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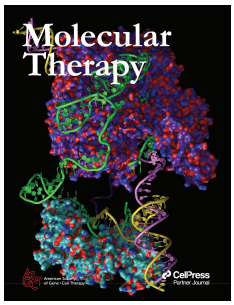

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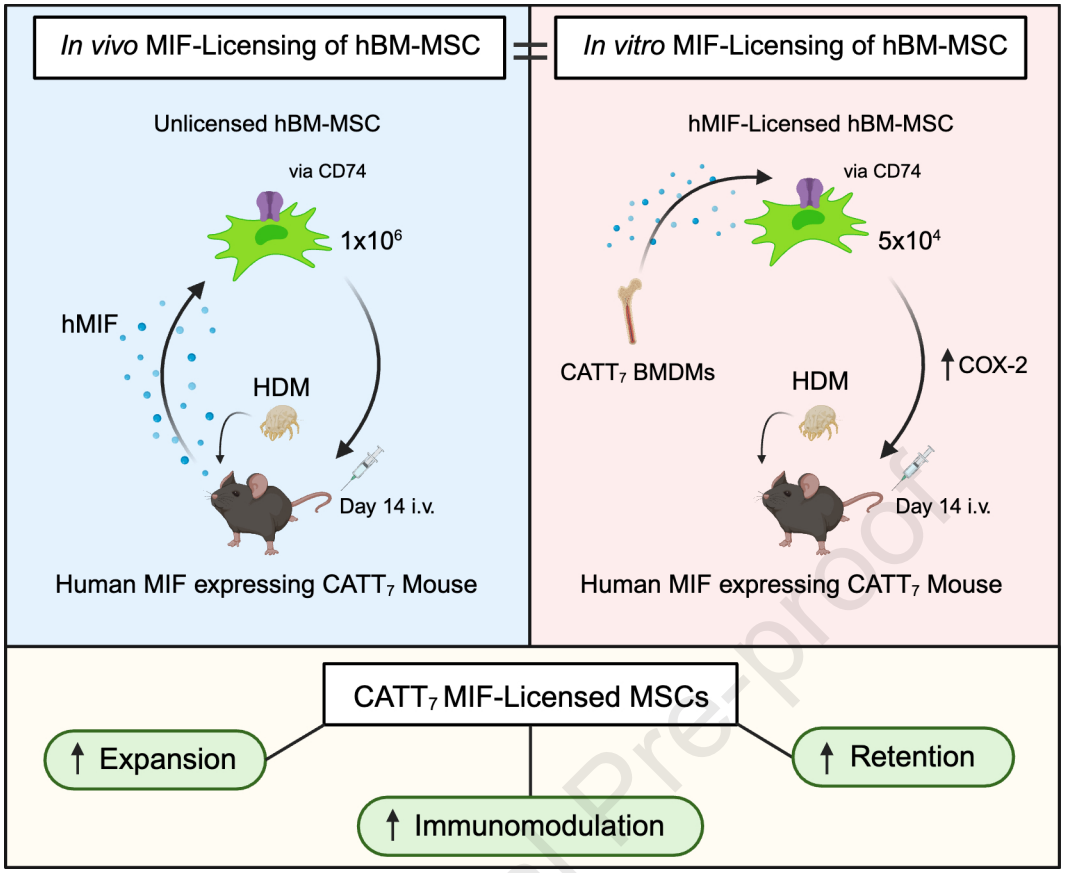
English and colleagues discuss how mesenchymal stromal cells significantly attenuated house dust mite-induced airway inflammation and airway remodelling in high MIF expressing $CATT_7$ mice. Blockade of CD74 or COX-2 function in MSCs prior to administration attenuated the efficacy of MIF-licensed MSCs *in vivo*.



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 ($CATT_{6/7/8}$).

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 IFNγ, NF-κB or TNFα 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, IFNγ, 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.

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 (Figure 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 reduced the level of goblet cell hyperplasia in all groups to almost background levels, with a significant reduction in the number of mucin secreting cells in the airways of HDM challenged $CATT_7$ mice (Figure 1B, 1C).

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 reduced the level of subepithelial fibrosis to almost background levels in all groups with significantly reduced subepithelial collagen deposition in HDM challenged $CATT_7$ mice (Figure 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 (Figure S1).

