Received: 20 April 2023 | Revised: 26 May 2023 | Accepted: 21 June 2023

DOI: 10.1096/fj.202300787R

RESEARCH ARTICLE

The FASEB Journal

Blockade of MIF biological activity ameliorates house dust mite-induced allergic airway inflammation in humanized MIF mice

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Funding information
Irish Research Council (An Chomhairle um Thaighde in Éirinn), Grant/Award Number: IRCLA/2017/288

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,

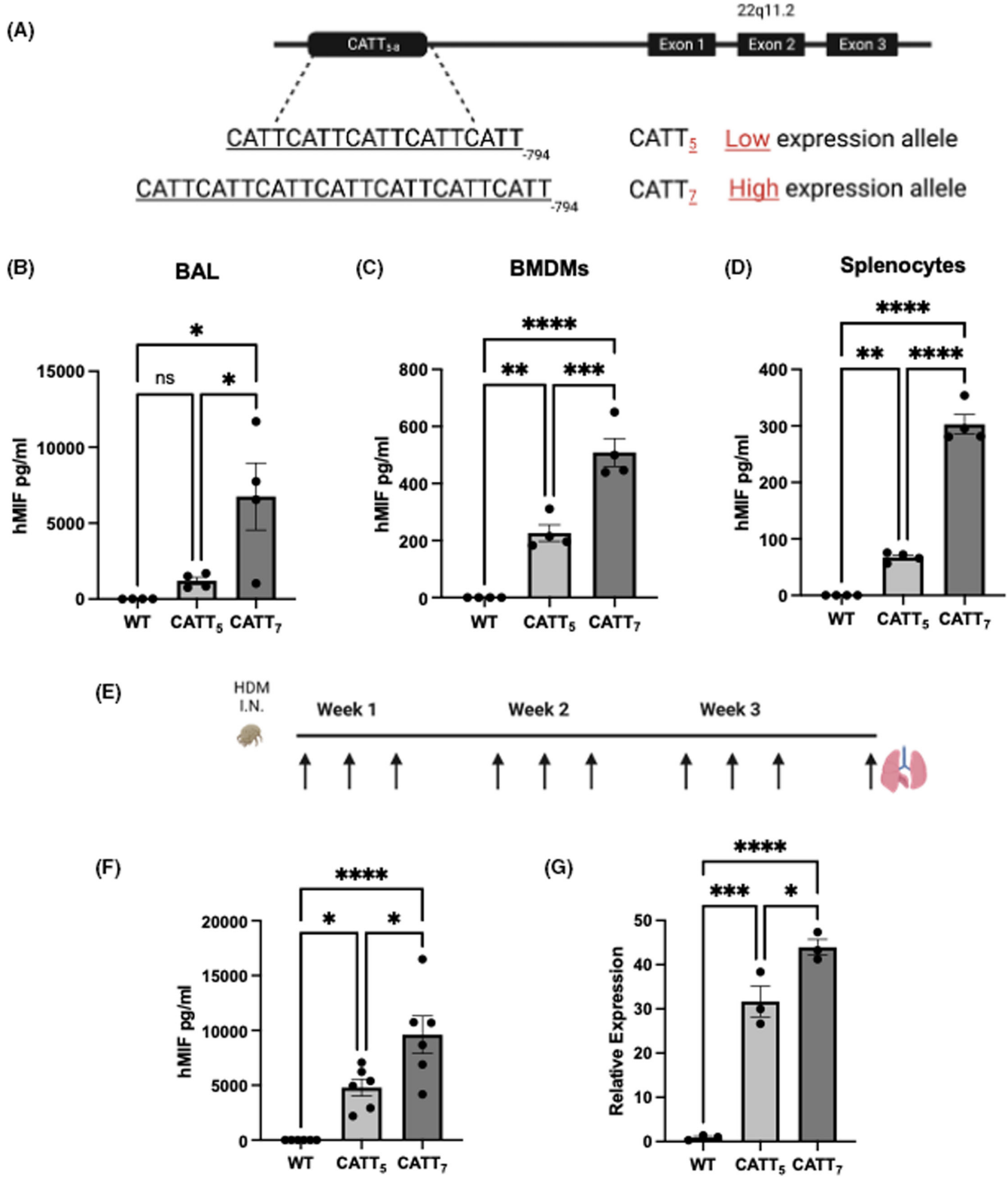

FIGURE 1 Functional –794CATT polymorphisms lead to different expression levels of hMIF under basal and disease conditions. (A) Human MIF gene containing a functional polymorphism in position 794 downstream of the promoter region, where the number of tetranucleotide repeats correlates with promoter activity 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. *$p < .05$; **$p < .01$; ****$p < .0001$.

