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Available online March 5, 2018.
<http://dx.doi.org/10.1016/j.jaci.2018.02.021>

The parasitic 68-mer peptide FhHDM-1 inhibits mixed granulocytic inflammation and airway hyperreactivity in experimental asthma



To the Editor:

During infection of their mammalian hosts, parasitic worms (helminths) secrete molecules that modulate the host immune response toward a regulatory phenotype. This ensures the long-term survival of the parasite within its host and also prevents excessive tissue damage mediated by inflammatory immune responses. We reported the identification of a novel immunomodulatory 68-mer peptide secreted by the animal and human parasite *Fasciola hepatica*, termed *F hepatica* helminth defense molecule 1 (FhHDM-1).¹ More recently, we showed that FhHDM-1 ameliorates disease in preclinical murine models of type 1 diabetes and multiple sclerosis and therefore represents a new bioactive peptide with potential as a novel anti-inflammatory pharmacological therapeutic.²

The mechanism by which FhHDM-1 protects against immune-related inflammatory disorders is yet to be fully elucidated. In studies thus far, we have shown that FhHDM-1 preferentially binds to macrophages over neutrophils and lymphocytes when administered into the peritoneal cavity of immune-competent mice.² Additionally, the peptide inhibits proinflammatory cytokine release in murine and human macrophages *in vitro*.^{2,3} To pinpoint the molecular pathways by which FhHDM-1 exerts its anti-inflammatory effects, we examined global transcriptional changes in murine (BALB/c) bone marrow–derived macrophages (BMDMs) treated with a synthetic form of FhHDM-1 for 1 hour prior to stimulation with bacterial LPS for either 6 or 24 hours. Gene expression was profiled on Agilent microarrays (Santa Clara, Calif) containing probes for 41,346 mouse coding transcripts. This analysis revealed that FhHDM-1 treatment enhanced the expression of 1,363 LPS-regulated genes, and decreased the expression of 1,491 genes by ≥ 2 fold at 6 hours. At 24 hours, the expression of 988 LPS-regulated genes was increased and 1,292 genes was decreased by ≥ 2 fold (Fig 1, A; see Table E1 in this article's Online Repository at www.jacionline.org). Differentially ($-2 > \text{fold change} > 2$, $P < .05$ with 5% false discovery rate) expressed genes were analyzed using Ingenuity Pathway Analysis (IPA) to identify signaling pathways putatively regulated by FhHDM-1 (Fig 1, B). Within the top 20 significantly altered pathways, 16 associated with the activation of proinflammatory responses were repressed by FhHDM-1, consistent with its potent anti-inflammatory activity. Notably, IPA analysis predicted that FhHDM-1 inhibits high-mobility group box-1 (HMGB1) signaling and IL-17–mediated allergic inflammation (Fig 1, B;

