, we examined the effect of the −794CATT polymorphism on airway remodeling. To determine the extent of remodeling, we stained lung tissue with Masson's trichrome to highlight subepithelial collagen. The trends in the Masson's trichrome staining mirrored that of the PAS, with $CATT_5$, and WT mice displaying similar levels of disease pathology. Both groups exhibit a significant yet small increase in collagen deposition compared to the PBS controls (Figure 3C,D). $CATT_7$ mice challenged with HDM display significantly higher levels of subepithelial fibrosis compared to the lower MIF expressing $CATT_5$ allele and WT groups (Figure 3D); suggesting that high levels of hMIF contribute to airway remodeling in a HDM model of allergic airway inflammation.

H&E staining revealed that HDM significantly induces cellular infiltration surrounding the airways in all groups compared to the PBS control (Figure 3E,F). However, $CATT_7$ HDM exhibited significantly higher airway immune cell infiltration compared to both $CATT_5$ and WT HDM (Figure 3E,F). There was no significant difference in immune cell infiltration between the $CATT_5$ and WT HDM groups according to the H&E score (Figure 3F).

These data suggest that high levels of human MIF exacerbate allergic airway pathophysiology by increasing mucin production through the induction of goblet cell hyperplasia, increasing deposition of subepithelial collagen thereby contributing to airway remodeling, and increasing the infiltration of immune cells surrounding the airways.

3.4 | High levels of human MIF alter HDM-induced lung mechanics in response to increasing concentrations of methacholine

Airway hyperresponsiveness (AHR) and remodeling are a major hallmark of asthma and allergic airway inflammation, and as such it is important for models to represent this. We examined HDM-induced AHR in response to aerosolized methacholine challenge using the FlexiVent system. $CATT_7$ HDM mice exhibited a marked increase in airway resistance (R_N) at 12.5 mg/mL and 25 mg/mL doses compared to the rest of the groups (Figure 4A). A trend of increased tissue damping (G) (Figure 4B) and tissue elastance (H) (Figure 4C) was demonstrated in the $CATT_7$ mice at the 25 mg/mL dose. It has been well documented

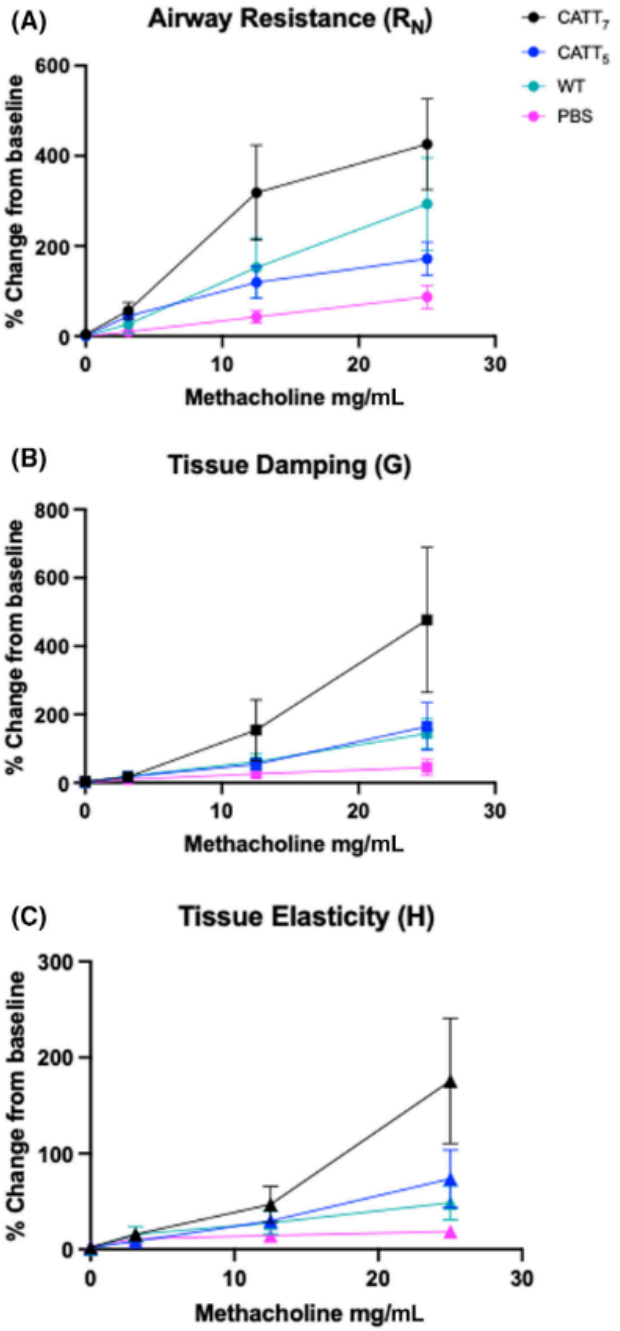

FIGURE 4 Changes in lung mechanics in response to increasing concentrations of methacholine in HDM-challenged $CATT_7$, $CATT_5$, and WT mice. Airway hyperresponsiveness determined (A) by airway resistance, (B) tissue damping, and (C) tissue elastance (R_N, G, and H respectively). Data are presented as peak response normalized to the baseline and expressed as % increase over baseline ± SEM; $N = 6$ per group.

that due to genetic differences, C57BL/6 mice have a higher resistance to airway hyperresponsiveness compared to the more sensitive BALB/c mice in acute airway inflammatory models[23,24] and this is reflected here in our

humanized MIF mice. These data show that $CATT_7$ mice challenged with HDM exhibit higher levels of airway remodeling and AHR compared to WT C57BL/6 mice.

3.5 | The MIF inhibitor SCD-19 decreases HDM-induced total cell counts and Th2 cytokines in BALF from $CATT_7$ mice

High human MIF expression has been demonstrated to play a role in driving the physiological hallmarks of allergic airway inflammation such as eosinophil infiltration (Figure 2A,B), increased Th2 cytokines (Figure 2D–F), goblet cell hyperplasia (Figure 3A,B), subepithelial collagen deposition (Figure 3C,D), and decreased lung function (Figure 4).

MIF is known to initiate its biological effects through its active site.[25] Therefore, to fully elucidate MIF's role in our model, the MIF antagonist SCD-19 was utilized to block this active site, as this alters the conformation of MIF and impairs its interaction with other molecules. Throughout the previous data, no significant difference was noted between the low MIF expressing $CATT_5$ mice and WT mice. Thus, the remainder of this study focused on comparing the high MIF expressing $CATT_7$ mice and WT mice. In addition to receiving HDM challenge, SCD-19 was administered intraperitoneally twice a week for three weeks.

Administration of SCD-19 to $CATT_7$ mice challenged with HDM significantly decreased human MIF production in BMDMs derived from $CATT_7$ mice (Supp. Figure 1). The total BALF cell count was decreased in $CATT_7$ HDM-challenged mice that received SCD-19 compared to HDM-challenged $CATT_7$ mice that received the vehicle control (Figure 5A). There was no significant difference between WT groups. BALF from SCD-19-treated $CATT_7$ mice had decreased numbers of eosinophils compared to $CATT_7$ mice that received the vehicle control (Figure 5B). There was no significant difference between the WT groups. Although not statistically significant, there is a visible trend

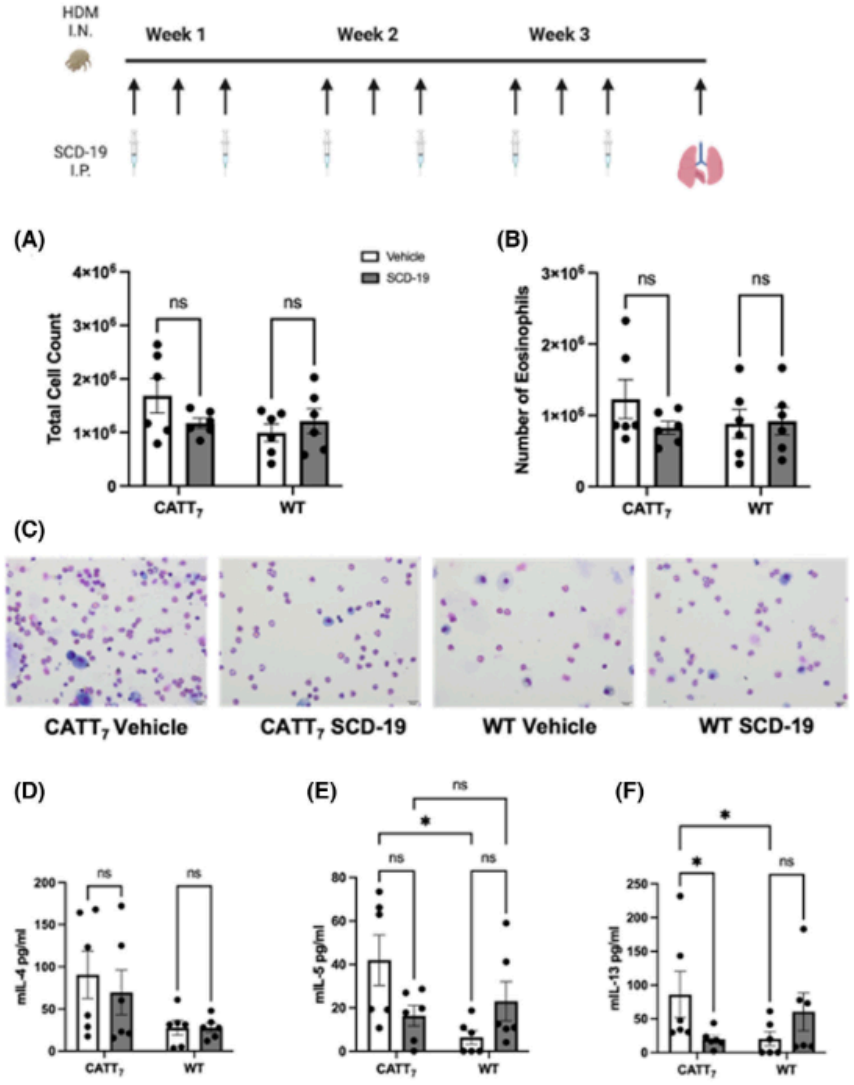

FIGURE 5 MIF inhibitor SCD-19 decreases total cell counts and Th2 cytokines in BALF from $CATT_7$ mice. Mice were challenged with 25 μg of HDM I.N. three times a week for three weeks, in addition to receiving 35 mg/kg of SCD-19 or vehicle control I.P. twice weekly for three weeks. Schematic created using Biorender.com. (A) Total cell count recovered from BAL fluid. (B) Number of Eosinophils from differential cell counts of BAL fluid from $CATT_7$ and WT mice. (C) 300 cells were counted and identified based on morphology. Cytokine levels of (D) IL-4, (E) IL-5, and (F) IL-13 in the BAL fluid determined by ELISA. Data are presented as mean ± SEM; $N = 6$ per group. *$p < .05$; **$p < .01$; ***$p < .001$; ****$p < .0001$.

of decreased total cellular infiltration in the BALF of SCD-19-treated $CATT_7$ mice compared to vehicle control mice.

No SCD-19-specific differences were observed in BALF IL-4 protein levels (Figure 5D). However, lower levels of IL-5 were detected in the BALF of SCD-19 $CATT_7$ mice compared to vehicle control $CATT_7$ mice (Figure 5E). There was no significant difference between SCD-19 and vehicle control WT groups, which was expected as we have previously noted that WT mice already have low levels of MIF production. Importantly, levels of BALF IL-13 were significantly decreased in SCD-19 $CATT_7$ mice compared to vehicle control $CATT_7$ mice (Figure 5F).

3.6 | SCD-19 significantly decreases HDM-induced lung pathology in $CATT_7$ mice

High levels of human MIF in the $CATT_7$ mice drive airway remodeling and inflammation following repeated exposure to the clinically relevant allergen HDM. Here we examined the capacity for SCD-19 to prevent this exacerbation-related pathology.

SCD-19 significantly abrogated the number of PAS-positive cells present compared to $CATT_7$ mice that received the vehicle control (Figure 6A,B). As expected, there was no significant difference between WT mice that received SCD-19 and the vehicle control (Figure 6A,B). In high human MIF expressing $CATT_7$ mice, SCD-19 significantly reduced the percentage of subepithelial collagen present after three weeks of intervention, compared to the vehicle control (Figure 6C,D). $CATT_7$ vehicle mouse lung sections had statistically significant higher levels of collagen deposition compared to WT vehicle mice. Moreover, SCD-19 had no effect in WT mice (Figure 6C,D). Lastly, lung sections were analyzed for cellular infiltration using H&E staining. High expression of human MIF amplified cellular infiltration, as $CATT_7$ mice that received SCD-19 had a significantly lower H&E score compared to those that received the vehicle control (Figure 6E,F). $CATT_7$

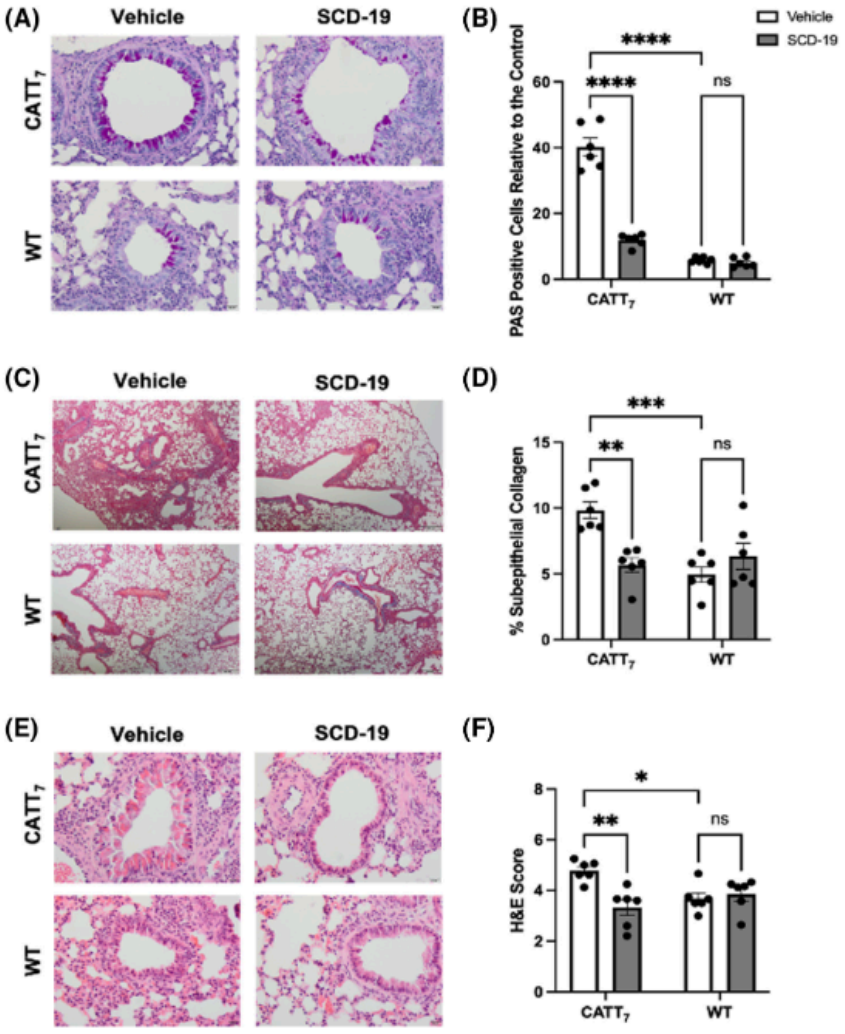

FIGURE 6 MIF antagonist SCD-19 significantly decreases HDM-induced allergic airway inflammation in $CATT_7$ mice. (A) Representative images of lung tissue stained with Periodic Acid Schiff at 20× magnification, scale bar = 20 μm. (B) Goblet cell hyperplasia was investigated through the quantitation of PAS-positive cells relative to the control. (C) Representative images of lung tissue stained with Masson's trichrome at 4× magnification, scale bar = 200 μm. (D) Quantitation of % subepithelial collagen. (E) Representative images of lung tissue stained with H&E from WT and $CATT_7$ mice challenged with HDM or PBS control and received SCD-19 or vehicle control at 20× magnification, scale bar = 20 μm. (F) Quantitation of airway inflammation in H&E-stained lung tissue. Data are presented as mean ± SEM; $N = 6$ per group. *$p < .05$; **$p < .01$; ***$p < .001$; ****$p < .0001$.

vehicle mice had significantly higher H&E scores compared to WT vehicle mice. Moreover, similarly to our previous histological findings, SCD-19 had no effect in WT mice (Figure 6E,F). In a similar manner, the MIF inhibitor; ISO-1 significantly reduced goblet cell hyperplasia, subepithelial collagen deposition and airway inflammation in CATT7 mice challenged with HDM (Supporting Information Figure S2). In WT mice, ISO-1 had no effect (Supporting Information Figure S2).

These data support our hypothesis that MIF is a key factor in driving acute airway inflammation in our house dust mite model, as blocking the biological activity of MIF reduced lung inflammation.