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 (Figure 2A). MSCs significantly reduced the number of total infiltrating cells in the

BALF of $CATT_7$ mice (Figure 2A). Differential cell counts identified eosinophils as the main cell infiltrating into the lung tissue following HDM challenge and MSCs significantly decreased infiltrating eosinophils in $CATT_7$ mice yet had no effect in the $CATT_5$ and WT groups (Figure 2B). IL-4 and IL-13 were significantly elevated in the BAL fluid of $CATT_7$ mice following HDM challenge (Figure 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 (Figure 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 (Figure 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 (Figure 3C, 3D). However, the number of clusters of labelled BM-MSCs within the lungs remained unchanged among the groups (Figure 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.

The influence of MIF on MSC expression of immunomodulatory factors and MSC cytokine licensing *in vitro*

MSCs mediate their therapeutic effects via expression or production of secreted factors *in vitro* and *in vivo*[64] and licensing with proinflammatory cytokines such as IFNγ or TNFα[65-69] can enhance expression of immunomodulatory mediators. Here, we characterised the effect of recombinant human MIF on the expression of IDO, COX-2, PTGES, ICAM-1 and HGF in untreated MSCs or MSCs licensed with IFNγ or TNFα. rhMIF (1ng/ml) stimulation alone did not increase expression of IDO, COX-2, PTGES, ICAM-1 or HGF (Figure 4A-E) in human

BM-MSCs. Following licensing with TNFα, MIF stimulation enhanced MSC expression of COX-2 and PTGES (Figure 4B-C). In IFNγ licensed MSCs, rhMIF stimulation did not enhance MSC expression of IDO or HGF and significantly reduced ICAM-1 expression (Figure 4A, 4D, 4E). We confirmed these findings at the protein level for IDO and COX-2 using intracellular flow cytometry (Figure 4F-G). Using increasing doses of rhMIF (1, 10 or 100 ng/ml), we showed that COX-2 expression is increased in a dose-dependent manner by rhMIF stimulation in TNFα licensed MSCs, with COX-2 expression plateauing at 10ng/ml of rhMIF (Figure 4H). The MIF receptor CD74 is expressed by MSCs, however, rhMIF stimulation (dose range 1, 10 or 100ng/ml) does not enhance CD74 expression (Figure 4I).

$CATT_7$ MIF licensing enhances MSC expansion and immunosuppressive function *in vitro*

In order to investigate the effect of endogenous MIF from $CATT_7$ mice on MSC expression of immunomodulatory factors, we generated bone marrow-derived macrophages (BMDMs) from $CATT_7$ mice and used the conditioned medium (CM) as a source of endogenous human MIF (Figure 5A) to license MSCs. The concentration of human MIF in $CATT_7$ BMDM CM ranged from ~3000–4000 pg/ml (Figure S2).

MIF may have a negative role in the regulation of IDO expression as $MIF^{-/-}$ mice produce more IDO,[70] however, MIF has an established role as an upstream positive regulator of cyclooxygenase 2 (COX-2) through the activation of the MAPK signalling pathway.[71,72] IDO, COX-2 and PGE2 are widely reported mediators of MSC immunosuppression.[39,73] MSCs constitutively express COX-2 but not IDO. IFNγ licensing of MSCs leads to expression of IDO while TNFα enhances MSC COX-2 expression.[68] Here we show that $CATT_7$ MIF stimulation reduces MSC IDO production (Figure 5B-C), however, the percentage of COX-2 expressing MSCs was significantly increased following $CATT_7$ MIF stimulation (Figure 5D-E). Human MSCs express the MIF receptor CD74 and this expression is maintained and not increased following exposure to $CATT_7$ MIF CM (Figure 5F). In line with other studies,[74] we show that IFNγ stimulation leads to significantly increased MSC CD74 expression and $CATT_7$ MIF CM does not significantly alter that (Figure 5F). This aligns with our data showing that MIF does not enhance IFNγ regulated IDO expression. Given the potentiating effect of MIF on the TNFα regulated gene COX-2 in MSCs, we examined the influence of $CATT_7$ MIF on MSC expression of the TNFα regulated genes TSG-6 and PTGS2. The presence of $CATT_7$ MIF CM significantly reduced TSG-6 in TNFα stimulated MSCs (Figure 5G), but did not significantly

alter the expression of PTGS2 (Figure 5H). 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 (Figure 5I) or the number of proliferating $CD3^+$ T cells (Figure 5J). 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 (Figure 5J).