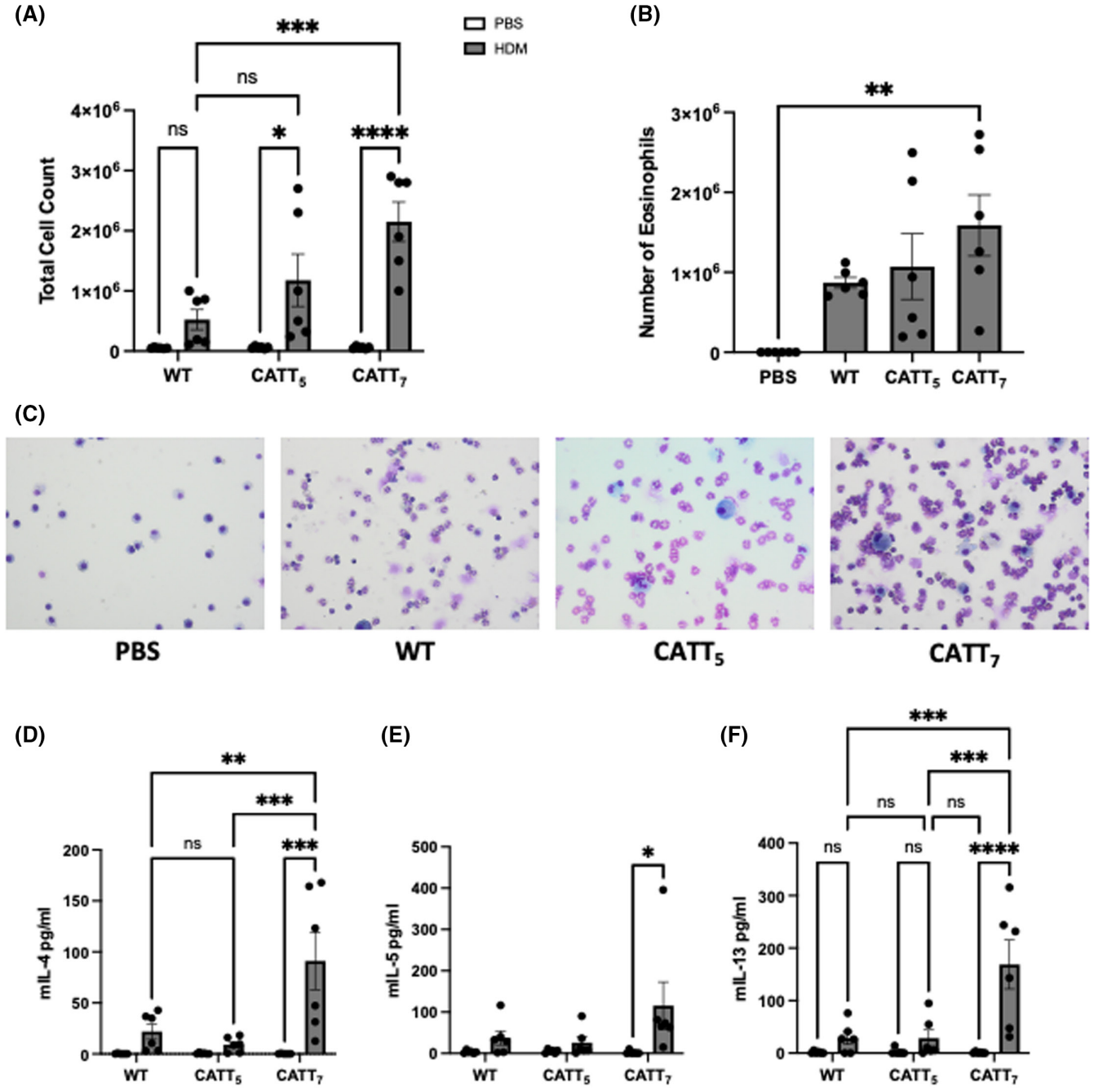

FIGURE 2 Human $CATT_7$ allele significantly increases the Th2 cytokine signature in a HDM model of allergic airway inflammation. (A) Total cell count recovered from the BAL. (B) BAL fluid eosinophil count determined by differential staining of cytospins. (C) Representative imgaes of cytosins. Cytokine 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