3.7 | SCD-19 improves lung function by decreasing airway resistance in $CATT_7$ mice in response to increasing concentrations of methacholine

We have previously shown that mice possessing the high human MIF expression allele ($CATT_7$) had increased airway resistance (Figure 4A), tissue damping (Figure 4B), and tissue elasticity (Figure 4C) after inhaling increasing concentrations of methacholine compared to mice possessing the low human MIF expression allele ($CATT_5$) and WT mice.

To further investigate our hypothesis that human MIF is driving the development of preclinical signs of allergic airway inflammation following HDM challenge in the $CATT_7$ mice, we measured the respiratory mechanics of mice that received SCD-19. In $CATT_7$ mice, SCD-19 had the ability to decrease HDM-induced inflammation at a mechanical level, by reducing the percentage increase of airway resistance (Figure 7A), tissue damping (Figure 7B), and tissue elasticity (Figure 7C) from baseline, compared to $CATT_7$ vehicle mice.

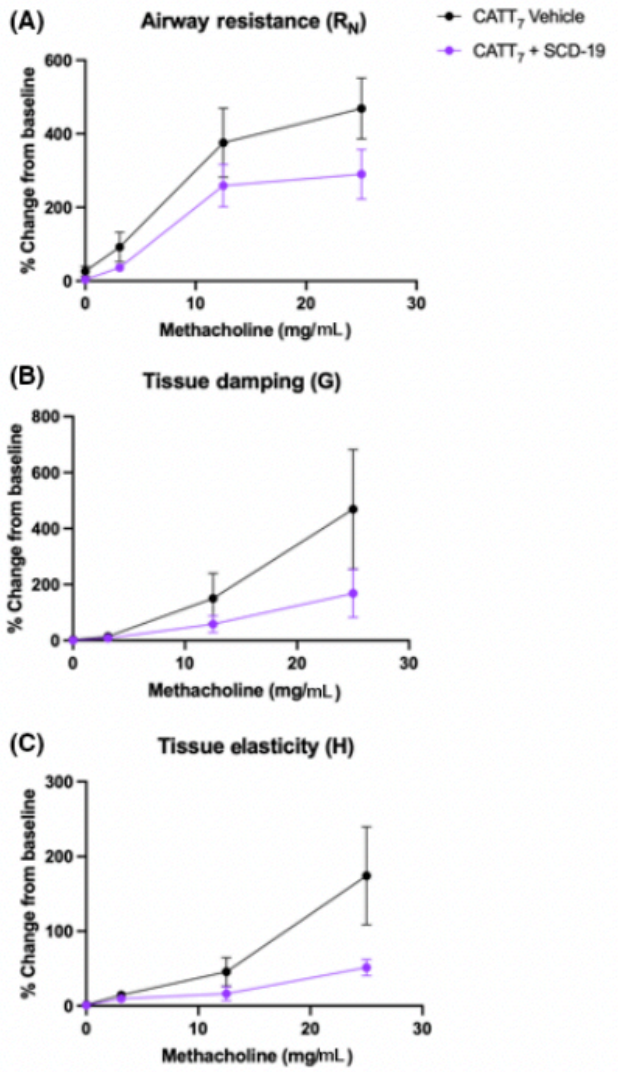

FIGURE 7 SCD-19 can decrease HDM-induced airway resistance (R_N), tissue damping (G), and tissue elasticity (H) in $CATT_7$ mice compared to vehicle control. $CATT_7$ mice were challenged with 25 μg of HDM or PBS control I.N. three times a week for three weeks, in addition to receiving 35 mg/kg of SCD-19 or vehicle control I.P. twice weekly for three weeks. 24 hr after last challenge, a tracheostomy was performed and lung function was measured using a FlexiVent® instrument (SCIREQ) in response to PBS or increasing concentrations of methacholine (3.125, 12.5, and 25 mg/mL). (A) Airway Resistance. (B) Tissue Damping. (C) Tissue Elasticity. Data are presented as peak response normalized to the baseline and expressed as % increase over baseline $N = 4–6$ per group.

4 | DISCUSSION

The biological role of macrophage migration inhibitory factor (MIF) has been previously documented in a plethora of inflammatory lung conditions,[19,26–31] including asthma.[10,13,16,32,33] The low human MIF expressing $CATT_5$ promoter polymorphism correlates with a milder manifestation of asthma symptoms.[10] We hypothesized that the $CATT_7$ promoter polymorphism which expresses high levels of human MIF could be linked to increased severity of allergic asthma. Using novel humanized MIF mice to create a physiological scale of allergic airway inflammation in response to the clinically relevant aeroallergen house dust mite, this study set out to investigate the biological role of this pro-inflammatory cytokine on key hallmarks associated with this atopic condition.

We demonstrate that intranasal challenge of HDM can drive enhanced MIF production in the lung of $CATT_7$ and $CATT_5$ mice, perhaps illustrating a positive feedback loop resulting in the exacerbations of physiological asthmatic characteristics. In a similar fashion, MIF is known to act

in an autocrine and paracrine fashion to promote downstream cytokine production.[18,21,34,35]

MIF's crucial contribution in this model of house dust mite-induced allergic airway inflammation was particularly clear at a histological level with significantly increased mucin-producing goblet cell hyperplasia and subepithelial collagen deposition in the presence of high levels of human MIF in $CATT_7$ mice. Airway remodeling occurs in uncontrolled cases of asthma, as repeated lung injury by inhaled insults and over-production of fibrotic tissue result in goblet cell hyperplasia and increased subepithelial collagen. This alteration in tissue architecture has consequences in the mechanical functioning of the lung, resulting in an increase in airway hyperresponsiveness, resistance, tissue damping, and elasticity. Our findings further clarified the physiological role of MIF in HDM-induced airway inflammation, as humanized high MIF expressing $CATT_7$ mice had increased airway resistance, tissue damping (energy dissipation into alveoli), and elastance (energy conservation in the alveoli) in response to increasing concentrations of the chemical bronchoconstrictor methacholine. This study provides new insights on the role of MIF in driving airway remodeling.

Blockade of MIF using the small molecule antagonist (S,R)3-(4-hy-droxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1) has been shown to significantly reduce the pathology associated with OVA-induced,[36] HDM-induced[14] airway inflammation and remodeling and in a neutrophilic experimental severe asthma model.[37] The MIF small molecule inhibitor SCD-19 has been tested in a range of disease systems including lung cancer[25] and infectious disease[38] studies. SCD-19 potently inhibits MIF activity but its therapeutic efficacy had not been tested in an allergic airway inflammation model. Although previous studies have utilized anti-MIF antibodies and the small molecule inhibitor ISO-1 in mouse models of inflammatory conditions,[14,15,36,39] this is the first study to do so in humanized MIF mice expressing human relevant MIF polymorphic alleles.

In this study, SCD-19 significantly mitigated the MIF-associated increase in inflammatory histopathology, in a dose-dependent manner, reiterating the importance of this cytokine in the induction and maintenance of experimental asthma, as described previously.[36] SCD-19 decreased the total cells present in bronchoalveolar lavage in $CATT_7$ mice. SCD-19 had no effect on IL-4 production, but decreased IL-5 and significantly decreased IL-13 production in the BALF from $CATT_7$ mice compared to vehicle control. In line with our data, administration of an anti-MIF antibody in an OVA model also did not affect IL-4 levels in BALF, but anti-MIF treatment during OVA sensitization significantly decreased eosinophil infiltration in BALB/c mice.[13] Conversely in C57BL/6 mice, MIF inhibition with SCD-19 did not decrease eosinophil infiltration in the BALF of HDM-challenged $CATT_7$. Transgenic mice are routinely generated on a C57BL/6 background, which may be a limitation of this study, as Th2 atopic allergy models are usually performed in BALB/c. As a result, readouts may have a lower baseline than those performed in BALB/c mice. Similarly, levels of cellular and eosinophil infiltration may be lower than suspected in our model, due to the mice used being generated of the C57BL/6 genetic background. Furthermore, SCD-19 may not decrease eosinophil infiltration as efficiently as seen with an anti-MIF antibody in an OVA model,[13] as our Th2 response was not high enough at baseline levels to be further suppressed by SCD-19. The timing of MIF inhibitor administration is fundamental, as the use of ISO-1 in a model of severe neutrophilic asthma could only abrogate HDM-induced airway inflammation when administered both 30 min prior and 6 h after allergen challenge.[37] Taking into account the SCD-19 dosing regimen, along with the suboptimal genetic background of the mice used, this may help to explain the incomplete effects of SCD-19 in this model. As previously mentioned, collagen deposition and goblet cell hyperplasia were seen to be significantly increased at this acute timepoint, but more HDM challenges might be required to see the full effects of chronic inflammation in our model. SCD-19 blocks MIF's conformationally sensitive tautomerase active site that overlaps functionally with MIF (CD74) receptor binding. Here we show that SCD-19 extensively diminishes HDM-induced histopathology in $CATT_7$ mice, along with having a subtle anti-inflammatory impact in the BALF of these mice when administered starting at first allergen challenge. Moreover, SCD-19 abrogated the airway resistance (R_N), tissue damping (G), and tissue elasticity (H) in $CATT_7$ mice compared to the vehicle control, showing a clear association between lower levels of tissue remodeling at a histological level and overall lung function.

In terms of asthma therapies, MIF is known to counter-regulate the effects of glucocorticoids, a steroidal treatment to manage severe asthmatic symptoms. In the future, small molecular weight MIF inhibitors may not only be used as a monotherapy for asthma patients with high MIF expression 7–7 genotypes (those genetically pre-dispositioned to secrete higher levels of this pro-inflammatory cytokine), but also as part of a synergistic regimen where they could initially work to inhibit MIF function, but also to enhance or restore the efficacy of glucocorticoids in the clinic.

This study demonstrated the ability of the MIF antagonist SCD-19 to abrogate HDM-induced cellular

infiltration, goblet cell hyperplasia, and subepithelial fibrosis. Furthermore, SCD-19 decreased airway hypersensitivity, but did not affect cell populations within the BALF retrieved from these novel humanized MIF mice.

Here we demonstrate that high MIF allele expression leads to enhanced severity of allergic airway inflammation driven by the clinically relevant allergen HDM. This study is the first to use novel humanized MIF mice to investigate the role of endogenous MIF expression on house dust mite-induced allergic asthma by utilizing small molecular weight inhibitors in vivo.

To conclude, this study demonstrates the important role of MIF in further driving allergic airway inflammation and potentially airway remodeling and provides a novel, clinically relevant, and reproducible model of allergic airway remodeling. Further experiments are required however, perhaps using therapeutic as opposed to prophylactic administration of SCD-19 to determine the ability of SCD-19 to reduce airway remodeling. These data pave the way for a new therapeutic avenue for the utilization of small molecule anti-MIF strategies that are both ant-inflammatory and that can potentially reduce airway remodeling in allergic asthma. This study is of high scientific and translational relevance given the obvious superiority of small molecules over biologic approaches (e.g., antibodies) to treating asthma. The validation of the humanized MIF mouse model is an additional advance, as it will enable the utility of this model in downstream pharmaceutical development, both in asthma and other MIF-dependent diseases in the sphere of airway disease, oncology, infection, and autoimmunity.

AUTHOR CONTRIBUTIONS

Hazel Dunbar performed research, data analysis, study design, and wrote the manuscript. Ian J. Hawthorne performed research, data analysis, study design and wrote the manuscript. Hazel Dunbar and Ian J. Hawthorne should be conjoint first authors. Courteney Tunstead performed research and data analysis. Seamas C. Donnelly and Michelle E. Armstrong provided reagents, contributed to study design and data analysis. Karen English designed and supervised the study and wrote the manuscript. All authors approved the final manuscript.

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DISCLOSURES

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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REFERENCES

1. Dharmage SC, Perret JL, Custovic A. Epidemiology of asthma in children and adults. *Front Pediatr*. 2019;7:246.
2. Djukanovic R, Roche WR, Wilson JW, et al. Mucosal inflammation in asthma. *Am Rev Respir Dis*. 1990;142:434-457.
3. Holgate ST, Wenzel S, Postma DS, Weiss ST, Renz H, Sly PD. Asthma. *Nat Rev Dis Primers*. 2015;1:15025.
4. Robays LJ, Lanckacker EA, Moerloose KB, et al. Concomitant inhalation of cigarette smoke and aerosolized protein activates airway dendritic cells and induces allergic airway inflammation in a TLR-independent way. *J Immunol*. 2009;183:2758-2766.
5. Custovic A. To what extent is allergen exposure a risk factor for the development of allergic disease? *Clin Exp Allergy*. 2015;45:54-62.
6. Dullaers M, Schuijs MJ, Willart M, et al. House dust mite-driven asthma and allergen-specific T cells depend on B cells when the amount of inhaled allergen is limiting. *J Allergy Clin Immunol*. 2017;140:76-88.e7.
7. Gregory LG, Lloyd CM. Orchestrating house dust mite-associated allergy in the lung. *Trends Immunol*. 2011;32:402-411.
8. Robinson DS, Hamid Q, Ying S, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med*. 1992;326:298-304.
9. Joseph C, Tatler AL. Pathobiology of airway remodeling in asthma: the emerging role of integrins. *J Asthma Allergy*. 2022;15:595-610.
10. Mizue Y, Ghani S, Leng L, et al. Role for macrophage migration inhibitory factor in asthma. *Proc Natl Acad Sci USA*. 2005;102:14410-14415.
11. Baugh JA, Chitnis S, Donnelly SC, et al. A functional promoter polymorphism in the macrophage migration inhibitory factor (MIF) gene associated with disease severity in rheumatoid arthritis. *Genes Immun*. 2002;3:170-176.
12. Plant BJ, Ghani S, O'Mahony MJ, et al. Sarcoidosis and MIF gene polymorphism: a case-control study in an Irish population. *Eur Respir J*. 2007;29:325-329.
13. Magalhaes ES, Mourao-Sa DS, Vieira-de-Abreu A, et al. Macrophage migration inhibitory factor is essential for allergic asthma but not for Th2 differentiation. *Eur J Immunol*. 2007;37:1097-1106.

14. Lan H, Luo L, Chen Y, Wang M, Yu Z, Gong Y. MIF signaling blocking alleviates airway inflammation and airway epithelial barrier disruption in a HDM-induced asthma model. *Cell Immunol*. 2020;347:103965.
15. Amano T, Nishihira J, Miki I. Blockade of macrophage migration inhibitory factor (MIF) prevents the antigen-induced response in a murine model of allergic airway inflammation. *Inflamm Res*. 2007;56:24-31.
16. Li R, Wang F, Wei J, et al. The role of macrophage migration inhibitory factor (MIF) in asthmatic airway remodeling. *Allergy Asthma Immunol Res*. 2021;13:88-105.
17. Bloom J, Metz C, Nalawade S, et al. Identification of Iguratimod as an inhibitor of macrophage migration inhibitory factor (MIF) with steroid-sparing potential. *J Biol Chem*. 2016;291:26502-26514.
18. Calandra T, Bucala R. Macrophage migration inhibitory factor: a counter-regulator of glucocorticoid action and critical mediator of septic shock. *J Inflamm*. 1995;47:39-51.
19. Shin JJ, Fan W, Par-Young J, et al. MIF Is a common genetic determinant of COVID-19 symptomatic infection and severity. *QJM*. 2022;116:205-212.
20. Hoegl SE, Ehrentraut H, Brodsky KS, et al. NK cells regulate CXCR2+ neutrophil recruitment during acute lung injury. *J Leukocyte Biol*. 2016;101:471-480.
21. Das R, Moss JE, Robinson E, et al. Role of macrophage migration inhibitory factor in the Th2 immune response to epicutaneous sensitization. *J Clin Immunol*. 2011;31:666-680.
22. Boucherat O, Boczkowski J, Jeannotte L, Delacourt C. Cellular and molecular mechanisms of goblet cell metaplasia in the respiratory airways. *Exp Lung Res*. 2013;39:207-216.
23. Atochina EN, Beers MF, Tomer Y, et al. Attenuated allergic airway hyperresponsiveness in C57BL/6 mice is associated with enhanced surfactant protein (SP)-D production following allergic sensitization. *Respir Res*. 2003;4:15.
24. Van Hove CL, Maes T, Cataldo DD, et al. Comparison of acute inflammatory and chronic structural asthma-like responses between C57BL/6 and BALB/c mice. *Int Arch Allergy Immunol*. 2009;149:195-207.
25. Mawhinney L, Armstrong ME, O'Reilly C, et al. Macrophage migration inhibitory factor (MIF) enzymatic activity and lung cancer. *Mol Med*. 2015;20:729-735.
26. Adamali H, Armstrong ME, McLaughlin AM, et al. Macrophage migration inhibitory factor enzymatic activity, lung inflammation, and cystic fibrosis. *Am J Respir Crit Care Med*. 2012;186:162-169.
27. Florez-Sampedro L, Soto-Gamez A, Poelarends GJ, Melgert BN. The role of MIF in chronic lung diseases: looking beyond inflammation. *Am J Physiol Lung Cell Mol Physiol*. 2020;318:L1183-L1197.
28. Florez-Sampedro L, Brandsma CA, de Vries M, et al. Genetic regulation of gene expression of MIF family members in lung tissue. *Sci Rep*. 2020;10:16980.
29. Melotti P, Mafficini A, Lebecque P, et al. Impact of MIF gene promoter polymorphism on F508del cystic fibrosis patients. *PLoS One*. 2014;9:e114274.
30. Plant BJ, Gallagher CG, Bucala R, et al. Cystic fibrosis, disease severity, and a macrophage migration inhibitory factor polymorphism. *Am J Respir Crit Care Med*. 2005;172:1412-1415.
31. Smith CA, Tyrell DJ, Kulkarni UA, et al. Macrophage migration inhibitory factor enhances influenza-associated mortality in mice. *JCI Insight*. 2019;4:e128034.
32. Kobayashi M, Nasuhara Y, Kamachi A, et al. Role of macrophage migration inhibitory factor in ovalbumin-induced airway inflammation in rats. *Eur Respir J*. 2006;27:726-734.
33. Rossi AG, Haslett C, Hirani N, et al. Human circulating eosinophils secrete macrophage migration inhibitory factor (MIF). Potential role in asthma. *J Clin Invest*. 1998;101:2869-2874.
34. Mitchell RA, Liao H, Chesney J, et al. Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p53: regulatory role in the innate immune response. *Proc Natl Acad Sci USA*. 2002;99:345-350.
35. Roger T, David J, Glauser MP, Calandra T. MIF regulates innate immune responses through modulation of Toll-like receptor 4. *Nature*. 2001;414:920-924.
36. Chen PF, Luo YL, Wang W, et al. ISO-1, a macrophage migration inhibitory factor antagonist, inhibits airway remodeling in a murine model of chronic asthma. *Mol Med*. 2010;16:400-408.
37. Allam V, Pavlidis S, Liu G, et al. Macrophage migration inhibitory factor promotes glucocorticoid resistance of neutrophilic inflammation in a murine model of severe asthma. *Thorax*. 2022;78:661-673.
38. Tynan A, Mawhinney L, Armstrong ME, et al. Macrophage migration inhibitory factor enhances Pseudomonas aeruginosa biofilm formation, potentially contributing to cystic fibrosis pathogenesis. *FASEB J*. 2017;31:5102-5110.
39. Luo Y, Yi H, Huang X, et al. Inhibition of macrophage migration inhibitory factor (MIF) as a therapeutic target in bleomycin-induced pulmonary fibrosis rats. *Am J Physiol Lung Cell Mol Physiol*. 2021;321:L6-L16.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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7.3