Previous studies have shown that MIF has the ability to support cell proliferation *in vitro*.[75-77] 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 (Figure 5K). Blockade of MIF using SCD-19 confirmed the role of MIF in driving MSC expansion (Figure 5K). This data might help to explain the enhanced retention of MSCs in $CATT_7$ HDM challenged mice (Figure 3) but further experiments would be required to confirm that.

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 (Figure 6A). MSCs maintained efficacy as low as $1x10^5$ cells with reduced immune cell infiltration (Fig. 6B, 6C) and reduced Th2 cytokines IL-4 (Figure 6D) and IL-13 (Figure 6E). 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 (Figure 6B, 6C) or regulate Th2 cytokine production (Figure 6D, 6E).

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 (Figure 7A). Only ^{CATT7}MSC administration

significantly reduced total cell counts and number of eosinophils in $CATT_7$ mice challenged with HDM (Figure 7B, 7C). ^{CATT7}MSCs markedly reduced IL-4 and IL-13 levels compared to control group although not significantly (Figure 7D, 7E). 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.[78] 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 (Figure 8A). 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 (Figure 8B, 8C). Additionally, the analysis of the Th2 cytokines in the BALF showed a marked increase in IL-4 (Figure 8D) and a significant increase in the levels of IL-13 (Figure 8E) 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 suppression of eosinophil infiltration and type 2 cytokines in the BAL (Figure 8). 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. Our data aligns with the literature demonstrating the ability of human MSCs to ameliorate eosinophil infiltration by reducing the levels of Th2 cytokines.[8,10-12,25,79] In addition to reducing inflammation, MSCs also alleviate features of airway remodelling in the $CATT_7$ mice. Interestingly while MSCs were effective at reducing the severity of goblet cell hyperplasia and subepithelial fibrosis in all groups, we did not observe the same changes in type 2 inflammatory markers in the BAL of WT and low MIF expressing $CATT_5$ mice suggesting that high levels of MIF may be 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.[80] 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. report 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. 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, however, the majority of the current literature demonstrates that MSCs can attenuate airway remodelling.[8,10,14,15,17,18,28] Others report a deficit in MSCs capacity to ameliorate goblet cell hyperplasia[14,19,23] or subepithelial collagen deposition.[9,28] Reasons for these discrepancies include source of MSCs,[8,23,81] genotypic mouse model differences, severity of mouse models, time of infusion, MSC fitness, dosing, and route of administration.[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.[39,82] 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. 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.[83] 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[84] 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[52,85,86] 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 microenvironments 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 IFNγ 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.[18,19,87]

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.[88] 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.[8,10,12,16,20-22,25,26,28,79,89-92] The efficacy observed with MIF licensed MSCs using $5x10^4$ 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. Given our use of human MSCs in a mouse host, the interspecies ligand/receptor non-functionality can raise questions about how human MSCs might mediate their effects in a mouse host. We and others have shown that indeed human MSCs can mediate protective effects in mouse hosts.[8,10-12,14-17,21,24,25,33,93] Four studies have tracked human MSC biodistribution following i.v. administration in patients with COPD,[94] liver cirrhosis,[95] haemophilia A[96] or breast cancer.[97] No MSCs were detected in blood at 1hr post infusion. MSCs were distributed mainly in the lung[95] or lungs and liver[96] at 48 hr post i.v. infusion with the signal decreasing thereafter. As such, these studies suggest that biodistribution of human MSCs following i.v. administration in humans aligns with the studies investigating MSC biodistribution in mouse models.[98]

In terms of mechanism, MIF mediated signal transduction is primarily initiated by binding to MIF's classical receptor CD74.[99] 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.[100-104] 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.[105] Interestingly MIF can upregulate COX-2 expression, a rate limiting step in the synthesis of prostaglandins such as PGE2,[71,72,106] however, MIF has been shown to have no effect on the expression of COX-1.[71]

The COX-2/PGE2 pathway has been extensively documented as being one of the key mediators driving MSC immunomodulation.[39,68,107,108] Our data shows that MIF stimulation enhances the expression of COX-2 but not TSG-6 or PTGS2 in untreated and TNFα licensed MSCs. 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.[109] 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*.[110-112] 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.[110-112] Furthermore, Zhang et al. demonstrated the ability of MIF to upregulate COX-2 expression and promote PGE2 production in astrocytes.[113] 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.

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 passaged once they achieved 80% confluency. All experiments were carried out between passages 2-5.