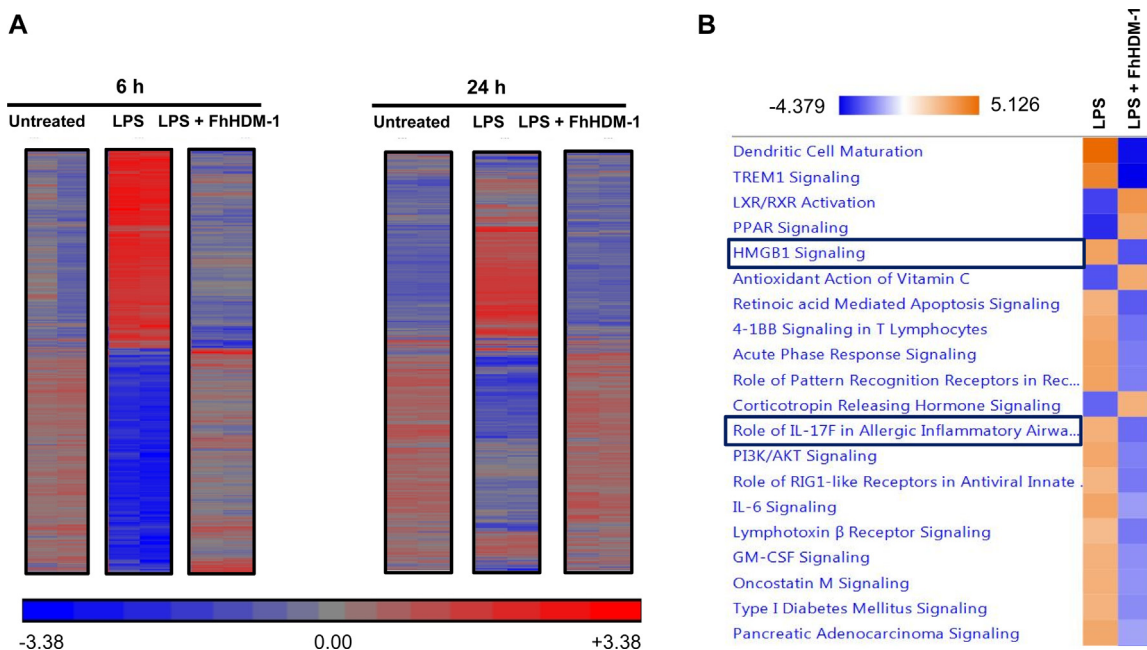


FIG 1. FhHDM-1 modulates macrophage gene expression. **A**, Clustered profiles for all genes expressed in macrophages that were untreated, treated with LPS (10 ng/mL) or treated with LPS (10 ng/mL) and FhHDM-1 (15 μ mol/L). Changes in fold expression are depicted for 2 experimental replicates. The color-code key indicates fold increases (red) or decreases (blue) in gene expression. **B**, Putative canonical pathways significantly altered by FhHDM-1 in macrophages treated with LPS for 6 hours, as determined by IPA analysis. The color-code key indicates the extent of activation (orange) or inhibition (blue) of a pathway. LXR/RXR, Liver X receptor/retinoid X receptor; PI3K, phosphoinositide 3-kinase; RIG1, retinoic acid inducible gene 1; TREM, triggering receptor expressed on myeloid cell.