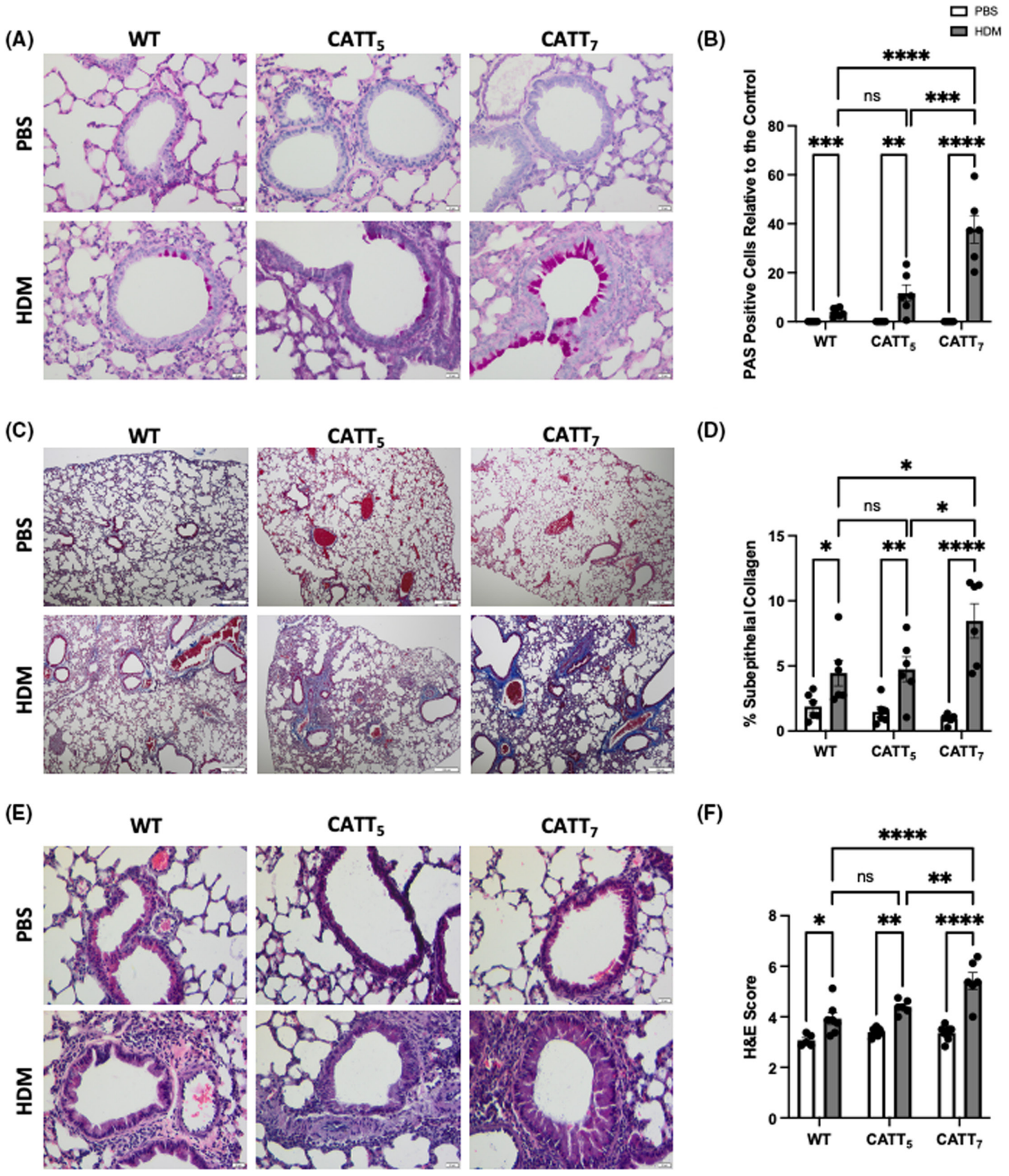

FIGURE 3 Human $CATT_7$ allele exacerbates airway inflammation in a house dust mite model of allergic asthma. (A) Representative images of lung tissue stained with periodic acid Schiff at 20× magnification, scale bar = 20 μm. (B) Goblet cell hyperplasia was investigated through the quantitation of PAS-positive 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