MSC Characterisation

Three different human bone marrow-derived MSC (BM-MSC) donors (identified as 001-177, 003-307 and 003-310) from RoosterBio Inc. (Frederick, MD, USA) were characterised by analysing the expression of cell surface markers. All MSCs donors were found to be negative for CD34 (FITC), CD45 (APC) and HLA-DR (PE) and positive for CD73 (APC), CD90 (FITC) and CD105 (PE) (BD Pharmingen, San Diego, CA, USA) by the Attune Nxt Flow Cytometer (Figure S3).

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)[114] (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.[115] 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 Systems, MN, USA) as described previously (Figure S2). [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 subepithelial collagen deposition respectively. H&E analysis was carried out as previously described.[116] Goblet cell hyperplasia was determined by the % of PAS positive cells in airways relative to airway diameter. Subepithelial collagen 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. 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]

Analysis of Gene Expression

Total RNA was extracted using TRIzol (Ambion Life Sciences, Cambridgeshire, UK) 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 (Quantabio, MA, USA). Real Time-Polymerase Chain Reaction (RT-PCR) was carried out using PerfeCta SYBR Green FastMix (Quantbio, MA, USA). Expression of human COX-2, PTGES, IDO, ICAM-1, HGF, TSG-6 and PTGS2 (primer sequence information is contained in Table S1) 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.

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 and IDO

MSCs were seeded at $1x10^5$ cells per well in 6 well plates. MSCs were stimulated with recombinant human IFNγ at low (5ng/ml) or high (40ng/ml) concentrations, TNFα (5-10ng/ml)

(PeproTech, London, UK), recombinant human MIF (1ng/ml) (provided by Rick Bucala, Yale) or endogenous MIF ($CATT_7$ 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) or IDO (APC) (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.

Surface Staining of CD74

MSCs were seeded at $1x10^5$ cells per well in 6 well plates. MSCs were stimulated with recombinant human IFNγ (5ng/ml) (PeproTech, London, UK), recombinant human MIF (1, 10 or 100ng/ml) or endogenous MIF ($CATT_7$ CM) for 24 hr. Cells were stained with CD74 (PE) (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™ human CD3/CD28 T cell activator antibody mix (STEMCELL Technologies, Cambridge, UK). After 4 days, PBMCs were harvested and the frequency (%) and number of proliferating $CD3^+$ T cells were analysed by flow cytometry (Attune Nxt Flow Cytometer).

Statistical Analysis

Mice were randomised. 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.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

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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 & TS performed research and data analysis; DJW, SRE & CCDS 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.

Declaration of Interest

The authors declare no conflict of interest.

Keywords

Mesenchymal Stromal Cells, House Dust Mite, Allergic Asthma, Macrophage Migration Inhibitory Factor, Cyclooxygenase

References

1. Sverrild A, Hansen S, Hvidtfeldt M, Clausson CM, Cozzolino O, Cerps S, Uller L, Backer V, Erjefält J, Porsbjerg C. 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, Sproule S, Gilmartin G, Aurivillius M, Werkström V, 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, Chipps BE, Wenzel SE, Thangavelu K, Rice MS, 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, Brightling CE, Pavord ID. 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, Steele C, Rincon M, Weiss DJ. 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, Diaz BL, Morales MM, Xisto DG, Rocco PRM. 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, Ferreira TPT, Weiss DJ, Martins MA, Morales MM, 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, Jung CK, Rhee CK. 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, Boyd RL, Heng TSP. 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, Olsen PC, Lopes-Pacheco M, Morales MM, Weiss DJ, 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, de Castro LL, Amorim NR, Martins V, Silva LHA, 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, Cheminant-Muller MA, Sagan C, Magnan A, Lemarchand P. 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, Lopes-Pacheco M, Cruz FF, Rocco PRM. 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, Sly PD, Phipps S. 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, Xisto DG, Martins MA, Morales MM, Rocco PRM, 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, Kang HR, Kim HY. 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, Antunes M, Robinson KL, Mitsialis SA, Kourembanas S, 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, Loi R, Cruz F, Dunaway CW, Steele C, 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, Zola Kitoko J, de Araújo AF, Dellatorre-Texeira L, Olsen PC, 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, Rodríguez Milla MÁ, García-Castro J, Ramos-Barbón D. 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, Zhang L. 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, Lataillade JJ, Bourin P, Holy X, Vernant JP, 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, 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. 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, 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 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, Betjes MGH, Baan CC, Weimar W, Hoogduijn MJ. 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, 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 Nov;10(11):1561–74.