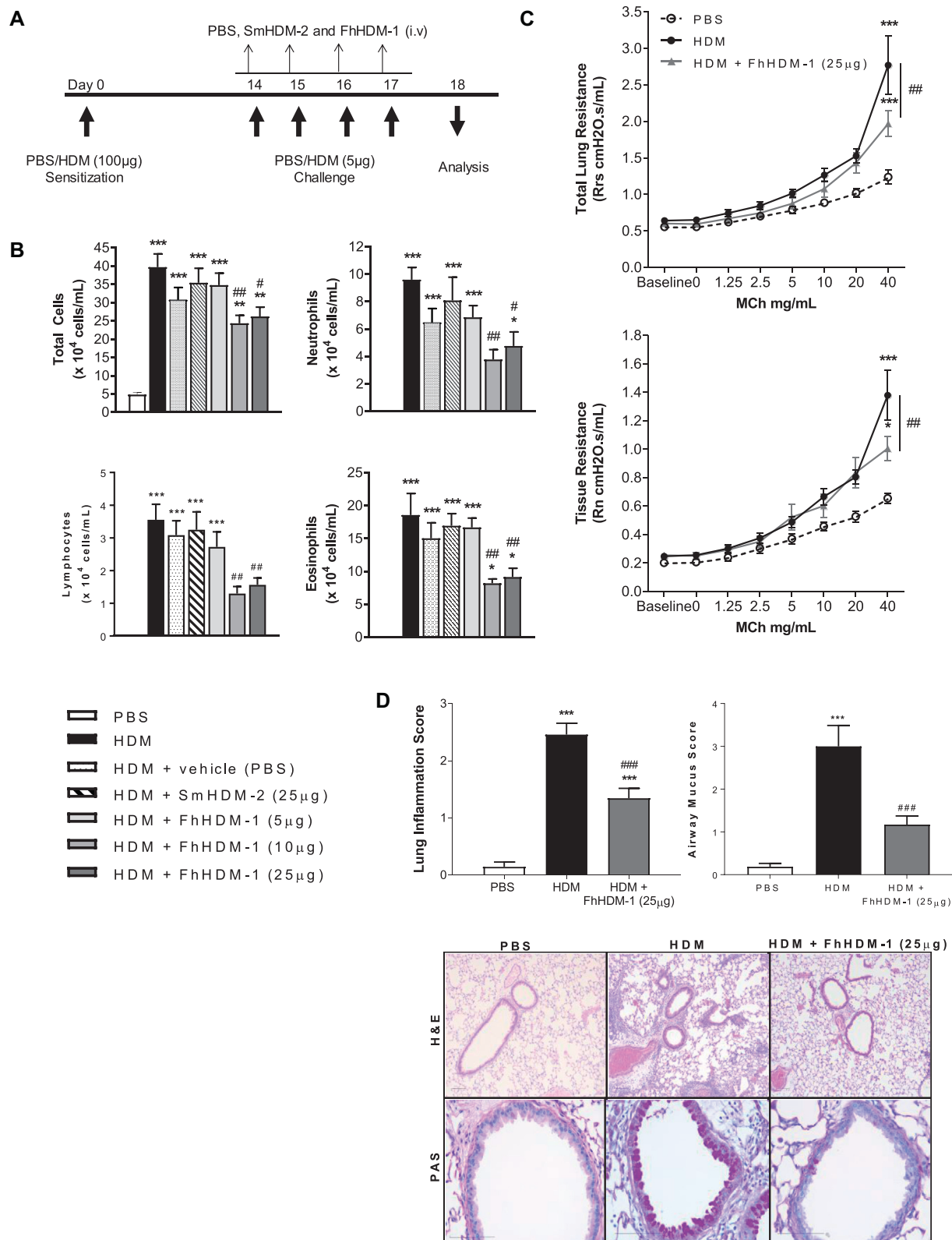


FIG 2. FhHDM-1 protects against experimental asthma. **A**, Study design. **B**, Total and differential cell counts in BALF. **C**, Total lung resistance and tissue resistance measured by forced oscillation technique using FlexiVent apparatus (SCIREQ, Montreal, Quebec, Canada). **D**, Lung inflammation score and airway mucus score as assessed by hematoxylin and eosin (H&E) and periodic-acid Schiff (PAS) staining, respectively. Representative images of H&E (upper panel, ×10 original magnification) and PAS (lower panel, ×40 original magnification) are shown. Bars = 60 μm. Data represent means ± SEMs. **P* < .05, ***P* < .01, and ****P* < .001 versus mice treated with PBS. #*P* < .05 and ##*P* < .01 versus mice treated with house dust mite (HDM). *n* = 6 to 8 mice per group. MCh, Methacholine.

see Fig E1, A and B in this article's Online Repository at www.jacionline.org). The anti-inflammatory effect of the peptide was not specific to BALB/c mice, as bone marrow-derived macrophages derived from C57BL/6 mice treated with FhHDM-1 showed the same pattern of cytokine suppression in response to LPS (see Fig E2 in this article's Online Repository at www.jacionline.org).

We have previously shown that HMGB1 is an upstream mediator of the allergic asthmatic response in mice,⁴ and that neutralization of this protein protects against allergen-induced eosinophilic and neutrophilic inflammation in experimental asthma.⁴ IL-17 mediates neutrophil recruitment to sites of inflammation and is implicated in the neutrophilic asthma phenotype, which is relatively resistant to treatment with corticosteroids.⁵ Accordingly, we hypothesized that FhHDM-1 has therapeutic potential in allergic asthma, and that it would protect against both eosinophilic and neutrophilic responses.