Human macrophage migration inhibitory factor potentiates mesenchymal stromal cell efficacy in a clinically relevant model of allergic asthma

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MIF enhances MSC efficacy in asthma

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Abstract

Current asthma therapies focus on reducing symptoms but fail to restore existing structural damage. Mesenchymal stromal cell (MSC) administration can ameliorate airway inflammation and reverse airway remodelling. However, differences in patient disease microenvironments seem to influence MSC therapeutic effects. Polymorphic CATT tetranucleotide repeat at position 794 of the human macrophage migration inhibitory factor (hMIF) gene has been associated with increased susceptibility and severity of asthma. We investigated the efficacy of human MSCs in high vs low hMIF environments and the impact of MIF pre-licensing of MSCs using humanised MIF mice in a clinically relevant house dust mite (HDM) model of allergic asthma. MSCs significantly attenuated airway inflammation and airway remodelling in high MIF expressing $CATT_7$ mice, but not in $CATT_5$ or wildtype littermates. Differences in efficacy correlated with increased MSC retention in the lungs of $CATT_7$ mice. MIF licensing potentiated MSC anti-inflammatory effects at a previously ineffective dose. Mechanistically, MIF binding to CD74 expressed on MSCs leads to upregulation of COX-2 expression. Blockade of CD74 or COX-2 function in MSCs prior to administration attenuated the efficacy of MIF-licensed MSCs *in vivo*. These findings suggest that MSC administration may be more efficacious in severe asthma patients with high MIF genotypes (CATT6/7/8).

Introduction

Allergic asthma is characterised by chronic airway inflammation and airway remodelling which refers to the structural changes in the airways. Currently, there is a heavy reliance on inhaled corticosteroids and long acting β2-adenoceptor agonists in the treatment of allergic asthma. The recent introduction of novel biologics such as benralizumab and dupilumab targeting Th2 cytokine receptors and tezepelumab targeting the alarmin thymic stromal lymphopoietin (TSLP) have been shown to significantly reduce allergic airway inflammation leading to reduced exacerbations and improved FEV_1 values (1–4). However, not all patients are responders, and evidence for biologics to reverse existing airway remodelling in patients is thus far limited (5). Thus, there is scope for novel therapeutics with capacity to attenuate inflammation and reverse remodelling to address the pitfalls in the current treatment and management of allergic asthma. Mesenchymal stromal cells (MSCs) have immunomodulatory and anti-fibrotic properties and have proven therapeutic effects in a range of allergic airway inflammation models and are currently under investigation in two clinical trials for asthma (NCT05147688, NCT05035862). Administration of MSCs intratracheally or intravenously has been shown to be effective in reducing airway inflammation and airway hyperresponsiveness in ovalbumin (OVA) (6–17), HDM (18–24), and aspergillus hyphal extract (25,26) models. However, other studies fail to demonstrate efficacy in experimental asthma models (7,14,23,24,27,28). To understand the mechanisms involved and to make MSCs a viable therapeutic in the clinic more focussed translational work is needed. Under basal conditions, for example in healthy animals or individuals, MSC administration does not seem to alter immunological status or function (homeostasis is preserved). MSCs only become licensed to an anti-inflammatory phenotype in the presence of extrinsic factors (29). Once licensed, MSCs modulate their surrounding microenvironment (30). Importantly, their therapeutic effect is blunted in the presence of IFN-γ, NF-κB or TNF-α receptor blockade/inhibition (31–33). Moreover, in the absence of appropriate signals to license anti-inflammatory functions, MSCs may even exacerbate disease (34–36). Licensing has been shown to improve MSC therapeutic efficacy by activating MSC anti-inflammatory characteristics prior to administration. Licensing through exposure to hypoxia (37,38), inflammatory cytokines (39,40), and pharmacological factors (41) have all been shown to improve MSC efficacy in a range of inflammatory diseases. Moreover, licensing of MSCs with serum from HDM-challenged mice (18) or with serum from ARDS patients (42) enhanced MSC therapeutic efficacy *in vivo* in pre-clinical lung disease models. However, there are also *in vitro* studies reporting differential and

in some cases negative effects of patient samples (acute respiratory distress syndrome (ARDS) versus cystic fibrosis (CF)) on MSC survival and function (42–44). Macrophage migration inhibitory factor (MIF) is an important regulator of host inflammatory responses demonstrated through its ability to promote the production of other inflammatory mediators. For example, MIF has been shown to amplify the expression of TNF, IFN-γ, IL-1β, IL-2, IL-6 and IL-8 from immune cells (45–48). This augmentation of immune signals contributes to MIF mediated pathogenesis by acting to sustain inflammatory responses. This has been shown in a range of inflammatory diseases where the absence of MIF is associated with lower levels of pro-inflammatory cytokines resulting in reduced pathology. For example, MIF knockout ($MIF^{-/-}$) mice display a less severe phenotype when exposed to OVA compared to control mice (49–52) and the use of anti-MIF antibodies or small molecule inhibitor (ISO-1) results in reduced Th2 cytokines in models of allergic airway inflammation (51,53–56). High levels of MIF as a result of longer CATT repeats such as $CATT_7$ have been shown to increase severity in a range of diseases including severe anaemia (57), pneumococcal meningitis (58), multiple sclerosis (59), tuberculosis (60) and COVID-19 (61). Importantly, associations between the CATT polymorphism and asthma incidence and severity have been observed (52). Not only do these studies show the pivotal role that MIF plays in pro-inflammatory diseases they also affirm the importance of differences in the MIF CATT polymorphism. Our previous work established a dominant role for MIF allelic variants in the severity of HDM-induced allergic asthma (62). Using humanised high-expressing and low-expressing MIF mice in a HDM model of allergic airway inflammation we demonstrated the pivotal role MIF plays in exacerbating asthma pathogenesis. High levels of human MIF resulted in a significant increase in airway inflammation as a result of elevated levels of Th2 cytokines promoting infiltration of eosinophils into the airways. Furthermore, high levels of MIF were associated with airway remodelling with significant mucus hyperplasia, subepithelial collagen deposition, and airway hyperresponsiveness generating a more severe asthma phenotype. MIF has been shown to promote MSC migration *in vitro* (63), however, the effect of MIF on MSC immunosuppressive function or therapeutic efficacy *in vivo* is unknown. Here, we sought to investigate the relationship between MIF and MSCs *in vivo* and to define conditions for optimal MSC therapeutic efficacy. The high MIF expressing $CATT_7$, low MIF expressing $CATT_5$, and WT mice were used as a platform to investigate the role of MIF on MSC efficacy in a clinically relevant HDM-induced mouse model of allergic airway inflammation.

Materials and Methods

Ethical Approval

All procedures involving the use of animals or human materials were carried out by licensed personnel. Ethical approval for all work was granted by the biological research ethics committee of Maynooth University (BRESC-2018-013). Project authorization was received from the scientific animal protection unit of the health products regulatory agency (HPRA) under AE19124/P022 whereby the terms of the animal experiments within this project were outlined and adhered to in accordance with the ARRIVE criteria.

Human bone marrow derived MSC culture

Three different human bone marrow-derived MSC (BM-MSC) donors were obtained from RoosterBio Inc. (Frederick, MD, USA). MSCs were first expanded in RoosterBio proprietary expansion medium (RoosterBasal and RoosterBooster) for the first two passages according to manufacturer's instructions. Following this MSCs were cultured and maintained in Dulbecco's Modified Eagles Media Low Glucose (DMEM, Sigma-Aldrich, Arklow, Wicklow, Ireland) supplemented with 10% (v/v) foetal bovine serum (FBS) (BioSera, Cholet, France) and 1% (v/v) Penicillin/Streptomycin (Sigma-Aldrich). Human MSCs were seeded at $1x10^6$ cells per T175 flask and cultured at 37 °c in 5% CO_2. Media was replenished every 2-3 days, and cells split once they achieved 80% confluency. All experiments were carried out between passages 2-5.

Animal Strains

Two C57BL/6 mouse strains expressing the human high- or low-expression *MIF* alleles (*MIF*^{CATT7} [(C57BL/6NTac-Miftm3884.1(MIF)Tac-Tg(CAG-Flpe)2Arte] and *MIF*^{CATT5} [C57BL/6NTac-Miftm3883.1(MIF)Tac-Tg(CAG-Flpe)2Arte] mice) were created using vector-based recombinant replacement of murine *Mif* by Taconic Biosciences (Rensselaer, NY) (Fig. 1). Validation of the expression of human and not murine *MIF* mRNA was verified by qPCR, and -794 CATT-length dependent stimulated MIF production was confirmed *in vivo* (61). Littermate wildtype (WT) and *Mif*$^{-\backslash-}$ (MIF KO) (64) (a kind donation from R. Bucala, Yale School of Medicine, Yale University, New Haven, CT, USA) mice were used as controls.

HDM-induced Airway Inflammation Model and Therapeutic Protocol

Both male and female *MIF*^{CATT7}, *MIF*^{CATT5} or WT mice aged 6-12 weeks were challenged with 25 µg HDM extract (*Dermatophagoides pteronyssinus,* Greer Laboratories, Lenoir, NC, USA) in 25 µl phosphate buffered saline (PBS) intranasally (i.n.) 3 days weekly for 3 weeks under light isoflurane anaesthesia. Control mice were challenged with 25 µl PBS under the same conditions. On day 14, after HDM challenge, mice received an intravenous (i.v.) injection of $1x10^6$ MSCs in 300 µl into the tail vein (65). For the dose curve $1x10^6$, $5x10^5$, $1x10^5$, and $5x10^4$ were administered i.v. into HDM challenged $CATT_7$ mice on day 14. $5x10^4$ was selected as the dose at which MSCs lose efficacy.

Licensing of MSCs with Endogenous Human MIF

Supernatants containing endogenous human MIF were generated from bone marrow- derived macrophages (BMDMs) of C57BL/6 mouse strains expressing the high-expressing MIF allele ($CATT_7$). $CATT_7$ mice were challenged with HDM in 25 µl phosphate buffered saline (PBS) intranasally 3 days weekly for 3 weeks under light isoflurane anaesthesia. 4 hr post final challenge, femurs and tibias were flushed with warm Roswell Park Memorial Institute (RPMI) 1640 medium GlutaMAX^{TM} (Gibco, Paisley, UK) supplemented with 10% (v/v) heat inactivated foetal bovine serum (FBS) (BioSera) and 1% (v/v) Penicillin/Streptomycin (Sigma-Aldrich). Cells were collected and seeded into T175 flasks in cRPMI supplemented with 10% L929 conditioned medium. L929 cell line produces high amounts of macrophage colony stimulating factor (M-CSF) and other proteins stimulating macrophage differentiation. After 96 hours, supernatants were collected, sterile filtered (0.22 µM pore size) and stored at -20°c. The conditioned media generated in this manner will be referred to as $CATT_7$ CM. Additionally, KO CM was generated from MIF KO mice as a control. Licensing MSCs was performed by removing existing media, washing with PBS, and incubating cells with $CATT_7$ CM (^{CATT7}MSC) or MIF KO CM (^{KO}MSC) for 24 hr. To account for variability of human MIF levels between $CATT_7$ mice and to ensure WT mice did not produce human MIF, $CATT_7$, $CATT_5$ and WT supernatants were measured by human MIF ELISA (R&D) as described previously (62). $5x10^4$ licensed MSCs were administered i.v. into HDM challenged $CATT_7$ mice on day 14. Where indicated, MSCs were treated with COX-2 inhibitor indomethacin (10 µM) for 30 min. Following pre-treatment, MSCs were licensed with $CATT_7$ CM for 24 hr as described above. Moreover, mouse anti-CD74 neutralising antibody and isotype control were

added to the assay. MSCs were pre-treated with anti-CD74 neutralising antibody (clone LN2) (10 μg/ml) or IgG1 κ isotype control (clone T8E5) (10 μg/ml) for 30 min. MSCs were then licensed with $CATT_7$ CM for 24 hr before administration.

Collection of Bronchoalveolar Lavage (BAL) Fluid

On day 18, 4 hr post final challenge, mice were sacrificed for cell and cytokine analysis of the BAL fluid. BAL fluid was obtained through 3 gentle aspirations of PBS. After centrifugation, protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany) was added to the supernatants before Th2 cytokine analysis. Total numbers of viable BAL cells were counted using ethidium bromide/acridine orange staining on a haemocytometer then pelleted onto microscope slides by cytocentrifugation. Slides were stained with Kwik Diff kit stain (Richard-Allan Scientific, Kalamazoo, MI, USA) and coverslips were mounted using DPX mounting medium (Sigma-Aldrich). Differential cells counts were derived by counting a minimum of 300 leukocytes on randomly selected fields under a light microscope at 20X magnification.

Enzyme-linked immunosorbent assay (ELISA)

Levels of mIL-4 (Biolegend, San Diego, CA, USA) and mIL-13 (eBioscience, San Diego, CA, USA) were determined using commercial ELISA kits, according to manufacturer's instructions.

Lung Histology

On day 21, mice were sacrificed for histological analysis. Lungs were removed and fixed in 10% neutral buffered formalin, paraffin embedded and 5 μm slices were mounted onto slides for histological analysis. Lung tissue was stained with haematoxylin and eosin (H&E), periodic acid-Schiff (PAS) or Masson's Trichrome to analyse immune cell infiltration, goblet cell hyperplasia or extracellular matrix deposition respectively. H&E analysis was carried out as previously described (66). Goblet cell hyperplasia was determined by the % of PAS positive cells in airways relative to airway diameter. Extracellular matrix deposition was calculated by analysing the % of positive staining using the trainable Weka segmentation plugin on Fiji open-source software.

Cryo-Imaging

$1x10^6$ MSCs were labelled with the Qtracker® 625 labelling kit (Invitrogen, Paisley, UK) according to manufacturer's instructions before being administered i.v. on day 14. On day 15, mice were humanely euthanised, and the lungs were embedded in mounting medium for cryotomy (O.C.T compound, VWR Chemicals, Leuven, Switzerland), frozen in liquid nitrogen and stored at -80°c. Sectioning. Lungs were sectioned into 40 μm slices and imaged with the automated CryoViz™ imaging system (BioInvision Inc., Cleveland, OH, USA). Images were then processed to generate 3D images using CryoViz™ processing, and the number of detected cells was quantified using cell detection software (BioInvision) (39).

MSC Expansion Assay

$1.4x10^5$ MSCs were seeded out into T25 flasks in cDMEM or 50:50 cDMEM and WT CM or $CATT_7$ CM for 72 hr. Cells were trypsinised and stained with ethidium bromide/ acridine orange and counted on a haemocytometer. MIF inhibitor SCD-19 (100 μM) was used to determine MIF specificity. In such cases, conditioned media was pre-incubated with SCD-19 1 hr before the expansion assay.