40. Corbett JM, Hawthorne I, Dunbar H, Coulter I, Chonghaile MN, Flynn CM, 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 Apr 14;12(1):238.

41. Silva JD, Lopes-Pacheco M, de Castro LL, Kitoko JZ, Trivelin SA, Amorim NR, Capelozzi VL, Morales MM, Gutfilen B, de Souza SAL, 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, Mroz L, McVerry BJ, Ellis BM, 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 Nov;2(11):884–95.

43. Rolandsson Enes S, Hampton TH, Barua J, McKenna DH, Dos Santos CC, Amiel E, Ashare A, Liu KD, Krasnodembskaya AD, English K, 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, Matthews DE, McKenna DH, 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 Sep 9;ajplung.00218.2020.

45. Bacher M, Metz CN, Calandra T, Mayer K, Chesney J, Lohoff M, Gemsa D, Donnelly T, Bucala R. 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, Cerami A, Bucala R. 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, Bruce LJ, Bucala R. 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, Leng L, McDonald C, Tigelaar RE, Herrick CA, 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, Ma L, Xu Q, Wu J, Lv Q, 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, Fonseca BPF, Viola JPB, Metz C, Martins MA, 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, Lane SJ, Craft J, Nishihira J, Donnelly SC, 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, Donnelly S, Guo YK, Hansbro PM, Phipps S, 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, Nishihira J, Nishimura M. 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, Cheng KF, Al-Abed Y. 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, Wang WK, Leng L, Ferrell RE, Bucala R, 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, van der Ende A, Bochud PY, van de Beek D, Calandra T. 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, Li J, Siu E, Frazer J, Piecychna M, 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, Qing H, Gu J, Zhao H, Schulz WL, 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 ME, 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. English K. Mechanisms of mesenchymal stromal cell immunomodulation. Immunol Cell Biol. 2013 Jan;91(1):19–26.

65. Chinnadurai R, Rajan D, Ng S, McCullough K, Arafat D, Waller EK, Anderson LJ, Gibson G, Galipeau J. Immune dysfunctionality of replicative senescent mesenchymal stromal cells is corrected by IFNγ priming. Blood Adv. 2017 Apr 25;1(11):628–43.

66. Chinnadurai R, Copland IB, Garcia MA, Petersen CT, Lewis CN, Waller EK, Kirk AD, Galipeau J. Cryopreserved mesenchymal stromal cells are susceptible to T-cell mediated apoptosis which is partly rescued by IFNγ licensing. Stem Cells Dayt Ohio. 2016 Sep;34(9):2429–42.

67. Chinnadurai R, Bates PD, Kunugi KA, Nickel KP, DeWerd LA, Capitini CM, Galipeau J, Kimple RJ. Dichotomic potency of IFNγ licensed allogeneic mesenchymal stromal cells in animal models of acute radiation syndrome and graft versus host disease. Front Immunol [Internet]. 2021 [cited 2023 Aug 28];12. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2021.708950

68. 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.

69. Murphy N, Treacy O, Lynch K, Morcos M, Lohan P, Howard L, Fahy G, Griffin MD, Ryan AE, 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 Off Publ Fed Am Soc Exp Biol. 2019 Aug;33(8):9404–21.

70. Gomes AO, Barbosa BF, Franco PS, Ribeiro M, Silva RJ, Gois PSG, Almeida KC, Angeloni MB, Castro AS, Guirelli PM, et al. Macrophage migration inhibitory factor (MIF) prevents maternal death, but contributes to poor fetal outcome during congenital toxoplasmosis. Front Microbiol. 2018;9:906.

71. 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.

72. Mitchell RA, Liao H, Chesney J, Fingerle-Rowson G, Baugh J, David J, Bucala R. 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.

73. Li D, Han Y, Zhuang Y, Fu J, Liu H, Shi Q, Ju X. 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.

74. Guan Q, Ezzati P, Spicer V, Krokhin O, Wall D, Wilkins JA. Interferon γ induced compositional changes in human bone marrow derived mesenchymal stem/stromal cells. Clin Proteomics. 2017 Jul 6;14(1):26.

75. Lan H, Wang N, Chen Y, Wang X, Gong Y, Qi X, Luo Y, Yao F. 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.

76. Ohta S, Misawa A, Fukaya R, Inoue S, Kanemura Y, Okano H, Kawakami Y, Toda M. 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.

77. 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.

78. 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.

79. Lin YD, Fan XL, Zhang H, Fang SB, Li CL, Deng MX, Qin ZL, Peng YQ, Zhang HY, Fu QL. 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.