To test our hypothesis, we employed an experimental mouse model of house dust mite-induced allergic asthma, as this model elicits a mixed granulocytic inflammatory response.⁴ C57BL/6 mice were sensitized to house dust mite extracts (100 µg) or saline intranasally, and after 2 weeks, were challenged daily with house dust mite extracts (5 µg) or saline, respectively, for 4 days (see study design, Fig 2, A). Mice received an intravenous injection of FhHDM-1 (5, 10, or 25 µg dose) 30 minutes prior to each house dust mite exposure during the challenge period only. For comparison, mice were also treated with a homologous helminth defense molecule derived from *Schistosoma mansoni* (SmHDM-2) or vehicle control (PBS). FhHDM-1, at both the 10 and 25 µg doses significantly attenuated allergen-induced eosinophil, neutrophil, and lymphocyte numbers in the bronchoalveolar lavage fluid (BALF) (Fig 2, B). This effect was specific to FhHDM-1, as neither SmHDM-2 nor PBS had any effect on airway inflammation.

Consistent with this anti-inflammatory effect, FhHDM-1 at both the 10 and 25 µg doses significantly attenuated allergen-induced airway hyperreactivity (Fig 2, C; see Fig E3 in this article's Online Repository at www.jacionline.org). FhHDM-1 also significantly reduced histological evidence of tissue inflammation and mucus production (Fig 2, D). Moreover, and consistent with data from the microarray analysis (see Table E1 in this article's Online Repository at www.jacionline.org) and predictions from the IPA analysis (Fig E1, A and B), treatment with FhHDM-1 significantly inhibited allergen-induced expression of macrophage-derived proinflammatory mediators, including IL-6, TNF, and chemokine C-C motif ligand 2, as well as cytokines/chemokines that mediate eosinophil (IL-5, GM-CSF) and neutrophil (chemokine C-X-C motif ligand 1, GM-CSF) recruitment (see Table E2 in this article's Online Repository at www.jacionline.org). Furthermore, although not statistically significant, FhHDM-1 reduced the expression of IL-4 and IL-17A (see Table E2).

Other research groups have identified parasite-derived proteins with therapeutic activity in mouse models of asthma. The most well-characterized of these parasite proteins is ES-62, a glycoprotein secreted by the nematode *Acanthocheilonema vitea*. Although effective in suppressing airway inflammation and features of airway remodeling in a mouse model of ovalbumin-induced asthma, which promotes eosinophilic inflammation, synthetic small molecule analogs of this glycoprotein failed to demonstrate efficacy in clinically relevant

models of allergic asthma.⁶ Indeed, our data are the first to demonstrate efficacy of a parasite-derived peptide in suppressing neutrophilic inflammation in response to clinically relevant allergens. To validate our finding, we tested the efficacy of FhHDM-1 in a model of LPS-induced neutrophilic inflammation. FhHDM-1 was administered via intraperitoneal injection 24 hours and 30 minutes prior to intratracheal delivery of LPS (1 mg/kg). In this model, treatment with FhHDM-1 also resulted in a significant reduction in the number of neutrophils in BALF 6 hours after LPS challenge (see Fig E4 in this article's Online Repository at www.jacionline.org).