Intracellular Staining of COX-2

MSCs were seeded at $1x10^5$ cells per well in 6 well plates. MSCs were stimulated with $CATT_7$ CM for 24 hr. Cells were prepared for intracellular staining using the Intracellular FoxP3 kit as per manufacturer's instructions. Cells were stained with COX-2 (PE; clone AS67) (BD Pharmingen, San Diego, CA, USA) for 45 min. Cells were then washed in flow cytometry staining buffer and acquired using the Attune Nxt Flow Cytometer.

T Cell Suppression assay

Human PBMCs were isolated from buffy packs (Irish Blood Transfusion Service), by Ficoll density gradient centrifugation. $5x10^4$ Carboxyfluorescein succinimidyl ester (CFSE) labelled PBMC were co-cultured (ThermoFisher Scientific, Eugene, OR, USA) with BM-MSC in a 1:20 ratio (2.5×10^3 cells/well). 24 hr prior to co-culture, BM-MSCs were incubated with $CATT_7$ CM or $CATT_7$ CM + SCD-19 (100 μM). After 24 hr, BM-MSCs were washed with PBS before adding the PBMCs. Activation and expansion of human T cells was carried out

using ImmunoCult^{TM} human CD3/CD28 T cell activator antibody mix (STEMCELL Technologies, Cambridge, UK). After 4 days, PBMCs were harvested and the % and number of proliferating $CD3^+$ cells were analysed by flow cytometry (Attune Nxt Flow Cytometer).

Statistical Analysis

Mice were randomized. Observers assessing end-points were blinded to group assignment. Data for individual animals and independent experiments are presented as individual symbols. All data are presented as mean ± SEM. Results of two or more groups were compared by one-way analysis of variance (ANOVA) followed by the *post-hoc* Tukey's multiple comparison test. GraphPad Prism (GraphPad Software Inc, San Diego, CA, USA) was used for all statistical analyses.

Results

Human BM-MSCs significantly reduce airway remodelling in $CATT_7$ mice challenged with HDM

Firstly, to investigate the impact of high- and low-expressing MIF alleles on MSC treatment of allergic airway inflammation we examined the lung histology. $CATT_7$, $CATT_5$, and WT were randomized to HDM or mock (saline) intranasally 3 times a week for 3 weeks. Mice were then further randomized to $1x10^6$ human BM-MSCs or equal volume saline administered via tail vein injection on day 14. On day 21, lung tissue was removed, formalin fixed and sectioned onto slides (Fig. 1A). Slides were stained with PAS to highlight mucin production to assess the level of goblet cell hyperplasia. $CATT_7$ mice exhibit significantly higher levels of goblet cell hyperplasia compared to WT and $CATT_5$ mice. Administration of BM-MSCs into HDM challenged $CATT_7$ mice significantly reduced the number of mucin secreting cells in the airways (Fig. 1B, 1C). There was a slight, not statistically significant, reduction in the number of PAS positive cells in the $CATT_5$ mice which received BM-MSCs. No change in goblet cell hyperplasia in WT mice treated with BM-MSC was noted (Fig. 1B, 1C). As MSCs only significantly decreased HDM-induced histopathology in $CATT_7$ mice, but not $CATT_5$ or WT mice, this suggests that MSCs may be more efficacious at reducing goblet cell hyperplasia in more severe inflammatory $CATT_7$ environment.

Subepithelial fibrosis was significantly increased in HDM challenged $CATT_7$ mice compared to the lower MIF expressing $CATT_5$ and WT groups. BM-MSC administration significantly reduced subepithelial collagen deposition in HDM challenged $CATT_7$ mice (Fig. 1D, 1E). In $CATT_5$ and WT mice challenged with HDM, BM-MSC administration had a small but not significant therapeutic effect. BM-MSC administration significantly mitigated increased inflammatory infiltrate and H&E pathological score in $CATT_7$ mice challenged with HDM (Supplementary Figure 1).

Human BM-MSCs significantly reduce airway inflammation in $CATT_7$ mice challenged with HDM

Total cell counts were significantly elevated in the BAL fluid of $CATT_7$ mice following HDM challenge (Fig. 2A). MSCs significantly reduced the number of total infiltrating cells in the BALF of $CATT_7$ mice (Fig. 2A). Differential cell counts identified eosinophils as the main cell infiltrating into the lung tissue following HDM challenge and MSCs significantly decrease infiltrating eosinophils in $CATT_7$ mice yet had no effect in the $CATT_5$ and WT groups (Fig. 2B). IL-4 and IL-13 were significantly elevated in the BAL fluid of $CATT_7$ mice following HDM challenge (Fig. 2C, 2D). These Th2 cytokines are not significantly upregulated in $CATT_5$ or WT mice. While MSCs significantly decreased IL-4 and IL-13 in $CATT_7$ mice, MSC treatment did not reduce and in some cases increased Th2 cytokines in the BAL fluid of $CATT_5$ and WT mice (Fig. 2C, 2D). These data show that BM-MSCs are effective at alleviating eosinophil infiltration and reducing Th2 cytokines in a high MIF expressing model of allergic asthma and that MSCs require a threshold level of inflammation to mediate their therapeutic effects.

High levels of hMIF significantly enhance BM-MSC retention in a HDM model of allergic asthma

Next, we analysed the biodistribution of MSCs following administration into HDM challenged WT, $CATT_5$ and $CATT_7$ mice. $1x10^6$ fluorescently labelled BM-MSCs were administered i.v. via tail vein injection on day 14. On day 15 mice were sacrificed and the lungs prepared for CryoViz imaging (Fig. 3A-C). Significantly higher numbers of labelled MSCs were detected in the lung of high MIF expressing $CATT_7$ mice compared to the low expressing $CATT_5$ or WT littermate control (Fig. 3C, 3D). However, the number of clusters of labelled BM-MSCs within the lungs remained unchanged among the groups (Fig. 3E). Taken together, these data

suggest prolonged MSC-pulmonary retention time increases the number of MSCs retained at the site of inflammation 24 hr post administration. These data suggest that high levels of MIF may provide a longer window for MSCs to carry out their therapeutic effects.

MIF licensing enhances MSC expansion and immunosuppressive function in vitro.

Human MSCs express the MIF receptor CD74 and this expression is maintained and not increased following exposure to human MIF containing supernatants (Fig. 4A). Previous studies have shown that MIF has the ability to support cell proliferation *in vitro* (67–69). Increasing the number of MSCs within the inflammatory niche could prove to be important in enhancing MSC immunoregulatory effects. High levels of MIF significantly enhanced MSC expansion *in vitro* compared to the complete medium control group (Fig. 4B). Blockade of MIF using SCD-19 confirmed the role of MIF in driving MSC expansion (Fig. 4B).

MIF has an established role as an upstream regulator of cyclooxygenase 2 (COX-2) through the activation of the MAPK signalling pathway (70,71). COX-2 and PGE2 are widely reported mediators of MSC immunosuppression (39,72). MSCs constitutively express COX-2 and the percentage of COX-2 expressing MSCs was significantly increased following MIF stimulation (Fig. 4C, 4D). MSCs licensed with high levels of hMIF from $CATT_7$ CM displayed enhanced suppression of T cell proliferation compared to the untreated MSCs and the presence of SCD-19 abrogated the enhanced suppression mediated by hMIF licensed MSCs (Fig. 4E, 4F). MIF has an established role as an upstream regulator of cyclooxygenase 2 (COX-2) through the activation of the MAPK signalling pathway (70,71). COX-2 and PGE2 are widely reported mediators of MSC immunosuppression (39,72). MSCs constitutively express COX-2 however the percentage of COX-2 expressing MSCs was significantly upregulated following MIF stimulation (Fig. 4C, 4D). MSCs licensed with high levels of hMIF from $CATT_7$ CM displayed enhanced suppression of T cell proliferation compared to the untreated MSCs, however this was not statistically significant in the frequency of proliferating $CD3^+$ T cells (Fig. 4E) or the number of proliferating $CD3^+$ T cells (Fig. 4F). The presence of SCD-19 abrogated the enhanced suppression mediated by hMIF licensed MSCs, as the number of proliferating $CD3^+$ T cells was significantly increased compared to the ^{CATT7}MSC group (Fig. 4F). The same trend was observed in the frequency of proliferating $CD3^+$ T cells after the addition of SCD-19, however this was not significant (Fig. 4E).

Titration of BM-MSC doses in $CATT_7$ mice challenged with HDM

Next, we investigated if MIF licensing could improve MSC efficacy in the high MIF expressing $CATT_7$ mice challenged with HDM. To do this we first investigated the dose at which MSCs lose efficacy. MSCs at doses of $1x10^6$, $5x10^5$, $1x10^5$, and $5x10^4$ were administered i.v. into HDM challenged $CATT_7$ on day 14 (Fig. 5A). MSCs maintained efficacy as low as $1x10^5$ cells with reduced immune cell infiltration (Fig. 5B, 5C) and reduced Th2 cytokines IL-4 (Fig. 5D) and IL-13 (Fig. 5E). We observed that BM-MSCs were no longer able to carry out their immunosuppressive effects at a dose of $5x10^4$. At $5x10^4$ BM-MSCs were unable to reduce the number of eosinophils infiltrating into the lungs (Fig. 5B, 5C) or regulate Th2 cytokine production (Fig. 5D, 5E).

MIF licensing restores MSC efficacy at low doses in $CATT_7$ mice

To investigate the effect of MIF licensing on MSC therapeutic efficacy MSCs were first licensed *in vitro* by stimulation with bone marrow derived macrophage (BMDM) conditioned media from $CATT_7$ or KO mice for 24 hr. $5x10^4$ MSCs, MIF licensed MSCs (^{CATT7}MSC), or MIF KO licensed MSCs (^{KO}MSC) were administered i.v. into $CATT_7$ mice via tail vein injection on day 14 in HDM challenged mice. On day 18, BAL fluid was collected, cell counts were performed and Th2 cytokines were measured (Fig. 6A). Only ^{CATT7}MSC administration significantly reduced total cell counts and number of eosinophils in CATT7 mice challenged with HDM (Fig. 6B, 6C). ^{CATT7}MSCs markedly reduced IL-4 and IL-13 levels compared to control group although not significant (Fig. 6D, 6E). The control MSC group and the ^{KO}MSC group displayed similar levels of immune cell infiltration and Th2 cytokine production suggesting the effects observed in the ^{CATT7}MSC group are specific to MIF licensed MSCs. These data show that MIF licensing can restore MSC immunosuppressive function at doses that would normally be ineffective.

Blocking COX-2 abrogates therapeutic efficacy of MIF licensed BM-MSCs

COX-2 is the rate-limiting enzyme involved in the synthesis of arachidonic acid to PGE2, a key mediator in the immunomodulatory effects of MSCs (73). To assess the role of COX-2 on MIF licensed MSCs we inhibited COX-2 with indomethacin. MSCs were treated with indomethacin (10 μM) for 30 min. Following the 30 min pre-treatment, cells were incubated with $CATT_7$ CM for 24 hours. To further validate the involvement of MIF in the improvement of MSC efficacy, MSCs were exposed to an anti-CD74 neutralising antibody (10 μg/ml) or

IgG1 isotype control (10 μg/ml) for 30 min. MSCs were then incubated with $CATT_7$ CM for 24 hours (Fig. 7A). Analysis of the BAL fluid cell counts showed that pre-treating MSCs with indomethacin before administration significantly reduces ^{CATT7}MSCs ability to suppress immune cell infiltration in the BAL fluid of $CATT_7$ mice challenged with HDM (Fig. 7B, 7C). Additionally, the analysis of the Th2 cytokines in the BALF showed a marked increase in IL-4 (Fig. 7D) and a significant increase in the levels of IL-13 (Fig. 7E) in the indomethacin group compared to the control MIF licensed MSC group. Taken together, these results show that COX-2 is an important mediator in the enhancement of therapeutic efficacy associated with MIF licensing. Furthermore, blocking of CD74 abrogates MIF licensed BM-MSC immunosuppression. The anti-CD74 group displayed significantly higher numbers of eosinophils in the BAL fluid than the non-treated control (Fig. 7B, 7C). Like the indomethacin group, blocking CD74 significantly abrogated MIF licensed MSCs ability to reduce cytokine levels in the BAL fluid. Markedly higher levels of IL-4 and IL-13 were detected (Fig. 7D, 7E). These data indicate that MIF enhances MSCs immunomodulatory capacity mainly through CD74 signalling to upregulate COX-2 production.

Discussion

Our main results advance the field of MSC-based therapeutics for asthma by demonstrating that (i) MSC treatment is highly effective in ameliorating airway inflammation; (ii) their therapeutic potential can be enhanced by MSC-MIF licensing as demonstrated in high MIF expressing $CATT_7$ mice; and finally (iii) that the mechanism of MIF-licensing is dependent upon MSC-CD74 expression levels that drive COX-2 expression in MSCs. We explored the effect of high and low levels of endogenous human MIF on MSC efficacy in a HDM model of allergic asthma. Our findings show that MSC administration significantly reduced airway inflammation in the high MIF expressing $CATT_7$ mice challenged with HDM. MSCs significantly decreased levels of IL-4 and IL-13 in the BAL fluid and in turn significantly abrogated pulmonary eosinophilia in $CATT_7$ mice. Our data aligns with the literature demonstrating the ability of human MSCs to ameliorate eosinophil infiltration by reducing the levels of Th2 cytokines (8,10–12,25,74). In addition to ameliorating inflammation, MSCs also alleviate features of airway remodelling in the $CATT_7$ mice, significantly reducing collagen deposition within the subepithelial space and goblet cell hyperplasia. Interestingly we did not observe the same changes in the WT and the low MIF expressing $CATT_5$ mice suggesting that

high levels of MIF were responsible for improving MSC efficacy. The reduced efficacy of MSCs in the WT and $CATT_5$ mice is likely attributed to lack of inflammation present associated with a bias towards Th1 immunity in C57BL/6 mice compared to the more Th2 bias in BALB/c mice, influencing the level of Th2 response in our HDM challenge model (75). There have been several instances where researchers also observed poor responses to MSC treatment of allergic airway inflammation in C57BL/6 mice (7,23). More recently, Castro et al. presented their findings on the requirement of at least 2 doses of human AD-MSCs to reverse airway remodelling and alleviate inflammation in HDM challenged C57BL/6 mice (21).

We show that a single human MSC dose is capable of significantly decreasing airway remodelling in $CATT_7$ mice by reducing subepithelial collagen deposition and goblet cell hyperplasia. This suggests that high levels of MIF may facilitate activation of MSCs improving their therapeutic efficacy and leading to reversal of airway remodelling. The literature surrounding MSC's effect on airway remodelling is conflicting. Overall, our data aligns with the majority of the current literature demonstrating that MSCs are able to attenuate airway remodelling by reducing collagen deposition and/ or goblet cell hyperplasia (8,10,14,15,17,18,28). However, it has also been reported in the literature that MSCs are ineffective at ameliorating goblet cell hyperplasia (14,19,23) or subepithelial collagen deposition (9,28). A reason for these discrepancies could be associated with the source of MSCs or genotypic differences in the mice. It is well documented that MSCs from different sources can behave in different ways and possess different immunomodulatory properties depending on the extracellular milieu (8,23,76). Other factors such as severity of model, time of infusion, MSC fitness, dosing, and route of administration may also be reasons for the contradictory findings (27).

Our previous studies have demonstrated that pro-inflammatory cytokine licensing of MSCs or MSC-like cells; multipotent adult progenitor cells (MAPCs) enhances their retention in inflammatory conditions and correlates with enhanced therapeutic efficacy (39,77). We detected significantly higher numbers of MSCs in the lungs of HDM challenged $CATT_7$ mice compared to $CATT_5$ or littermate controls 24 hours following administration (Fig. 2). It is suggested that short-term effects of MSCs are mediated by their diverse secretome and the longer-term effects of MSC therapy are a result of direct interaction with other cell types (78). Increased longevity at the site of injury allows MSCs a longer period to secrete soluble factors and interact with cells in the inflammatory microenvironment. MSC retention in the $CATT_7$

HDM challenged mice is an important observation and future work will determine if enhanced retention is also involved in the enhanced MSC efficacy observed.

Taken together these data suggest that MSCs are more efficacious in the high MIF environment of $CATT_7$ mice. By investigating the effects of different concentrations of a human cytokine on the efficacy of human MSCs in a model of allergic asthma using a clinically relevant allergen, we have identified a specific disease microenvironment which supports and enhances MSC efficacy. The use of our humanised model aims to provide a more accurate depiction of how human MSCs would interact in subsets of patients compared to conventional murine models. Of course, despite exploring the effect of a human cytokine on human MSCs there are still limitations as we are unable to fully mimic clinical severe allergic asthma and the use of transgenic MIF mice on a C57BL/6 background meant that control WT mice do not develop a high level of type 2 inflammation. However, these results may have implications in tailoring MSC treatment in cases of severe asthma. Our results have demonstrated that MSCs are less efficacious in low MIF environments. Patients with 5/5 haplotypes tend to have lower levels of circulating MIF and therefore may not respond as well to MSC treatment. Whereas patients with 6/6/, 7/7 or 8/8 haplotypes are more likely to have high levels of circulating MIF which may lead to greater MSC activation and enhanced therapeutic efficacy.