80. 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.

81. 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.

82. Carty F, Corbett JM, Cunha JPMCM, Reading JL, Tree TIM, Ting AE, Stubblefield SR, 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 Apr 23;9:645.

83. de Witte SFH, Merino AM, Franquesa M, Strini T, van Zoggel JAA, Korevaar SS, Luk F, Gargesha M, O'Flynn L, Roy D, 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.

842 84. Baugh JA, Chitnis S, Donnelly SC, Monteiro J, Lin X, Plant BJ, Wolfe F, Gregersen PK,
843 Bucala R. A functional promoter polymorphism in the macrophage migration inhibitory
844 factor (MIF) gene associated with disease severity in rheumatoid arthritis. Genes Immun.
845 2002 May;3(3):170–6.

846 85. Renner P, Roger T, Calandra T. Macrophage migration inhibitory factor: gene
847 polymorphisms and susceptibility to inflammatory diseases. Clin Infect Dis Off Publ
848 Infect Dis Soc Am. 2005 Nov 15;41 Suppl 7:S513-519.

849 86. Plant BJ, Gallagher CG, Bucala R, Baugh JA, Chappell S, Morgan L, O'Connor CM,
850 Morgan K, Donnelly SC. Cystic fibrosis, disease severity, and a macrophage migration
851 inhibitory factor polymorphism. Am J Respir Crit Care Med. 2005 Dec 1;172(11):1412–
852 5.

853 87. Kim RL, Bang JY, Kim J, Mo Y, Kim Y, Lee CG, Elias JA, Kim HY, Kang HR.
854 Mesenchymal stem cells exert their anti-asthmatic effects through macrophage
855 modulation in a murine chronic asthma model. Sci Rep. 2022 Jun 13;12:9811.

856 88. Kabat M, Bobkov I, Kumar S, Grumet M. Trends in mesenchymal stem cell clinical trials
857 2004-2018: Is efficacy optimal in a narrow dose range? Stem Cells Transl Med. 2019 Dec
858 5;9(1):17–27.

859 89. Malaquias M a. S, Oyama LA, Jericó PC, Costa I, Padilha G, Nagashima S, Lopes-
860 Pacheco M, Rebelatto CLK, Michelotto PV, Xisto DG, et al. Effects of mesenchymal
861 stromal cells play a role the oxidant/antioxidant balance in a murine model of asthma.
862 Allergol Immunopathol (Madr). 2018 Apr;46(2):136–43.

863 90. Kwak J, Choi SJ, Oh W, Yang YS, Jeon HB, Jeon ES. Cobalt chloride enhances the anti-
864 inflammatory potency of human umbilical cord blood-derived mesenchymal stem cells
865 through the ERK-HIF-1α-microRNA-146a-mediated signaling pathway. Stem Cells Int.
866 2018;2018:4978763.

867 91. Hong GH, Kwon HS, Lee KY, Ha EH, Moon KA, Kim SW, Oh W, Kim TB, Moon HB,
868 Cho YS. hMSCs suppress neutrophil-dominant airway inflammation in a murine model
869 of asthma. Exp Mol Med. 2017 27;49(1):e288.

870 92. Cruz FF, Borg ZD, Goodwin M, Coffey AL, Wagner DE, Rocco PRM, Weiss DJ.
871 CD11b+ and Sca-1+ cells exert the main beneficial effects of systemically administered
872 bone marrow-derived mononuclear cells in a murine model of mixed Th2/Th17 allergic
873 airway inflammation. STEM CELLS Transl Med. 2016;5(4):488–99.

874 93. Zhilai Z, Biling M, Sujun Q, Chao D, Benchao S, Shuai H, Shun Y, Hui Z.
875 Preconditioning in lowered oxygen enhances the therapeutic potential of human umbilical
876 mesenchymal stem cells in a rat model of spinal cord injury. Brain Res. 2016 Jul
877 1;1642:426–35.

878 94. Armitage J, Tan DBA, Troedson R, Young P, Lam KV, Shaw K, Sturm M, Weiss DJ,
879 Moodley YP. Mesenchymal stromal cell infusion modulates systemic immunological
880 responses in stable COPD patients: a phase I pilot study. Eur Respir J. 2018
881 Mar;51(3):1702369.