Asthma is a complex and heterogeneous disease in which multiple molecular pathways are at play. Current therapies based on inhaled corticosteroids are effective in patients in which eosinophilic inflammation is a primary feature, but have limited efficacy in patients with neutrophil-dominant inflammation, or mixed granulocytic inflammation.⁵ Recent studies have identified the NLRP3 inflammasome as an important driver of neutrophilic inflammation in asthma.⁷ Notably, however, the NLRP3 inflammasome also acts as a transcriptional regulator of T_H2 cell differentiation,⁸ which is critical to the development of the eosinophilic response. We have previously shown that FhHDM-1 inhibits lysosomal-associated NLRP3 inflammasome activation in murine and human macrophages *in vitro*.³ Thus, the protective effects of FhHDM-1 against mixed granulocytic inflammation may be attributed to its capacity to impair the NLRP3 inflammasome.³ We did not detect increased levels of secreted HMGB1 in BALF at the time point examined in this study (data not shown). However, our transcriptomic studies indicated that FhHDM-1 inhibits HMGB1 signaling. Since HMGB1 is released in response to inflammasome activation in macrophages,⁹ FhHDM-1 may potentially modulate inflammasome-dependent regulation of HMGB1 signaling in asthma. Certainly, this is an important area for further research. In conclusion, the data support our proposal that the immune modulatory activity of FhHDM-1 is sufficient to prevent granulocytic inflammation and airway hyperreactivity in asthma and provide a compelling basis for its investigation as a novel therapeutic in this disease.

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A.T. is supported by an Australian Government Research Training Program Scholarship. V.S.R.R.A. is supported by a University of Technology Sydney Postgraduate Research Scholarship. N.T. is supported by the Swiss National Science Foundation

(grant P300PA_164715). C.T. and S.W. are supported by the Medical Research Council. J.P.D. is funded by grants from a European Research Council Advanced Grant (HELVAC, 322725) and is a member of the Horizon 2020-funded Consortium PARAGONE. S.D. is funded by the National Health and Medical Research Council Australia (APP1087341).

Disclosure of potential conflict of interest: S. Donnelly and J. Dalton are both named inventors on a patent application covering the use of FhHDM-1 as an immune-modulating agent. The rest of the authors declare that they have no relevant conflicts of interest.

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Available online March 6, 2018.
<http://dx.doi.org/10.1016/j.jaci.2018.01.050>

Non-posttransplant lymphoproliferative disorder malignancy after hematopoietic stem cell transplantation in patients with primary immunodeficiency: UK experience



To the Editor:

Secondary malignancy after hematopoietic stem cell transplantation (HSCT) for malignant disorders related to cytotoxic treatment, radiotherapy, and preexisting genetic predisposition is well recognized. Data regarding the incidence of malignancy in normal children and in those post-HSCT for hematological disorders can be found in this article's Online Repository at www.jacionline.org.

Some nonmalignant disorders may predispose to malignancy as seen in cases with Fanconi anemia developing squamous cell carcinoma of head, neck, and anogenital mucosal surfaces particularly if graft-versus-host disease (GvHD) occurs. Posttransplant lymphoproliferative disorders (PTLDs) usually in association with EBV may occur after HSCT for any underlying diagnosis, usually within 2 years posttransplant, most often associated with T-lymphocyte depletion and intense immunosuppression, for example, in the context of GvHD.¹ Data are sparse on the occurrence of non-PTLD malignancy post-HSCT in patients with primary immunodeficiencies (PIDs).

PIDs are a heterogeneous group of disorders affecting the regulation and function of the immune system. To date, it has been found that there are more than 300 genetic defects that cause different disease phenotypes. It is estimated that the risk of malignancy in children with PIDs is about 4%, 10,000 times greater than in age-matched controls.² HSCT can cure PIDs, and reduce the risk of malignancy, but it is unknown whether HSCT itself might increase the incidence of malignancy compared with the normal population. Fifty-two post-HSCT malignancies were confirmed in a large study of 2266 patients with PIDs including 45 PTLDs. Three patients developed myelodysplastic syndrome, all of whom had received total body irradiation and T-lymphocyte depletion grafts, 1 patient developed acute myeloid leukemia (AML), and 3 developed solid tumors, giving an incidence of 0.3% of non-PTLD malignancy.³ Six malignancies were reported from 318 patients who underwent allogeneic HSCT for nonmalignant disorders, including 2 solid tumors in 130 patients with PIDs (1 with severe combined immunodeficiency and 1 with chronic granulomatous disease).⁴

We report a retrospective analysis of 944 children who underwent HSCT for PID at 2 UK centers. Diagnosis, timing and type of transplant, conditioning regimen, GvHD, viral reactivation, type of malignancy, and outcome were recorded.