Following on from the discovery that MSC administration into $CATT_7$ mice led to improved MSC efficacy, we investigated strategies to use the high MIF microenvironment to potentiate the effects of MSCs. Past work in our lab has focussed on different licensing strategies of MSCs to enhance MSC efficacy. Previously, we have demonstrated how IFN-γ licensing can improve MSC efficacy in a humanised model of acute GvHD and how endogenous factors such as peroxisome proliferator-activated receptor (PPAR)δ ligands or treatments like cyclosporine A can influence this (39,40). Other studies have shown how licensing with pharmacological agents or endogenous factors can further enhance the effects of MSC therapy in preclinical models of asthma (18,19,79). We sought to investigate whether licensing MSCs with CM derived from the high MIF expressing $CATT_7$ mice could potentiate their immunomodulatory effects. Using a low MSC dose ($5x10^4$ cells) which failed to protect against HDM-driven allergic airway inflammation, we showed that pre-licensing low dose MSCs with $CATT_7$ CM enhanced low dose MSC therapeutic efficacy. Licensing with BMDM CM from MIF knockout mice failed to restore MSCs immunosuppressive function suggesting that our findings were MIF specific. Our findings agree with the current literature as doses reported for the systemic

administration of MSCs in preclinical models of asthma range from $1x10^6$ to $1x10^5$ cells per mouse (17,24).

One of the main criticisms of preclinical research is the use of doses which far exceed what would be reasonable in the clinic. Analysis of clinical trials using i.v. injection of MSCs reveals that the minimal effective dose used ranges from 1-2 million cells/kg (80). Studies which have investigated the i.v. administration of MSCs in preclinical models of allergic asthma administer doses which equate to 4-40 million cells/kg with the majority at the higher end of the scale (8,10,12,16,20–22,25,26,28,74,81–84). The efficacy observed with MIF licensed MSCs using $5x10^5$ cells per mouse results in an effective dose of 2 million cells/kg. This shows that through MIF licensing we are able to restore MSC efficacy at a dose akin to what is used in clinical trials.

We then sought to elucidate the mechanisms involved. MIF mediated signal transduction is primarily initiated by binding to MIF's classical receptor CD74 (85). We showed that blocking CD74 on the surface of MSCs ultimately abolished their immunosuppressive abilities. These findings not only reaffirmed that the licensing with $CATT_7$ CM was MIF mediated but it also showed that these effects were dependent on binding to CD74. MIF signal transduction through CD74 binding has been shown to initiate a range of signalling pathways which induce cell proliferation, resistance to apoptosis, and the promotion of repair (86–90). Furthermore, MIF binding to CD74 has been shown to activate cytosolic phospholipase A2 ($cPLA_2$). Moreover, $cPLA_2$ activation results in the mobilisation of arachidonic acid from membrane phospholipids which is a precursor to the synthesis of prostaglandins (91). Interestingly MIF can upregulate COX-2 expression, a rate limiting step in the synthesis of prostaglandins such as PGE2 (70,71,92), however, MIF has been shown to have no effect on the expression of COX-1 (70).

The COX-2/PGE2 pathway has been extensively documented as being one of the key mediators driving MSC immunomodulation (39,93–95). We hypothesised that the COX-2/PGE2 pathway could be involved in the restoration of MSCs immunomodulatory capacity following $CATT_7$ licensing. To investigate, we pre-treated MSCs with indomethacin prior to licensing. Indomethacin is a potent non-selective inhibitor of COX-1 and COX-2 (96). We showed that blocking COX-2 abrogated therapeutic efficacy of $CATT_7$ licensed MSCs. Interestingly we observed that blocking of COX-2 via indomethacin had more pronounced effect than blocking CD74. COX-2 is constitutively expressed in human MSCs, therefore, inhibition with

indomethacin also blocks basal COX-2 expression which will contribute to the effects observed.

These data show that MIF licensing can improve MSC therapeutic efficacy through the upregulation of COX-2 which likely drives PGE2 production. Our data agrees with several studies in the literature which also reveal the ability of MIF to improve MSC efficacy *in vivo* (97–99). The Zhang group demonstrated the ability of MIF to improve MSC therapeutic efficacy by transducing MSCs with a lentiviral vector containing *Mif* cDNA thus promoting MIF overexpression (97–99). Furthermore, Zhang et al. demonstrated the ability of MIF to upregulate COX-2 expression and promote PGE2 production in astrocytes (100). Here, we further demonstrate the effects of *ex vivo* MIF licensing on MSC therapeutic efficacy by showing binding to CD74 and increased COX-2 expression enhances MSCs immunomodulatory abilities.

The knowledge gained from this study can be used to further optimise MSCs as a therapy and provide a basis for future studies regarding the effects of MSCs on the immune response in high MIF environments such as in asthma patients exhibiting the $CATT_7$ polymorphism.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

Conflict of Interest Statement

The authors declare no conflict of interest.

Author's Contributions

IJH performed research, data analysis, study design and wrote the manuscript. HD performed research, data analysis, study design and wrote the manuscript. CT performed research and data analysis; DJW, SRE & CDS contributed to study design and data analysis. SCD & MEA provided reagents, contributed to study design and data analysis. KE designed and supervised the study and wrote the manuscript. All authors approved the final manuscript.

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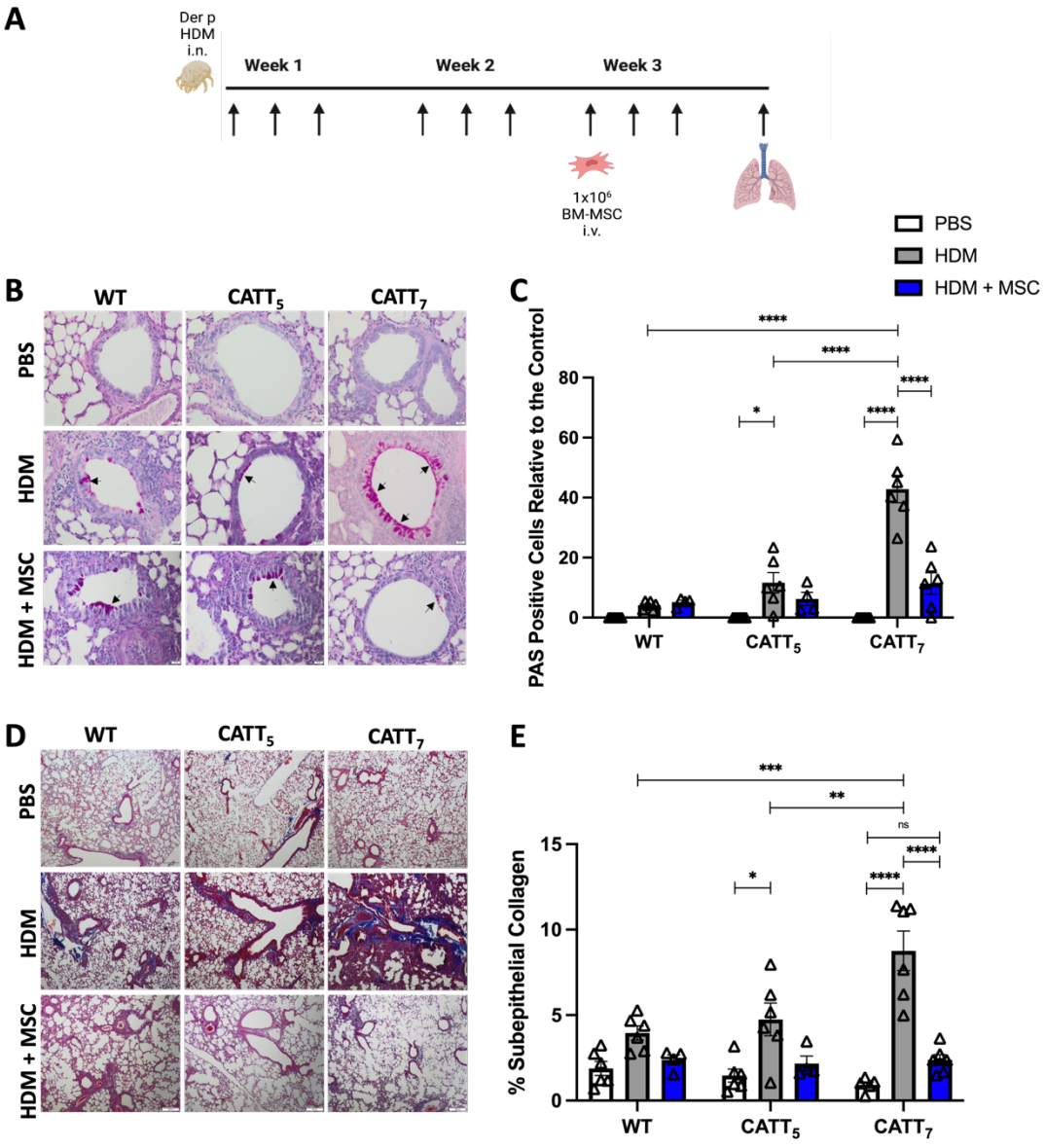


Figure 1. Human BM-MSCs significantly reduce goblet cell metaplasia and collagen deposition in CATT$_7$ mice challenged with HDM. A PBS and HDM groups received PBS or HDM i.n. 3 times a week for 3 consecutive weeks. 1x10^6 human BM-MSCs were administered i.v. to the HDM+MSC groups on day 14. Mice were sacrificed on day 21 (Schematic created with BioRender.com). **B** Representative images of lung tissue from WT, CATT$_5$ and CATT$_7$ mice stained with Periodic acid Schiff (PAS) at 20X magnification, scale bar = 20 µm. Arrows show examples of mucin-containing goblet cells. **C** Goblet cell hyperplasia was investigated through the quantitation of PAS positive cells. **D** Representative images of lung tissue stained with Masson's trichome at 4X magnification, scale bar = 200 µm. **E** Quantitation of % subepithelial collagen. Data are presented as mean ± SEM; N=6 per group. Human BM-MSC donors 001-177 and 003-310 were used (RoosterBio Inc., Frederick, MD, USA). Statistical analysis was carried out using one-way ANOVA followed by the *post-hoc* Tukey's multiple comparison test where *p<0.05, ***p<0.001, ****p<0.0001 ns non significant.

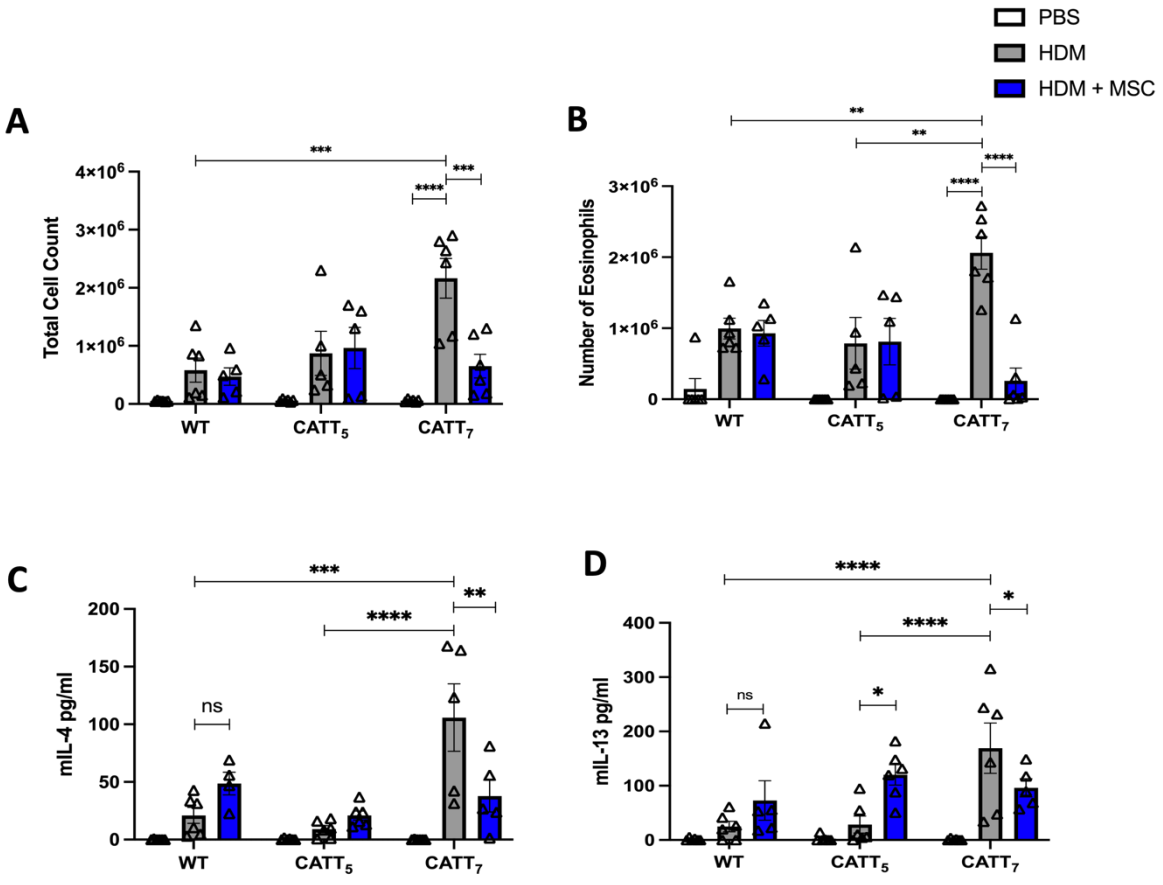


Figure 2. Human BM-MSCs significantly reduce levels of Th2 cytokines in the BAL fluid of CATT$_7$ mice challenged with HDM. PBS and HDM groups received PBS or HDM i.n. 3 times a week for 3 consecutive weeks. 1x10^6 human BM-MSCs were administered i.v. to the HDM+MSC groups on day 14. BAL was performed 4 hr post final HDM challenge on day 18. **A** Total cell count recovered from the BAL. **B** BAL fluid eosinophil count determined by differential staining of cytospins. Cytokine levels of **C** IL-4 and **D** IL-13 in the BAL fluid

determined by ELISA. White bar: PBS; Grey bar: HDM; Blue bar: HDM+MSC. Data are presented as mean ± SEM; N=5-6 per group. Human BM-MSC donors 001-177 and 003-310 were used (RoosterBio Inc., Frederick, MD, USA). Statistical analysis was carried out using one-way ANOVA followed by the *post-hoc* Tukey's multiple comparison test where *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns non significant.

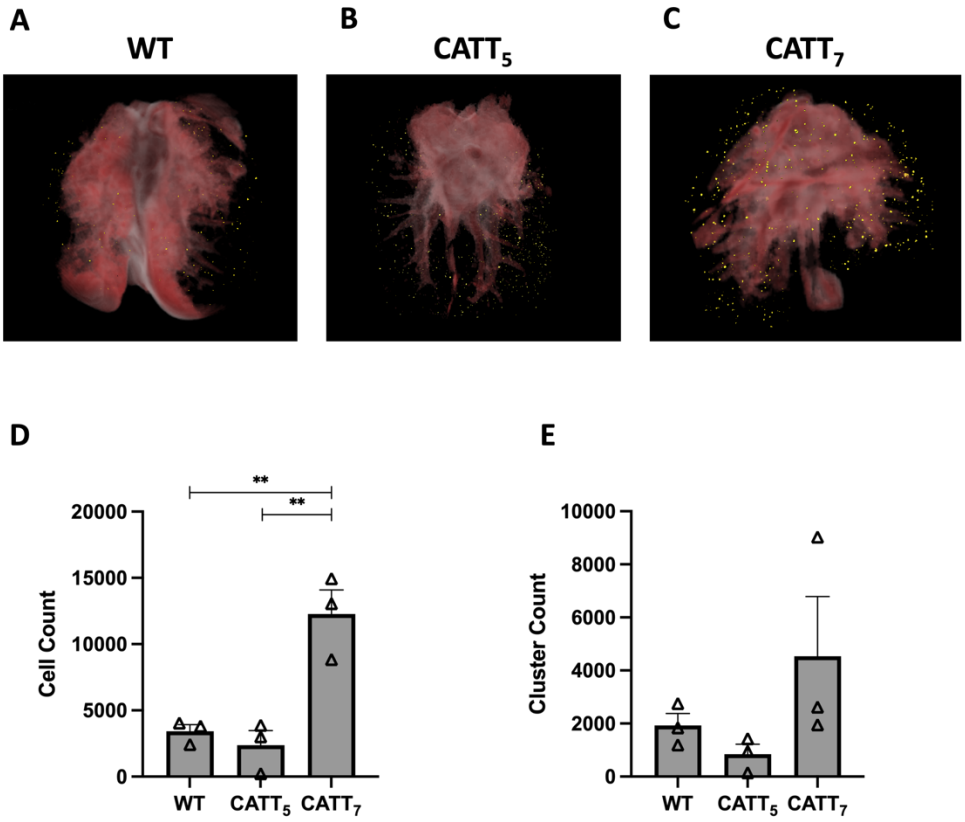


Figure 3. High levels of hMIF significantly enhance BM-MSC retention in a HDM model of allergic asthma. HDM was administered i.n. 3 times a week for 2 weeks. On day 14, $1x10^6$ Qtracker 625- labelled hMSCs were administered i.v. in WT, $CATT_5$ or $CATT_7$ mice. 24 hr later the lungs were harvested, embedded in OCT compound and frozen at -80. Tissue blocks were sectioned and imaged using the CryoViz™ (Bioinvision, Cleveland, OH, USA) imaging system. 3D images show representative lung images from **A** WT, **B** $CATT_5$ and **C** $CATT_7$ mice with detected MSCs shown in yellow. **D** Total number of MSCs detected in the lung and **E** number of clusters were quantified using CryoVizTM Quantification software. Data are presented as mean ± SEM; N=3 per group. Human BM-MSC donor 001-177 was used (RoosterBio Inc., Frederick, MD, USA). Statistical analysis was carried out using one-way ANOVA followed by the *post-hoc* Tukey's multiple comparison test where **p<0.01.