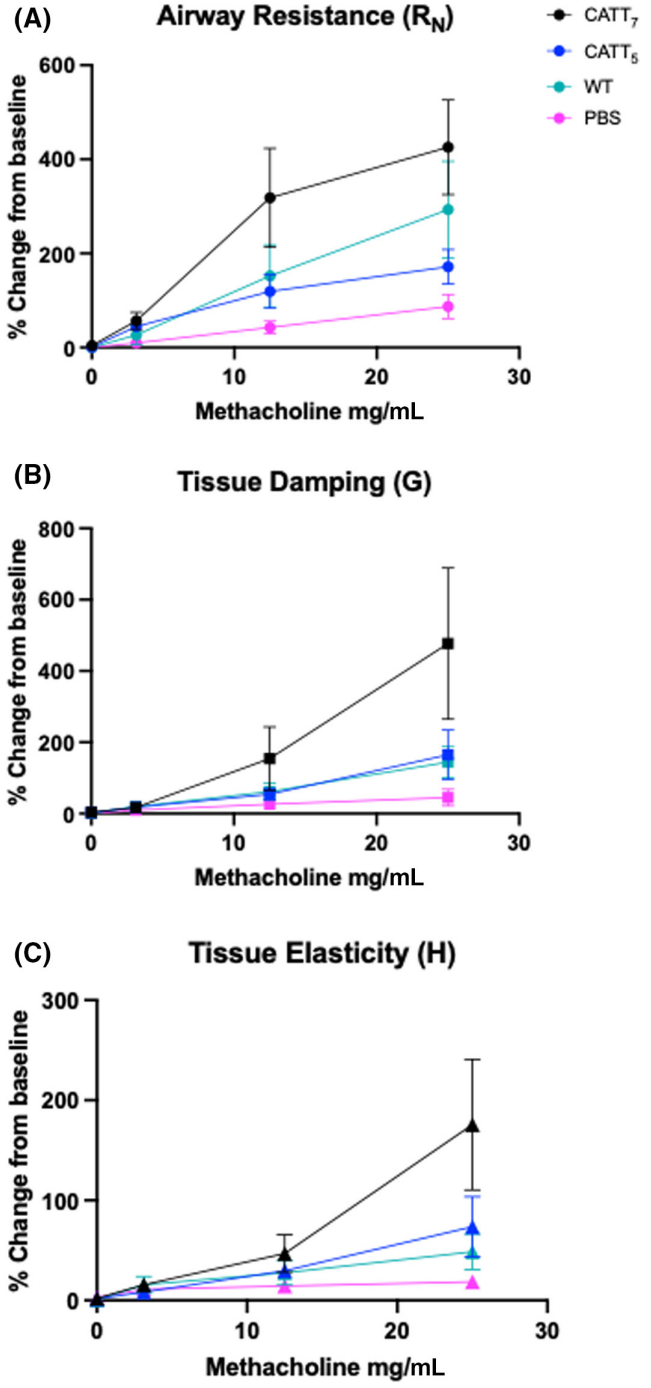

FIGURE 4 Changes in lung mechanics in response to increasing concentrations of methacholine in HDM-challenged $CATT_7$, $CATT_5$, and WT mice. Airway hyperresponsiveness determined (A) by airway resistance, (B) tissue damping, and (C) tissue elastance (R_N, G, and H respectively). Data are presented 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