95. Gholamrezanezhad A, Mirpour S, Bagheri M, Mohamadnejad M, Alimoghaddam K, Abdolahzadeh L, Saghari M, Malekzadeh R. In vivo tracking of 111In-oxine labeled mesenchymal stem cells following infusion in patients with advanced cirrhosis. Nucl Med Biol. 2011 Oct;38(7):961–7.

96. Sokal EM, Lombard CA, Roelants V, Najimi M, Varma S, Sargiacomo C, Ravau J, Mazza G, Jamar F, Versavau J, et al. Biodistribution of liver-derived mesenchymal stem cells after peripheral injection in a hemophilia A patient. Transplantation. 2017 Aug;101(8):1845–51.

97. Koç ON, Gerson SL, Cooper BW, Dyhouse SM, Haynesworth SE, Caplan AI, Lazarus HM. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. J Clin Oncol Off J Am Soc Clin Oncol. 2000 Jan;18(2):307–16.

98. Sanchez-Diaz M, Quiñones-Vico MI, Sanabria de la Torre R, Montero-Vílchez T, Sierra-Sánchez A, Molina-Leyva A, Arias-Santiago S. Biodistribution of mesenchymal stromal cells after administration in animal models and humans: a systematic review. J Clin Med. 2021 Jun 29;10(13):2925.

99. Kang I, Bucala R. The immunobiology of MIF: function, genetics and prospects for precision medicine. Nat Rev Rheumatol. 2019 Jul;15(7):427–37.

100. 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.

101. 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.

102. 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.

103. Klasen C, Ziehm T, Huber M, Asare Y, Kapurniotu A, Shachar I, Bernhagen J, El Bounkari O. LPS-mediated cell surface expression of CD74 promotes the proliferation of B cells in response to MIF. Cell Signal. 2018;46:32–42.

104. Soppert J, Kraemer S, Beckers C, Averdunk L, Möllmann J, Denecke B, Goetzenich A, Marx G, Bernhagen J, Stoppe C. Soluble CD74 reroutes MIF/CXCR4/AKT-mediated survival of cardiac myofibroblasts to necroptosis. J Am Heart Assoc. 2018 04;7(17):e009384.

105. 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.

106. 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.

107. Duffy MM, Pindjakova J, Hanley SA, McCarthy C, Weidhofer GA, Sweeney EM, English K, Shaw G, Murphy JM, Barry FP, 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.

108. 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.

109. Sun L, Chen K, Jiang Z, Chen X, Ma J, Ma Q, Duan W. 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.

110. Zhu W, Sun L, Zhao P, Liu Y, Zhang J, Zhang Y, Hong Y, Zhu Y, Lu Y, Zhao W, 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.

111. Liu X, Li X, Zhu W, Zhang Y, Hong Y, Liang X, Fan B, Zhao H, He H, Zhang F. Exosomes from mesenchymal stem cells overexpressing MIF enhance myocardial repair. J Cell Physiol. 2020 Jan 20;

112. Zhang Y, Zhu W, He H, Fan B, Deng R, Hong Y, Liang X, Zhao H, Li X, Zhang F. Macrophage migration inhibitory factor rejuvenates aged human mesenchymal stem cells and improves myocardial repair. Aging. 2019 27;11(24):12641–60.

113. Zhang Y, Zhou Y, Chen S, Hu Y, Zhu Z, Wang Y, Du N, Song T, Yang Y, Guo A, 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.

114. Fingerle-Rowson G, Petrenko O, Metz CN, Forsthuber TG, Mitchell R, Huss R, Moll U, Müller W, Bucala R. 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.

115. 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.

116. Ehrentraut H, Clambey ET, McNamee EN, Brodsky KS, Ehrentraut SF, Poth JM, Riegel AK, Westrich JA, Colgan SP, Eltzschig HK. 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.

List of Figure Captions

959 **Figure 1. Human BM-MSCs significantly reduce goblet cell metaplasia and collagen**
960 **deposition in CATT$_7$ mice challenged with HDM. A** PBS and HDM groups received PBS or
961 HDM i.n. 3 times a week for 3 consecutive weeks. 1x10^6 human BM-MSCs were administered
962 i.v. to the HDM+MSC groups on day 14. Mice were sacrificed on day 21 (Schematic created
963 with BioRender.com). **B** Representative images of lung tissue from WT, CATT$_5$ and CATT$_7$
964 mice stained with Periodic acid Schiff (PAS) at 20X magnification, scale bar = 20 µm. Arrows
965 show examples of mucin-containing goblet cells. **C** Goblet cell hyperplasia was investigated
966 through the quantitation of PAS positive cells. **D** Representative images of lung tissue stained
967 with Masson's trichome at 4X magnification, scale bar = 200 µm. **E** Quantitation of %
968 subepithelial collagen. Data are presented as mean ± SEM; N=6 per group. Human BM-MSC
969 donors 001-177 and 003-310 were used (RoosterBio Inc., Frederick, MD, USA). Statistical
970 analysis was carried out using one-way ANOVA followed by the *post-hoc* Tukey's multiple
971 comparison test where *p<0.05, ***p<0.001, ****p<0.0001, ns: non significant.