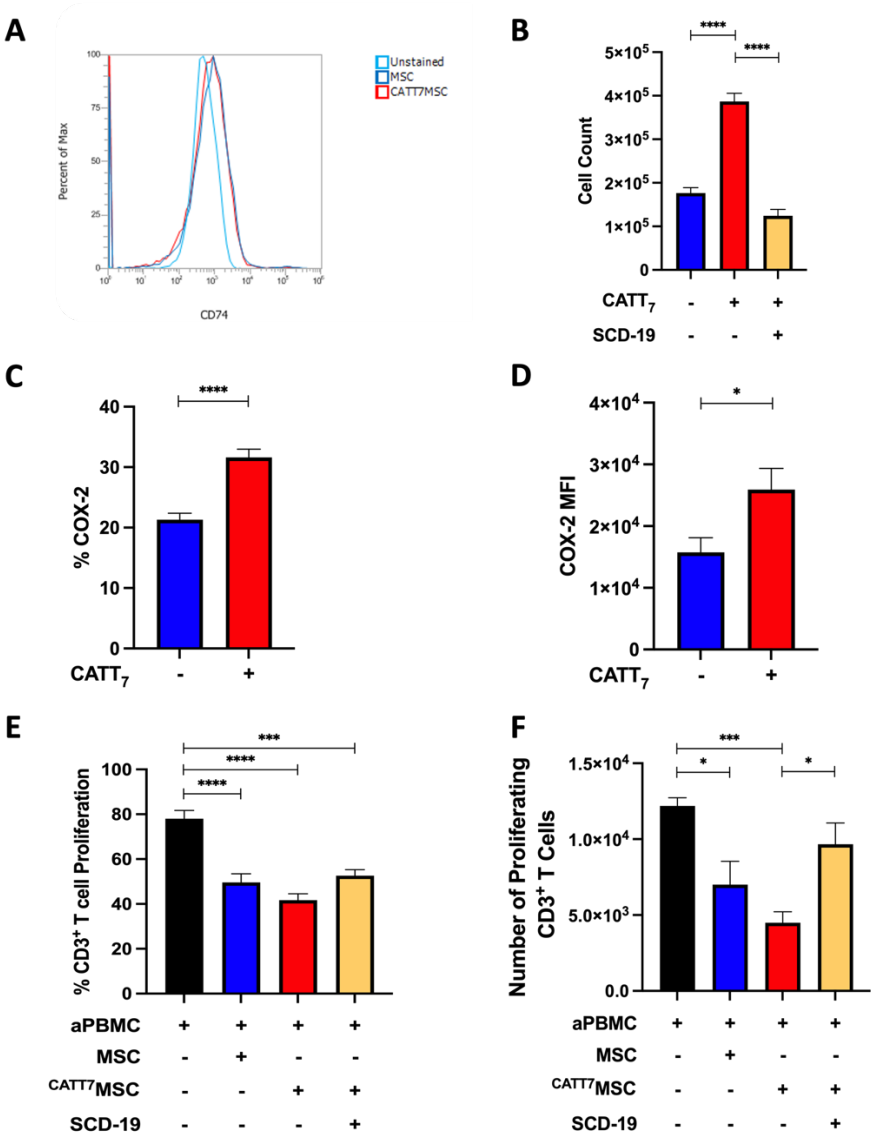


Figure 4. MIF licensing enhances MSC expansion and immunosuppressive function in vitro. A Human BM-MSC express the canonical MIF receptor CD74 in the presence or absence of endogenous hMIF generated from BMDMs from $CATT_7$ HDM challenged mice. **B** Licensing of MSCs with supernatants generated from BMDMs from $CATT_7$ HDM challenged mice but not WT HDM challenged mice enhances MSC expansion in vitro. Addition of MIF inhibitor SCD-19 (100 µM) to supernatants generated from BMDMs from $CATT_7$ HDM challenged mice 1 hr before MSC licensing prevents MIF enhanced MSC expansion. Licensing of MSCs with supernatants generated from BMDMs from $CATT_7$ HDM challenged mice significantly increases % MSC expression of **C** COX-2 and **D** COX-2 MFI. Licensing of MSCs with supernatants generated from BMDMs from $CATT_7$ HDM challenged mice enhances MSC suppression of **E** frequency (%) and **F** number of CD3+ T cell proliferation. Blockade of MIF using SCD-19 (100 µM) in the BMDM supernatants 1 hr before addition to MSCs abrogates the enhanced effect of MIF on MSC suppression of T cell proliferation (**E** & **F**). Data are presented as mean ± SEM and are representative of 3 independent experiments. Human BM-MSC donors 001-177, 003-310 and 003-307 were used (RoosterBio Inc., Frederick, MD, USA). Statistical analysis was carried out using unpaired t test where *p<0.05, ***p<0.001, ****p<0.0001

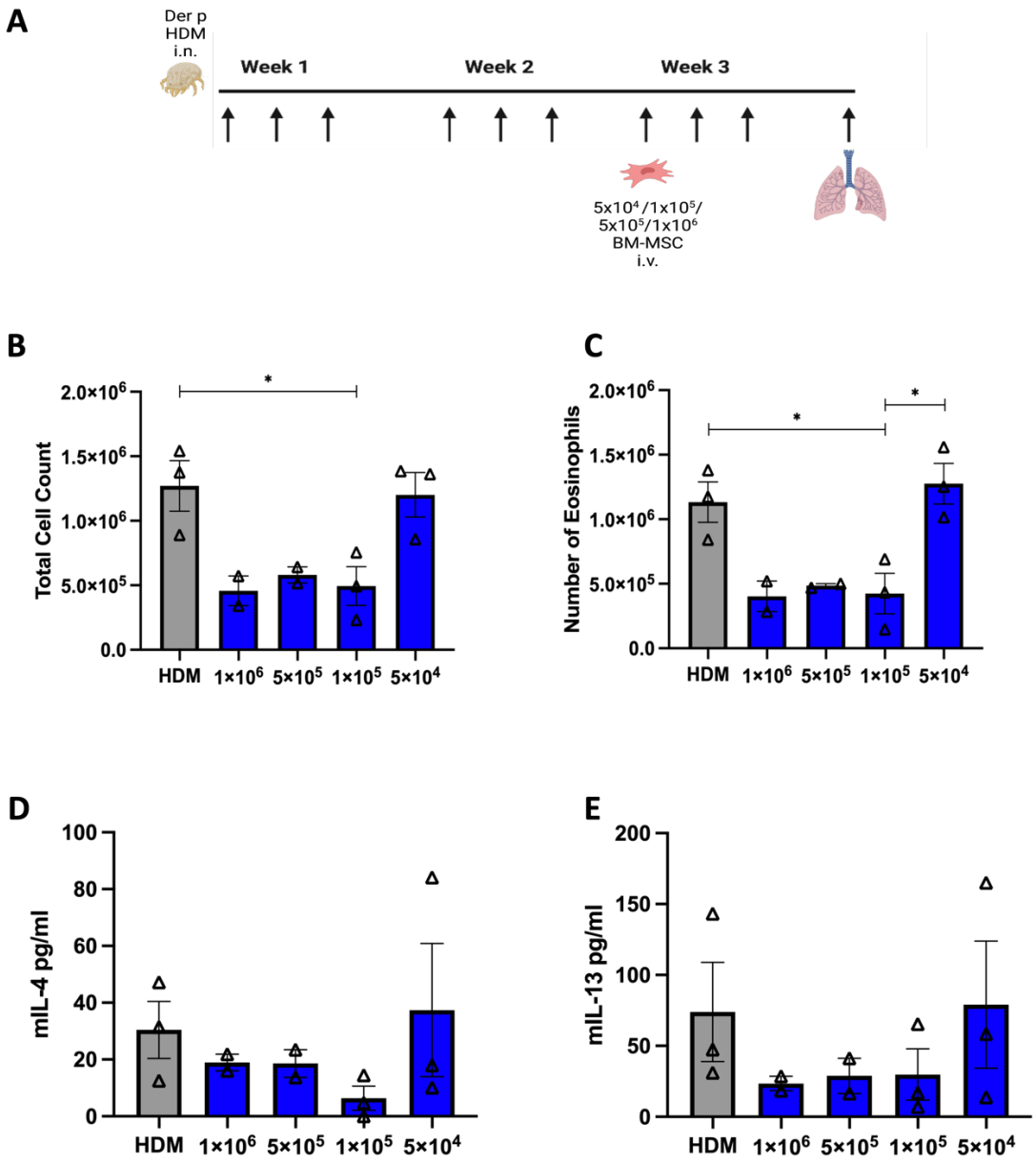


Figure 5. Titration of BM-MSC doses in CATT$_7$ mice challenged with HDM. A To determine the point where MSCs lose efficacy in CATT$_7$ mice, a range of doses were administered on day 14. BAL was performed 4 hr post final HDM challenge on day 18 (Schematic created with BioRender.com). **B** Total cell count recovered from the BAL. **C** Number of eosinophils obtained from the BAL fluid. Cytokine levels of **D** IL-4 and **E** IL-13 in the BAL fluid determined by ELISA. Data are presented as mean ± SEM; N=2-3 per group. Human BM-MSC donors 001-177 and 003-310 were used (RoosterBio Inc., Frederick, MD, USA). Statistical analysis was carried out using one-way ANOVA followed by the *post-hoc* Tukey's multiple comparison test where *p<0.05.

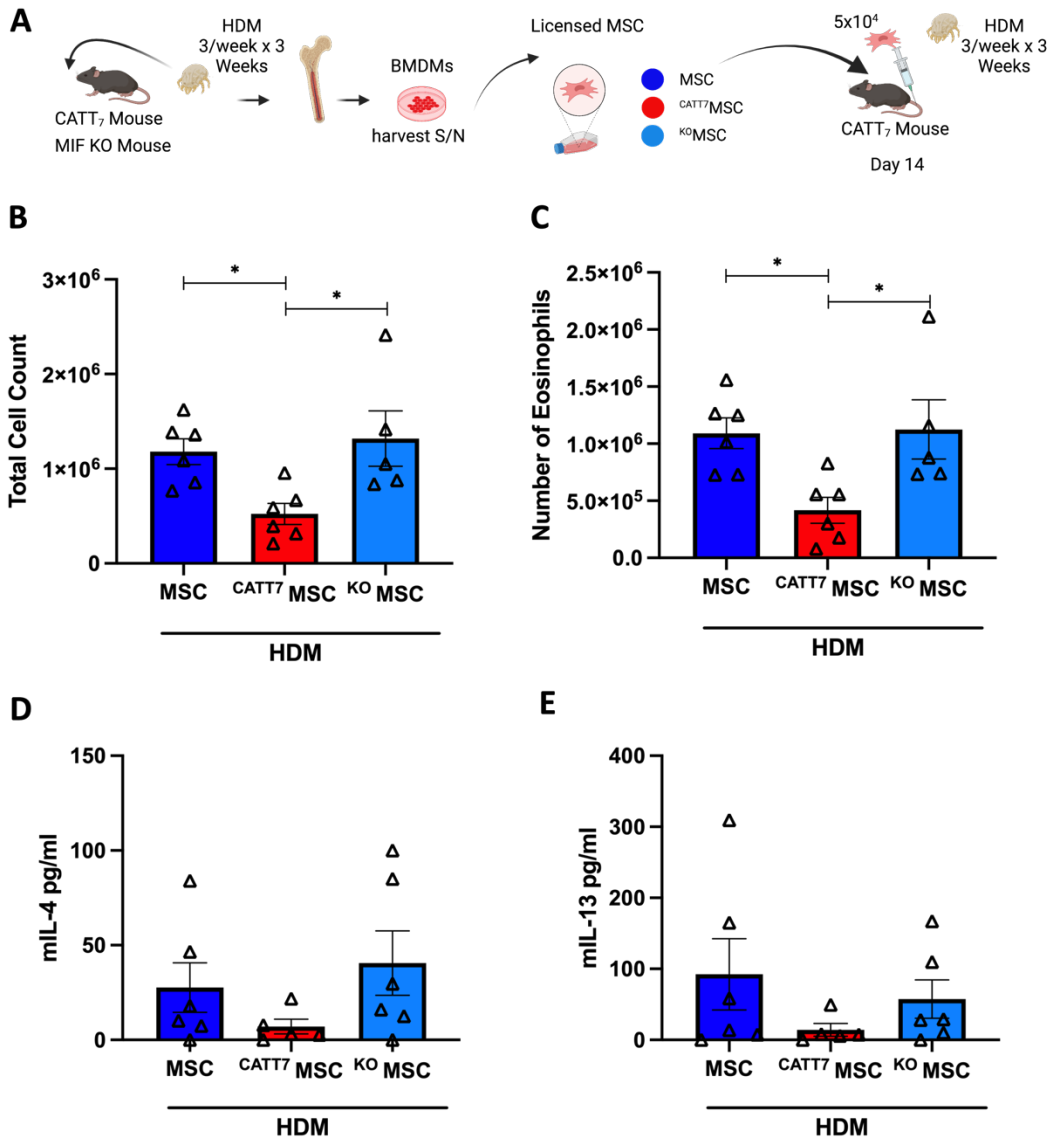


Figure 6. MIF licensing restores MSC efficacy at low doses in CATT$_7$ mice. A Once it was determined that MSCs lose their efficacy in the HDM model at 5x10^4, we used that dose as a benchmark for licensing. 5x10^4 MSCs were administered to HDM challenged CATT$_7$ mice on day 14. ^{CATT7}MSCs were licensed with CATT$_7$ BMDM supernatant for 24 hr prior to i.v. administration. The control group ^{KO}MSCs were generated by licensing MSCs with BMDM supernatant from MIF KO mice 24 Hr prior to i.v. administration. BAL was performed 4 Hr post final HDM challenge on day 18 (Schematic created with BioRender.com). **B** Total number of cells in the BAL were determined and differential cell counts were performed on the collected cells to determine the numbers of **C** eosinophils. Cytokine levels of **D** IL-4 and **E** IL-13 in the BAL fluid determined by ELISA. Data are presented as mean ± SEM; N=5-6 per group. Human BM-MSC donors 001-177 and 003-310 were used (RoosterBio Inc., Frederick, MD, USA). Statistical analysis was carried out using one-way ANOVA followed by the *post-hoc* Tukey's multiple comparison test where *p<0.05.