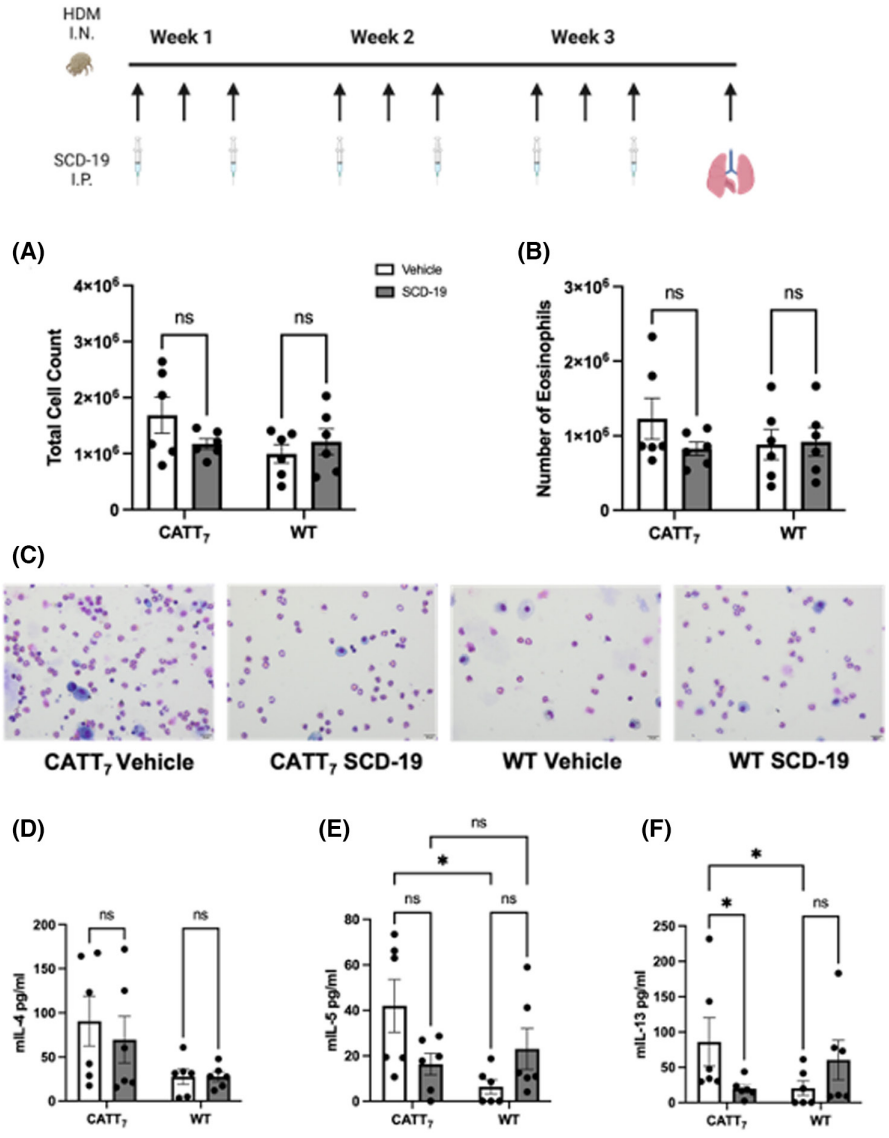

FIGURE 5 MIF inhibitor SCD-19 decreases total cell counts and Th2 cytokines in BALF from $CATT_7$ mice. Mice were challenged with 25 μg of HDM I.N. three times a week for three weeks, in addition to receiving 35 mg/kg of SCD-19 or vehicle control I.P. twice weekly for three weeks. Schematic created using 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). $CATT_7$