Twelve patients (1.27%) developed non-PTLD malignancy (Table 1). Median interval from HSCT to malignancy diagnosis was 3.75 years (3 months to 11.2 years). Mean age at transplant in those who developed malignancy was 85 months (4-204 months). Four received an HLA-matched sibling donor bone marrow transplant, 4 had matched and 1 mismatched unrelated donor bone marrow, 2 matched unrelated donor peripheral blood stem cell, and 1 matched cord blood transplant.

None of the patients received radiotherapy. Seven had reduced-intensity conditioning with fludarabine and melphalan, 3 had reduced toxicity myeloablative conditioning with treosulfan and fludarabine, and 2 had myeloablative doses of busulfan, one with cyclophosphamide and the other with fludarabine. Ten received serotherapy with alemtuzumab and 2 matched sibling donor recipients had no serotherapy. All patients received cyclosporine-based GvHD prophylaxis either alone (n = 2) or with mycophenolate mofetil (P1-P5, 7, 8, and 11) or methotrexate (P9 and P12).

Two patients died from malignancy. The rest were successfully treated and are alive with a median follow-up of 13.2 years (2 years 1 month to 18 years).

Both P1 and P12 had lost donor engraftment in whole blood or B- cell/myeloid subsets before the onset of Philadelphia positive acute lymphoblastic leukemia (P1) and juvenile myelomonocytic leukemia (P12), respectively. Both malignancies were confirmed to be recipient in origin. Both had an underlying immune defect that predisposes to malignancy (RAG 2 in P1, Griscelli syndrome in P12). This poses the question as to whether full donor chimerism might have abolished this risk. Alternatively, recipient stem cells surviving chemotherapy may have acquired genotoxic insults.

P9 developed AML (M4) 11 years post-HSCT, which is uncommon. Unfortunately, it was undetermined whether leukemic cells were donor or recipient origin. A European Bone Marrow Transplantation group survey estimated the incidence of myelodysplastic syndrome/AML to be 1.2:1000 transplants, mostly occurring within 4 years of HSCT (see this article's Online Repository at www.jacionline.org).^{E6} Multiple hit

1 **Materials and Methods**

3 **Synthesis of parasite peptides**

4 FhHDM-1 and SmHDM-2 were synthesised to 95% purity, with trifluoroacetic acid removed and
5 verified to be endotoxin free (Genscript, USA).

7 **Preparation and stimulation of bone-marrow derived macrophages (BMDMs)**

8 BALB/c and C57BL6 mice were purchased from the Australian Resource Centre (Perth, Australia).
9 All mice were housed under specific pathogen free conditions and procedures were performed at
10 the University of Technology Sydney (UTS), under protocols compliant with the Australian Code
11 for the Care and Use of Animals for Scientific Purposes and approved by the UTS Animal Care and
12 Ethics Committee. Bone marrow cells isolated from BALB/c mice were differentiated for 6 d in
13 RPMI 1640 supplemented with FBS (10%v/v), recombinant M-CSF (50 ng/ml; eBioscience, USA),
14 2-mercaptoethanol (50µM; Sigma Aldrich) and Penicillin-Streptomycin (100U/ml). BMDMs were
15 confirmed to be F4/80⁺ CD11b⁺ by flow cytometry. Cells were resuspended at 1x10⁶ cells/mL and
16 allowed to adhere for 2 h. Cells were then treated with FhHDM-1 (15µM) for 1 h and/or stimulated
17 with LPS (from *E. Coli*, 0111.B4; Sigma Aldrich) at a concentration of 10 ng/ml, for 6 h or 24 h at
18 37°C in 5% CO₂.