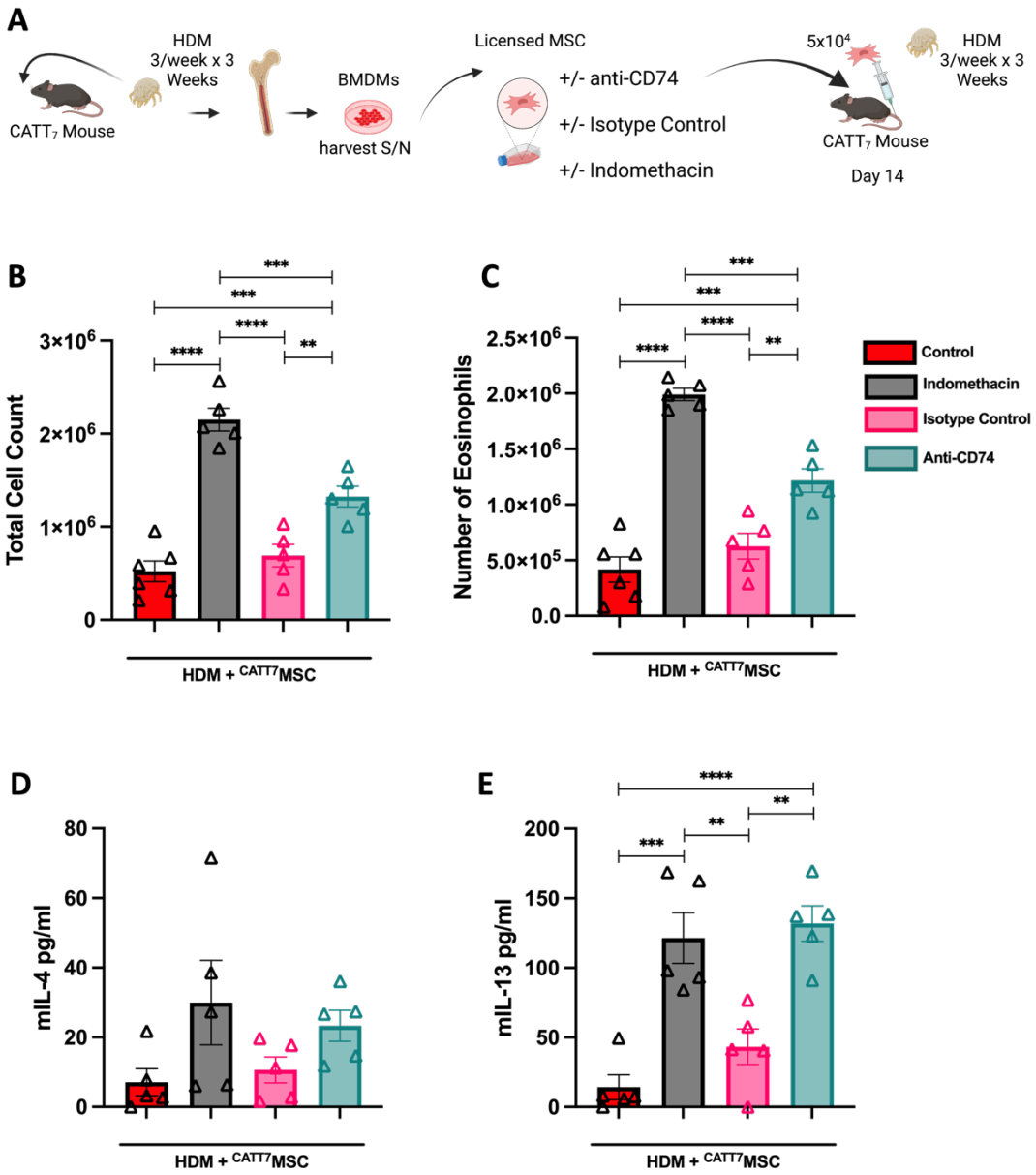
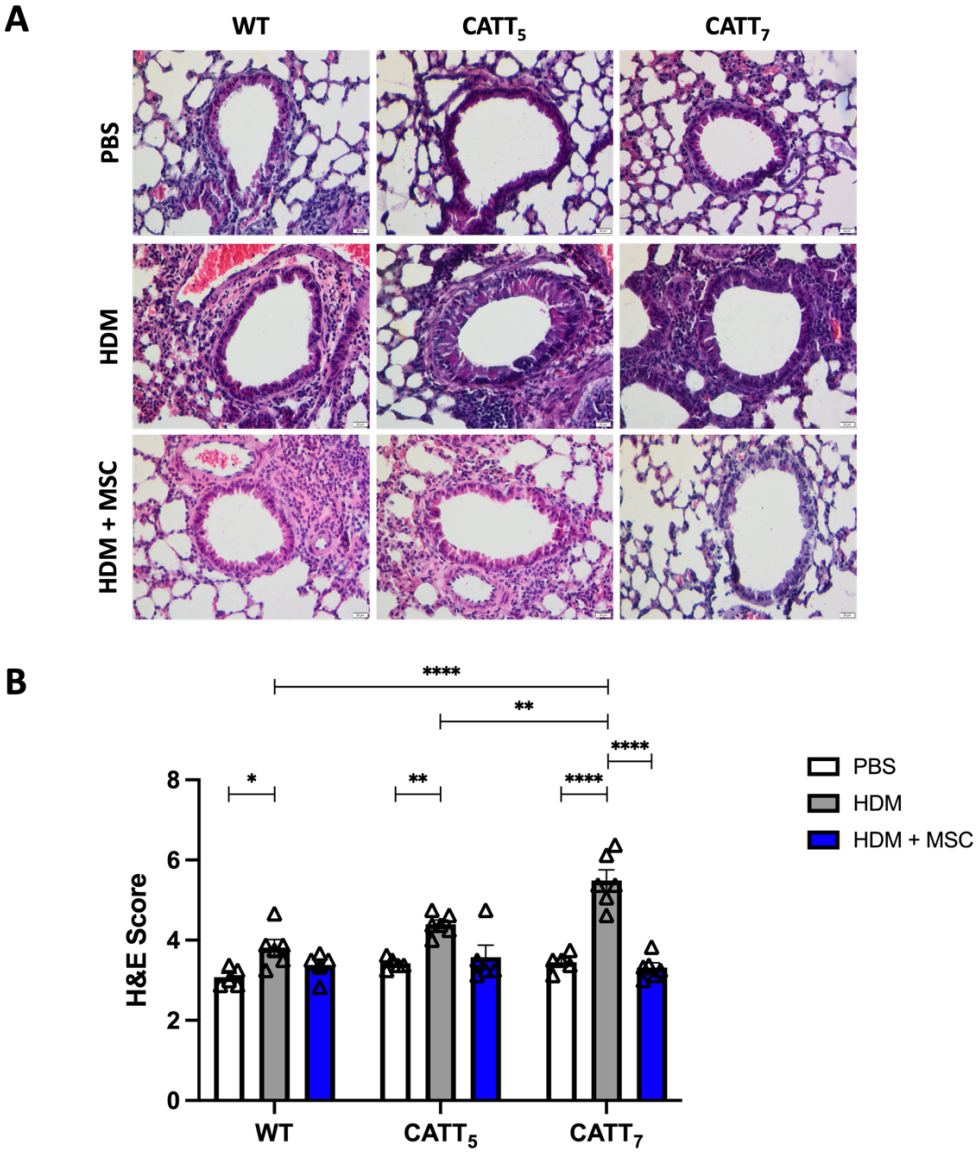


Figure 7. MIF-Licensed MSCs mediate their protective effects in HDM-induced allergic airway inflammation in a CD74 and COX-2 dependent manner in CATT7 mice. A 5x10^4 MSCs were exposed to the COX-2 inhibitor indomethacin, an anti-CD74 neutralising antibody or an isotype control antibody for 24 hr in vitro. All MSCs were licensed with CATT7 BMDM supernatant for 24 hr prior to i.v. administration. BAL was performed 4 hr post final HDM challenge on day 18 (Schematic created with BioRender.com). **B** Total number of cells in the BAL were determined and differential cell counts were performed on the collected cells to determine the numbers of **C** eosinophils. Cytokine levels of **D** IL-4 and **E** IL-13 in the BAL fluid determined by ELISA. Data are presented as mean ± SEM; N=5-6 per group. Human BM-MSC donors 001-177 and 003-310 were used (RoosterBio Inc., Frederick, MD, USA). Statistical analysis was carried out using one-way ANOVA followed by the *post-hoc* Tukey's multiple comparison test where *p<0.05.



Supplementary Figure 1. Human BM-MSCs significantly reduce inflammatory infiltrate surrounding airways in CATT$_7$ mice challenged with HDM. Haematoxylin and eosin (H&E) staining of lung tissue from WT, CATT$_5$ and CATT$_7$ mice. PBS and HDM groups received PBS or HDM i.n. 3 times a week for 3 consecutive weeks. 1x10^6 human BM-MSCs were administered i.v. to the HDM+MSC groups on day 14. Mice were sacrificed on day 21. **A** Representative images of lung tissue stained with H&E at 20X magnification. **B** Quantitation of airway inflammation in H&E-stained lung tissue. Data are presented as mean ± SEM; N=6 per group. Human BM-MSC donors 001-177 and 003-310 were used (RoosterBio Inc., Frederick, MD, USA). Statistical analysis was carried out using one-way ANOVA followed by the *post-hoc* Tukey's multiple comparison test where *p<0.05, **p<0.01, ****p<0.0001.

References

1. Sverrild A, Hansen S, Hvidtfeldt M, Clausson CM, Cozzolino O, Cerps S, et al. The effect of tezepelumab on airway hyperresponsiveness to mannitol in asthma (UPSTREAM). Eur Respir J. 2022 Jan;59(1):2101296.
2. Bleecker ER, FitzGerald JM, Chanez P, Papi A, Weinstein SF, Barker P, et al. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting β2-agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial. Lancet Lond Engl. 2016 Oct 29;388(10056):2115–27.
3. Corren J, Castro M, O'Riordan T, Hanania NA, Pavord ID, Quirce S, et al. Dupilumab efficacy in patients with uncontrolled, moderate-to-severe allergic asthma. J Allergy Clin Immunol Pract. 2020 Feb;8(2):516–26.
4. Ortega HG, Yancey SW, Mayer B, Gunsoy NB, Keene ON, Bleecker ER, et al. Severe eosinophilic asthma treated with mepolizumab stratified by baseline eosinophil thresholds: a secondary analysis of the DREAM and MENSA studies. Lancet Respir Med. 2016 Jul 1;4(7):549–56.
5. Chan R, Lipworth B. Efficacy of biologic therapy on airway hyperresponsiveness in asthma. Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol. 2023 Feb 23;S1081-1206(23)00121-7.
6. Goodwin M, Sueblinvong V, Eisenhauer P, Ziats NP, LeClair L, Poynter ME, et al. Bone marrow-derived mesenchymal stromal cells inhibit Th2-mediated allergic airways inflammation in mice. Stem Cells Dayt Ohio. 2011 Jul;29(7):1137–48.
7. Abreu SC, Antunes MA, de Castro JC, de Oliveira MV, Bandeira E, Ornellas DS, et al. Bone marrow-derived mononuclear cells vs. mesenchymal stromal cells in experimental allergic asthma. Respir Physiol Neurobiol. 2013 Jun 15;187(2):190–8.
8. Choi JY, Hur J, Jeon S, Jung CK, Rhee CK. Effects of human adipose tissue- and bone marrow-derived mesenchymal stem cells on airway inflammation and remodeling in a murine model of chronic asthma. Sci Rep. 2022 Jul 14;12(1):12032.
9. Dai R, Yu Y, Yan G, Hou X, Ni Y, Shi G. Intratracheal administration of adipose derived mesenchymal stem cells alleviates chronic asthma in a mouse model. BMC Pulm Med. 2018 Aug 8;18(1):131.
10. de Castro LL, Xisto DG, Kitoko JZ, Cruz FF, Olsen PC, Redondo PAG, et al. Human adipose tissue mesenchymal stromal cells and their extracellular vesicles act differentially on lung mechanics and inflammation in experimental allergic asthma. Stem Cell Res Ther. 2017 24;8(1):151.
11. Hur J, Kang JY, Kim YK, Lee SY, Jeon S, Kim Y, et al. Evaluation of human MSCs treatment frequency on airway inflammation in a mouse model of acute asthma. J Korean Med Sci. 2020 May 12;35(23):e188.
12. Mathias LJ, Khong SML, Spyroglou L, Payne NL, Siatskas C, Thorburn AN, et al. Alveolar macrophages are critical for the inhibition of allergic asthma by mesenchymal stromal cells. J Immunol Baltim Md 1950. 2013 Dec 15;191(12):5914–24.
13. Ou-Yang HF, Huang Y, Hu XB, Wu CG. Suppression of allergic airway inflammation in a mouse model of asthma by exogenous mesenchymal stem cells. Exp Biol Med Maywood NJ. 2011 Dec;236(12):1461–7.
14. Royce SG, Mao W, Lim R, Kelly K, Samuel CS. iPSC- and mesenchymoangioblast-derived mesenchymal stem cells provide greater protection against experimental chronic allergic airways disease compared with a clinically used corticosteroid. FASEB J Off Publ Fed Am Soc Exp Biol. 2019 May;33(5):6402–11.
15. Royce SG, Rele S, Broughton BRS, Kelly K, Samuel CS. Intranasal administration of mesenchymoangioblast-derived mesenchymal stem cells abrogates airway fibrosis and airway hyperresponsiveness associated with chronic allergic airways disease. FASEB J. 2017;31(9):4168–78.
16. Song X, Xie S, Lu K, Wang C. Mesenchymal stem cells alleviate experimental asthma by inducing polarization of alveolar macrophages. Inflammation. 2015 Apr;38(2):485–92.
17. Zhong H, Fan XL, Fang SB, Lin YD, Wen W, Fu QL. Human pluripotent stem cell-derived mesenchymal stem cells prevent chronic allergic airway inflammation via TGF-β1-Smad2/Smad3 signaling pathway in mice. Mol Immunol. 2019 May 1;109:51–7.

18. Abreu SC, Xisto DG, de Oliveira TB, Blanco NG, de Castro LL, Kitoko JZ, et al. Serum from asthmatic mice potentiates the therapeutic effects of mesenchymal stromal cells in experimental allergic asthma. Stem Cells Transl Med. 2019 Mar;8(3):301–12.
19. Abreu SC, Lopes-Pacheco M, da Silva AL, Xisto DG, de Oliveira TB, Kitoko JZ, et al. Eicosapentaenoic acid enhances the effects of mesenchymal stromal cell therapy in experimental allergic asthma. Front Immunol. 2018;9:1147.
20. Braza F, Dirou S, Forest V, Sauzeau V, Hassoun D, Chesné J, et al. Mesenchymal stem cells induce suppressive macrophages through phagocytosis in a mouse model of asthma. STEM CELLS. 2016;34(7):1836–45.
21. Castro LL, Kitoko JZ, Xisto DG, Olsen PC, Guedes HLM, Morales MM, et al. Multiple doses of adipose tissue-derived mesenchymal stromal cells induce immunosuppression in experimental asthma. Stem Cells Transl Med. 2020 Feb;9(2):250–60.
22. Duong KM, Arikkatt J, Ullah MA, Lynch JP, Zhang V, Atkinson K, et al. Immunomodulation of airway epithelium cell activation by mesenchymal stromal cells ameliorates house dust mite-induced airway inflammation in mice. Am J Respir Cell Mol Biol. 2015 Nov;53(5):615–24.
23. Kitoko JZ, Castro LL de, Nascimento AP, Abreu SC, Cruz FF, Arantes AC, et al. Therapeutic administration of bone marrow-derived mesenchymal stromal cells reduces airway inflammation without up-regulating Tregs in experimental asthma. Clin Exp Allergy. 2018;48(2):205–16.
24. Shin JW, Ryu S, Ham J, Jung K, Lee S, Chung DH, et al. Mesenchymal stem cells suppress severe asthma by directly regulating th2 cells and type 2 innate lymphoid cells. Mol Cells. 2021 Aug 31;44(8):580–90.
25. Cruz FF, Borg ZD, Goodwin M, Sokocevic D, Wagner DE, Coffey A, et al. Systemic administration of human bone marrow-derived mesenchymal stromal cell extracellular vesicles ameliorates aspergillus hyphal extract-induced allergic airway inflammation in immunocompetent mice. Stem Cells Transl Med. 2015 Nov;4(11):1302–16.
26. Lathrop MJ, Brooks EM, Bonenfant NR, Sokocevic D, Borg ZD, Goodwin M, et al. Mesenchymal stromal cells mediate Aspergillus hyphal extract-induced allergic airway inflammation by inhibition of the Th17 signaling pathway. Stem Cells Transl Med. 2014 Feb;3(2):194–205.
27. Abreu SC, Antunes MA, Xisto DG, Cruz FF, Branco VC, Bandeira E, et al. Bone marrow, adipose, and lung tissue-derived murine mesenchymal stromal cells release different mediators and differentially affect airway and lung parenchyma in experimental asthma. Stem Cells Transl Med. 2017 Jun;6(6):1557–67.
28. Mariñas-Pardo L, Mirones I, Amor-Carro Ó, Fraga-Iriso R, Lema-Costa B, Cubillo I, et al. Mesenchymal stem cells regulate airway contractile tissue remodeling in murine experimental asthma. Allergy. 2014 Jun;69(6):730–40.
29. Dunbar H, Weiss DJ, Rolandsson Enes S, Laffey JG, English K. The inflammatory lung microenvironment; a key mediator in MSC licensing. Cells. 2021 Nov 2;10(11):2982.
30. Liu J, Gao J, Liang Z, Gao C, Niu Q, Wu F, et al. Mesenchymal stem cells and their microenvironment. Stem Cell Res Ther. 2022 Aug 20;13(1):429.
31. Sudres M, Norol F, Trenado A, Grégoire S, Charlotte F, Levacher B, et al. Bone marrow mesenchymal stem cells suppress lymphocyte proliferation in vitro but fail to prevent graft-versus-host disease in mice. J Immunol Baltim Md 1950. 2006 Jun 15;176(12):7761–7.
32. Dorronsoro A, Ferrin I, Salcedo JM, Jakobsson E, Fernández-Rueda J, Lang V, et al. Human mesenchymal stromal cells modulate T-cell responses through TNF-α-mediated activation of NF-κB. Eur J Immunol. 2014 Feb;44(2):480–8.
33. Tobin LM, Healy ME, English K, Mahon BP. Human mesenchymal stem cells suppress donor CD4(+) T cell proliferation and reduce pathology in a humanized mouse model of acute graft-versus-host disease. Clin Exp Immunol. 2013 May;172(2):333–48.
34. Weiss DJ, English K, Krasnodembskaya A, Isaza-Correa JM, Hawthorne IJ, Mahon BP. The necrobiology of mesenchymal stromal cells affects therapeutic efficacy. Front Immunol. 2019;10:1228.
35. Islam D, Huang Y, Fanelli V, Delsedime L, Wu S, Khang J, et al. Identification and modulation of microenvironment is crucial for effective mesenchymal stromal cell therapy in acute lung injury. Am J Respir Crit Care Med. 2019 May 15;199(10):1214–24.
36. Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. Nat Biotechnol. 2014 Mar;32(3):252–60.