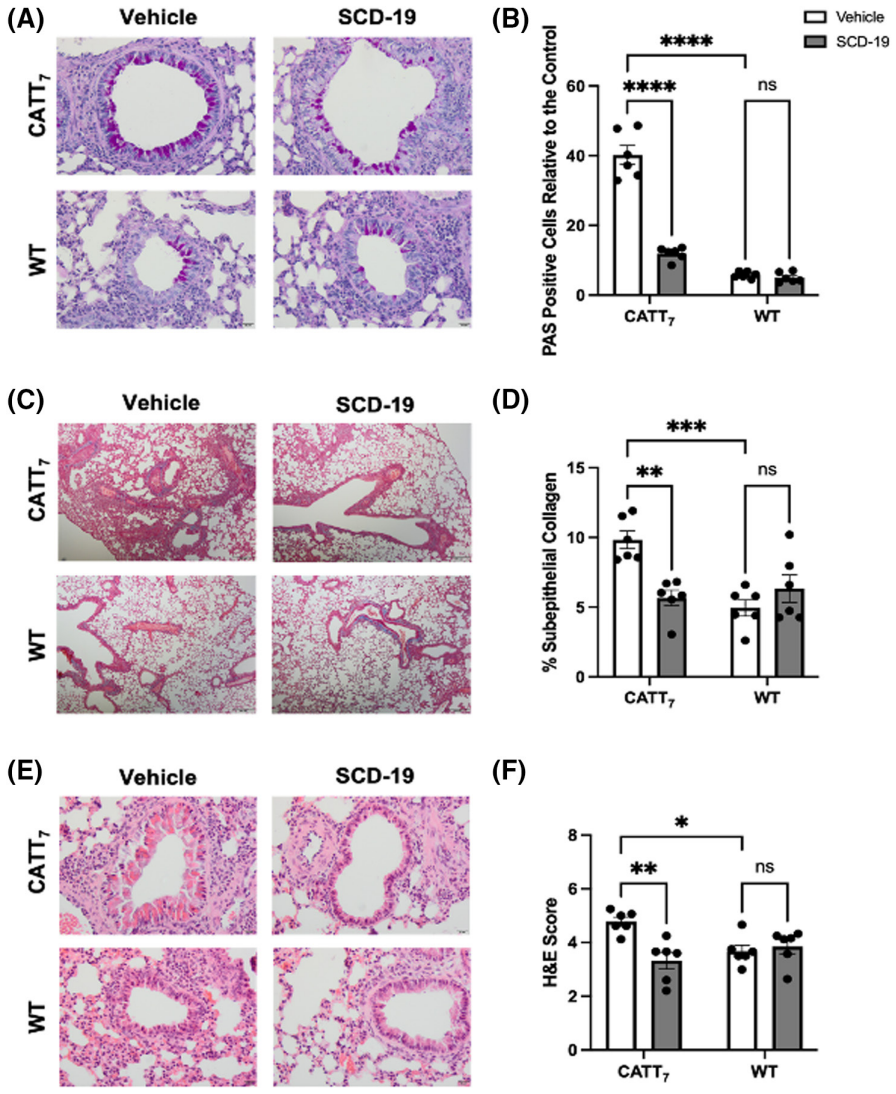

FIGURE 6 MIF antagonist SCD-19 significantly decreases HDM-induced allergic airway inflammation in $CATT_7$ mice. (A) Representative images of lung tissue stained with Periodic Acid Schiff at 20× magnification, scale bar = 20 μm. (B) Goblet cell hyperplasia was investigated through the quantitation of 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.

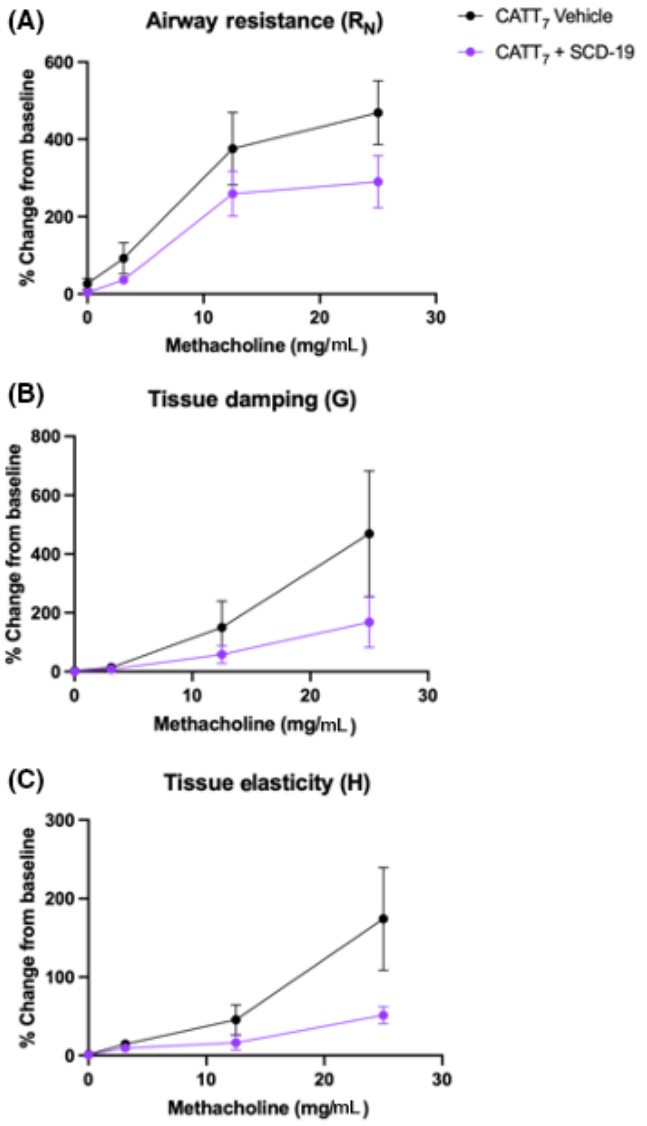

FIGURE 7 SCD-19 can decrease HDM-induced airway resistance (R_N), tissue damping (G), and tissue elasticity (H) in $CATT_7$ mice compared to vehicle control. $CATT_7$ mice were challenged with 25 µg of HDM or PBS control I.N. three times a week for three weeks, in addition to receiving 35 mg/kg of SCD-19 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.

ACKNOWLEDGMENTS

We would like to thank Deirdre Daly, Gillian O'Meara, and Shannon Grellan for their exceptional care of our animals used in this study. Open access funding provided by IReL.

FUNDING INFORMATION

This research was supported by an Irish Research Council Laureate Award to KE (IRCLA/2017/288).

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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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: 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 humanized MIF mice. *The FASEB Journal*. 2023;37:e23072. doi:10.1096/fj.202300787R