972

973 **Figure 2. Human BM-MSCs significantly reduce levels of Th2 cytokines in the BAL fluid**
974 **of CATT$_7$ mice challenged with HDM.** PBS and HDM groups received PBS or HDM i.n. 3
975 times a week for 3 consecutive weeks. 1x10^6 human BM-MSCs were administered i.v. to the
976 HDM+MSC groups on day 14. BAL was performed 4 hr post final HDM challenge on day 18.
977 **A** Total cell count recovered from the BAL. **B** BAL fluid eosinophil count determined by
978 differential staining of cytospins. Cytokine levels of **C** IL-4 and **D** IL-13 in the BAL fluid
979 determined by ELISA. White bar: PBS; Grey bar: HDM; Blue bar: HDM+MSC. Data are
980 presented as mean ± SEM; N=5-6 per group. Human BM-MSC donors 001-177 and 003-310
981 were used (RoosterBio Inc., Frederick, MD, USA). Statistical analysis was carried out using
982 one-way ANOVA. followed by the *post-hoc* Tukey's multiple comparison test where *p<0.05,
983 **p<0.01, ***p<0.001, ****p<0.0001, ns: non significant.

984

985 **Figure 3. High levels of hMIF significantly enhance BM-MSC retention in a HDM model**
986 **of allergic asthma.** HDM was administered i.n. 3 times a week for 2 weeks. On day 14, 1x10^6
987 Qtracker 625- labelled hMSCs were administered i.v. in WT, CATT$_5$ or CATT$_7$ mice. 24 hr
988 later the lungs were harvested, embedded in OCT compound and frozen at -80. Tissue blocks
989 were sectioned and imaged using the CryoViz^{TM} (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. Influence of rhMIF licensing on MSC expression of immunomodulatory factors *in vitro*. A-E Gene expression of IDO, COX-2, PTGES, ICAM-1 and HGF by hBM-MSCs after stimulation with recombinant human MIF (1ng/ml), human TNFα or human IFNγ for 24hr. 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 one-way ANOVA where *p<0.05, **p<0.01, ****p<0.0001, ns: non significant.

Figure 5. $CATT_7$ MIF licensing enhances MSC expansion and immunosuppressive function *in vitro*.

A Schematic (created using Biorender.com) depicting the generation of $CATT_7$ MIF CM and experimental design. **B-E** Percentage or mean fluorescence intensity (MFI) of IDO or COX-2 expression in human BM-MSCs measured by flow cytometry after cells were stimulated with $CATT_7$ MIF CM, human TNFα or human IFNγ for 24hr. **F** Percentage expression and representative histogram plots of CD74 surface expression on human MSCs measured by flow cytometry after cells were stimulated with $CATT_7$ MIF CM and human IFNγ for 24hr. **G-H** Relative gene expression of TSG-6 and PTGS2 by hBM-MSCs after cells were stimulated with endogenous human MIF ($CATT_7$ CM) and human TNFα for 6hr. **I-J** Licensing of MSCs with supernatants generated from BMDMs from $CATT_7$ HDM challenged mice enhances MSC suppression of **(I)** frequency (%) and **(J)** absolute number of $CD3^+$ T cells proliferating. 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 **(I-J)**. **K** Licensing of MSCs with $CATT_7$ MIF CM enhances MSC expansion *in vitro*. Addition of MIF inhibitor SCD-19 (100 µM) to $CATT_7$ MIF CM 1 hr before MSC licensing prevents MIF enhanced MSC expansion. 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 a one-way ANOVA or unpaired t test where *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns: non significant.

Figure 6. 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 7. MIF licensing restores MSC efficacy at low doses in $CATT_7$ mice. A $5x10^4$ 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 8. MIF-Licensed MSCs mediate their protective effects in HDM-induced allergic airway inflammation in a CD74 and COX-2 dependent manner in $CATT_7$ 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 $CATT_7$ 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.