37. Mathew SA, Chandravanshi B, Bhonde R. Hypoxia primed placental mesenchymal stem cells for wound healing. Life Sci. 2017 Aug 1;182:85–92.
38. Roemeling-van Rhijn M, Mensah FKF, Korevaar SS, Leijs MJ, van Osch GJVM, Ijzermans JNM, et al. Effects of hypoxia on the immunomodulatory properties of adipose tissue-derived mesenchymal stem cells. Front Immunol. 2013;4:203.
39. Carty F, Dunbar H, Hawthorne IJ, Ting AE, Stubblefield SR, Van't Hof W, et al. IFN-γ and PPARδ influence the efficacy and retention of multipotent adult progenitor cells in graft vs host disease. Stem Cells Transl Med. 2021 Nov;10(11):1561–74.
40. Corbett JM, Hawthorne I, Dunbar H, Coulter I, Chonghaile MN, Flynn CM, et al. Cyclosporine A and IFNγ licencing enhances human mesenchymal stromal cell potency in a humanised mouse model of acute graft versus host disease. Stem Cell Res Ther. 2021 Apr 14;12(1):238.
41. Silva JD, Lopes-Pacheco M, de Castro LL, Kitoko JZ, Trivelin SA, Amorim NR, et al. Eicosapentaenoic acid potentiates the therapeutic effects of adipose tissue-derived mesenchymal stromal cells on lung and distal organ injury in experimental sepsis. Stem Cell Res Ther. 2019 23;10(1):264.
42. Bustos ML, Huleihel L, Meyer EM, Donnenberg AD, Donnenberg VS, Sciurba JD, et al. Activation of human mesenchymal stem cells impacts their therapeutic abilities in lung injury by increasing interleukin (IL)-10 and IL-1RN levels. Stem Cells Transl Med. 2013 Nov;2(11):884–95.
43. Rolandsson Enes S, Hampton TH, Barua J, McKenna DH, Dos Santos CC, Amiel E, et al. Healthy versus inflamed lung environments differentially affect mesenchymal stromal cells. Eur Respir J. 2021 Oct;58(4):2004149.
44. Abreu SC, Hampton TH, Hoffman E, Dearborn J, Ashare A, Singh Sidhu K, et al. Differential effects of the cystic fibrosis lung inflammatory environment on mesenchymal stromal cells. Am J Physiol-Lung Cell Mol Physiol. 2020 Sep 9;ajplung.00218.2020.
45. Bacher M, Metz CN, Calandra T, Mayer K, Chesney J, Lohoff M, et al. An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. Proc Natl Acad Sci U S A. 1996 Jul 23;93(15):7849–54.
46. Calandra T, Bernhagen J, Metz CN, Spiegel LA, Bacher M, Donnelly T, et al. MIF as a glucocorticoid-induced modulator of cytokine production. Nature. 1995 Sep 7;377(6544):68–71.
47. Calandra T, Bernhagen J, Mitchell RA, Bucala R. The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. J Exp Med. 1994 Jun 1;179(6):1895–902.
48. Donnelly SC, Haslett C, Reid PT, Grant IS, Wallace WA, Metz CN, et al. Regulatory role for macrophage migration inhibitory factor in acute respiratory distress syndrome. Nat Med. 1997 Mar;3(3):320–3.
49. Das R, Moss JE, Robinson E, Roberts S, Levy R, Mizue Y, et al. Role of macrophage migration inhibitory factor in the Th2 immune response to epicutaneous sensitization. J Clin Immunol. 2011 Aug;31(4):666–80.
50. Li R, Wang F, Wei J, Lin Y, Tang G, Rao L, et al. The role of macrophage migration inhibitory factor (mif) in asthmatic airway remodeling. Allergy Asthma Immunol Res. 2021 Jan;13(1):88–105.
51. Magalhães ES, Mourao-Sa DS, Vieira-de-Abreu A, Figueiredo RT, Pires AL, Farias-Filho FA, et al. Macrophage migration inhibitory factor is essential for allergic asthma but not for Th2 differentiation. Eur J Immunol. 2007 Apr;37(4):1097–106.
52. Mizue Y, Ghani S, Leng L, McDonald C, Kong P, Baugh J, et al. Role for macrophage migration inhibitory factor in asthma. Proc Natl Acad Sci U S A. 2005 Oct 4;102(40):14410–5.
53. Allam VSRR, Pavlidis S, Liu G, Kermani NZ, Simpson J, To J, et al. Macrophage migration inhibitory factor promotes glucocorticoid resistance of neutrophilic inflammation in a murine model of severe asthma. Thorax. 2022 Nov 7;thoraxjnl-2021-218555.
54. Amano T, Nishihira J, Miki I. Blockade of macrophage migration inhibitory factor (MIF) prevents the antigen-induced response in a murine model of allergic airway inflammation. Inflamm Res Off J Eur Histamine Res Soc Al. 2007 Jan;56(1):24–31.
55. Kobayashi M, Nasuhara Y, Kamachi A, Tanino Y, Betsuyaku T, Yamaguchi E, et al. Role of macrophage migration inhibitory factor in ovalbumin-induced airway inflammation in rats. Eur Respir J. 2006 Apr;27(4):726–34.
56. Chen PF, Luo Y ling, Wang W, Wang J xin, Lai W yan, Hu S ming, et al. ISO-1, a macrophage migration inhibitory factor antagonist, inhibits airway remodeling in a murine model of chronic asthma. Mol Med Camb Mass. 2010 Oct;16(9–10):400–8.

57. Awandare GA, Martinson JJ, Were T, Ouma C, Davenport GC, Ong'echa JM, et al. Macrophage migration inhibitory factor (MIF) promoter polymorphisms and susceptibility to severe malarial anemia. J Infect Dis. 2009 Aug 15;200(4):629–37.
58. Savva A, Brouwer MC, Roger T, Valls Serón M, Le Roy D, Ferwerda B, et al. Functional polymorphisms of macrophage migration inhibitory factor as predictors of morbidity and mortality of pneumococcal meningitis. Proc Natl Acad Sci. 2016 Mar 29;113(13):3597–602.
59. Benedek G, Meza-Romero R, Jordan K, Zhang Y, Nguyen H, Kent G, et al. MIF and D-DT are potential disease severity modifiers in male MS subjects. Proc Natl Acad Sci U S A. 2017 Oct 3;114(40):E8421–9.
60. Liu A, Bao F, Voravuthikunchai SP. CATT polymorphism in MIF gene promoter is closely related to human pulmonary tuberculosis in a southwestern China population. Int J Immunopathol Pharmacol. 2018 May 29;32:2058738418777108.
61. Shin JJ, Fan W, Par-Young J, Piecychna M, Leng L, Israni-Winger K, et al. MIF is a common genetic determinant of COVID-19 symptomatic infection and severity. QJM Mon J Assoc Physicians. 2022 Oct 12;hcac234.
62. Dunbar H, Hawthorne IJ, Tunstead C, Armstrong M, Donnelly SC, English K. Blockade of MIF biological activity ameliorates house dust mite-induced allergic airway inflammation in humanised MIF mice. FASEB J. 2023;37.
63. Lourenco S, Teixeira VH, Kalber T, Jose RJ, Floto RA, Janes SM. Macrophage Migration Inhibitory Factor - CXCR4 is the dominant chemotactic axis in human mesenchymal stem cell recruitment to tumors. J Immunol Baltim Md 1950. 2015 Apr 1;194(7):3463–74.
64. Fingerle-Rowson G, Petrenko O, Metz CN, Forsthuber TG, Mitchell R, Huss R, et al. The p53-dependent effects of macrophage migration inhibitory factor revealed by gene targeting. Proc Natl Acad Sci U S A. 2003 Aug 5;100(16):9354–9.
65. Cahill EF, Tobin LM, Carty F, Mahon BP, English K. Jagged-1 is required for the expansion of CD4+ CD25+ FoxP3+ regulatory T cells and tolerogenic dendritic cells by murine mesenchymal stromal cells. Stem Cell Res Ther. 2015 Mar 11;6(1):19.
66. Ehrentraut H, Clambey ET, McNamee EN, Brodsky KS, Ehrentraut SF, Poth JM, et al. CD73+ regulatory T cells contribute to adenosine-mediated resolution of acute lung injury. FASEB J Off Publ Fed Am Soc Exp Biol. 2013 Jun;27(6):2207–19.
67. Lan H, Wang N, Chen Y, Wang X, Gong Y, Qi X, et al. Macrophage migration inhibitory factor (MIF) promotes rat airway muscle cell proliferation and migration mediated by ERK1/2 and FAK signaling. Cell Biol Int. 2018;42(1):75–83.
68. Ohta S, Misawa A, Fukaya R, Inoue S, Kanemura Y, Okano H, et al. Macrophage migration inhibitory factor (MIF) promotes cell survival and proliferation of neural stem/progenitor cells. J Cell Sci. 2012 Jul 1;125(Pt 13):3210–20.
69. Utispan K, Koontongkaew S. Macrophage migration inhibitory factor modulates proliferation, cell cycle, and apoptotic activity in head and neck cancer cell lines. J Dent Sci. 2021 Jan;16(1):342–8.
70. Carli C, Metz CN, Al-Abed Y, Naccache PH, Akoum A. Up-regulation of cyclooxygenase-2 expression and prostaglandin E2 production in human endometriotic cells by macrophage migration inhibitory factor: involvement of novel kinase signaling pathways. Endocrinology. 2009 Jul;150(7):3128–37.
71. Mitchell RA, Liao H, Chesney J, Fingerle-Rowson G, Baugh J, David J, et al. Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p53: regulatory role in the innate immune response. Proc Natl Acad Sci U S A. 2002 Jan 8;99(1):345–50.
72. Li D, Han Y, Zhuang Y, Fu J, Liu H, Shi Q, et al. Overexpression of COX-2 but not indoleamine 2,3-dioxygenase-1 enhances the immunosuppressive ability of human umbilical cord-derived mesenchymal stem cells. Int J Mol Med. 2015 May;35(5):1309–16.
73. Kulesza A, Paczek L, Burdzinska A. The role of COX-2 and PGE2 in the regulation of immunomodulation and other functions of mesenchymal stromal cells. Biomedicines. 2023 Feb;11(2):445.
74. Lin YD, Fan XL, Zhang H, Fang SB, Li CL, Deng MX, et al. The genes involved in asthma with the treatment of human embryonic stem cell-derived mesenchymal stem cells. Mol Immunol. 2018 Mar 1;95:47–55.
75. Fallon PG, Schwartz C. The high and lows of type 2 asthma and mouse models. J Allergy Clin Immunol. 2020 Feb 1;145(2):496–8.

76. Hass R, Kasper C, Böhm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. Cell Commun Signal CCS. 2011 May 14;9:12.
77. Carty F, Corbett JM, Cunha JPMCM, Reading JL, Tree TIM, Ting AE, et al. Multipotent adult progenitor cells suppress T cell activation in in vivo models of homeostatic proliferation in a prostaglandin E2-dependent manner. Front Immunol. 2018 Apr 23;9:645.
78. de Witte SFH, Merino AM, Franquesa M, Strini T, van Zoggel JAA, Korevaar SS, et al. Cytokine treatment optimises the immunotherapeutic effects of umbilical cord-derived MSC for treatment of inflammatory liver disease. Stem Cell Res Ther. 2017 Jun 8;8(1):140.
79. Kim RL, Bang JY, Kim J, Mo Y, Kim Y, Lee CG, et al. Mesenchymal stem cells exert their anti-asthmatic effects through macrophage modulation in a murine chronic asthma model. Sci Rep. 2022 Jun 13;12:9811.
80. Kabat M, Bobkov I, Kumar S, Grumet M. Trends in mesenchymal stem cell clinical trials 2004-2018: Is efficacy optimal in a narrow dose range? Stem Cells Transl Med. 2019 Dec 5;9(1):17–27.
81. Malaquias M a. S, Oyama LA, Jericó PC, Costa I, Padilha G, Nagashima S, et al. Effects of mesenchymal stromal cells play a role the oxidant/antioxidant balance in a murine model of asthma. Allergol Immunopathol (Madr). 2018 Apr;46(2):136–43.
82. Kwak J, Choi SJ, Oh W, Yang YS, Jeon HB, Jeon ES. Cobalt chloride enhances the anti-inflammatory potency of human umbilical cord blood-derived mesenchymal stem cells through the ERK-HIF-1α-microRNA-146a-mediated signaling pathway. Stem Cells Int. 2018;2018:4978763.
83. Hong GH, Kwon HS, Lee KY, Ha EH, Moon KA, Kim SW, et al. hMSCs suppress neutrophil-dominant airway inflammation in a murine model of asthma. Exp Mol Med. 2017 27;49(1):e288.
84. Cruz FF, Borg ZD, Goodwin M, Coffey AL, Wagner DE, Rocco PRM, et al. CD11b+ and Sca-1+ cells exert the main beneficial effects of systemically administered bone marrow-derived mononuclear cells in a murine model of mixed Th2/Th17 allergic airway inflammation. STEM CELLS Transl Med. 2016;5(4):488–99.
85. Kang I, Bucala R. The immunobiology of MIF: function, genetics and prospects for precision medicine. Nat Rev Rheumatol. 2019 Jul;15(7):427–37.
86. Farr L, Ghosh S, Moonah S. Role of MIF Cytokine/CD74 receptor pathway in protecting against injury and promoting repair. Front Immunol. 2020;11:1273.
87. Bucala R, Shachar I. The integral role of CD74 in antigen presentation, MIF signal transduction, and B cell survival and homeostasis. Mini Rev Med Chem. 2014;14(14):1132–8.
88. Imaoka M, Tanese K, Masugi Y, Hayashi M, Sakamoto M. Macrophage migration inhibitory factor-CD74 interaction regulates the expression of programmed cell death ligand 1 in melanoma cells. Cancer Sci. 2019 Jul;110(7):2273–83.
89. Klasen C, Ziehm T, Huber M, Asare Y, Kapurniotu A, Shachar I, et al. LPS-mediated cell surface expression of CD74 promotes the proliferation of B cells in response to MIF. Cell Signal. 2018;46:32–42.
90. Soppert J, Kraemer S, Beckers C, Averdunk L, Möllmann J, Denecke B, et al. Soluble CD74 reroutes MIF/CXCR4/AKT-mediated survival of cardiac myofibroblasts to necroptosis. J Am Heart Assoc. 2018 04;7(17):e009384.
91. Flaster H, Bernhagen J, Calandra T, Bucala R. The macrophage migration inhibitory factor-glucocorticoid dyad: regulation of inflammation and immunity. Mol Endocrinol. 2007 Jun 1;21(6):1267–80.
92. Sampey AV, Hall PH, Mitchell RA, Metz CN, Morand EF. Regulation of synoviocyte phospholipase A2 and cyclooxygenase 2 by macrophage migration inhibitory factor. Arthritis Rheum. 2001 Jun;44(6):1273–80.
93. Duffy MM, Pindjakova J, Hanley SA, McCarthy C, Weidhofer GA, Sweeney EM, et al. Mesenchymal stem cell inhibition of T-helper 17 cell- differentiation is triggered by cell–cell contact and mediated by prostaglandin E2 via the EP4 receptor. Eur J Immunol. 2011;41(10):2840–51.
94. Cahill EF, Kennelly H, Carty F, Mahon BP, English K. Hepatocyte growth factor is required for mesenchymal stromal cell protection against bleomycin-induced pulmonary fibrosis. Stem Cells Transl Med. 2016 Oct;5(10):1307–18.
95. English K, Barry FP, Field-Corbett CP, Mahon BP. IFN-γ and TNF-α differentially regulate Immunomodulation by murine mesenchymal stem cells. Immunol Lett. 2007 Jun 15;110(2):91–100.

96. Sun L, Chen K, Jiang Z, Chen X, Ma J, Ma Q, et al. Indometacin inhibits the proliferation and activation of human pancreatic stellate cells through the downregulation of COX-2. Oncol Rep. 2018 May;39(5):2243–51.
97. Zhu W, Sun L, Zhao P, Liu Y, Zhang J, Zhang Y, et al. Macrophage migration inhibitory factor facilitates the therapeutic efficacy of mesenchymal stem cells derived exosomes in acute myocardial infarction through upregulating miR-133a-3p. J Nanobiotechnology. 2021 Feb 27;19:61.
98. Liu X, Li X, Zhu W, Zhang Y, Hong Y, Liang X, et al. Exosomes from mesenchymal stem cells overexpressing MIF enhance myocardial repair. J Cell Physiol. 2020 Jan 20;
99. Zhang Y, Zhu W, He H, Fan B, Deng R, Hong Y, et al. Macrophage migration inhibitory factor rejuvenates aged human mesenchymal stem cells and improves myocardial repair. Aging. 2019 27;11(24):12641–60.
100. Zhang Y, Zhou Y, Chen S, Hu Y, Zhu Z, Wang Y, et al. Macrophage migration inhibitory factor facilitates prostaglandin E2 production of astrocytes to tune inflammatory milieu following spinal cord injury. J Neuroinflammation. 2019 Apr 13;16(1):85.