20 **Gene expression profiling and Ingenuity Pathway Analysis[®]**

21 After removal of supernatants, cells were homogenised using TRIzol (Life Technologies), as per the
22 manufacturer's protocol. The aqueous phase was then transferred into a genomic DNA eliminating
23 column from the RNeasy Mini Plus kit (Qiagen). RNA was subsequently isolated as per the
24 manufacturer's instructions. Gene expression was assessed using the Affymetrix Mouse Gene 2.1ST
25 Array and performed at the Ramaciotti Centre for Genomics (University of New South Wales,
26 Australia). Analysis of fold changes in gene expression was completed using Partek Genomics
27 Suite (Partek Inc. USA). The generated data files were grouped according to treatment (untreated,
28 LPS or LPS+FhHDM-1), and individual gene lists were generated by one way ANOVA comparison
29 of untreated vs LPS, and LPS vs LPS+FhHDM-1 at 6 h and 24 h time points (-2>fold-change>2, *P*
30 <0.05 with 5% false discovery rate). Generated gene lists were further analysed using Ingenuity
31 Pathway Analysis (IPA)[®] software (Ingenuity Systems, Redwood City, CA), where a comparison
32 analysis was conducted. The analysis produced a list of canonical pathways that were assigned an
33 activation z-score, which infers the activation states of predicted transcriptional events as either
34 activated or inhibited.

36 **Quantification of cytokines secreted by macrophages**

37 After 24 h stimulation with LPS, supernatants of C57BL/6 BMDMs were harvested and the levels of
38 CCL2, IL-6 and TNF quantified by ELISA (BD Pharmingen, Australia).

40 **Mouse model of allergic asthma**

41 C57BL/6 mice were purchased from the Australian Resource Centre (Perth, Australia). All mice
42 were housed under specific pathogen free conditions and procedures were performed at the
43 University of Technology Sydney (UTS), under protocols compliant with the Australian Code for
44 the Care and Use of Animals for Scientific Purposes and approved by the UTS Animal Care and
45 Ethics Committee. Mice (8 weeks of age) were sensitized to house dust mite allergen (100µg)
46 (Dermatophagoides pteronyssinus, Greer Laboratories, Lenoir, NC, USA) intranasally. After 2
47 weeks, mice were challenged with house dust mite (5µg) intranasally for 4 days (days 14-17).
48 Control mice were sensitized and challenged with PBS only. Synthetic FhHDM-1 (5, 10, 25µg),
49 SmHDM-1 (25µg) or PBS was administered by intravenous injection through the tail vein 30 min
50 prior to allergen challenge on days 14-17. Mice were exsanguinated with an overdose of
51 pentobarbital (100mg/kg) 24 h after the final allergen challenge on day 18. Study design is shown in
52 Fig 2A.

54 **Analysis of Bronchoalveolar lavage fluid**

55 The lungs were lavaged twice with 0.5mL sterile Hanks Balanced Salt Solution (HBSS). The
56 collected fluid was spun at 3000 rpm for 10 min at 4°C. Cell supernatants were retained for analysis
57 of cytokine/chemokine expression, while cell pellets were resuspended in sterile HBSS for
58 enumeration of total and differential cell counts. To perform differential cell counts, cells were spun
59 on glass slides using a Cytospin 4 Cytocentrifuge (Thermo Fisher Scientific) and were stained with
60 Diff-Quik®. A total of 200 cells were counted to enumerate the differential leukocyte count.
61 Cytokine and chemokine concentrations in BALF were measured using a customized
62 MILLIPLEX® Multiplex Mouse Cytokine/Chemokine assay (Millipore, Billerica, MA) according
63 to manufacturer instructions. The cytokines/chemokines measured were IL-25/IL-17E, IL-22, IL-
64 23, IL-33, eotaxin-1, GM-CSF, IFN-γ, IL-1β, IL-4, IL-5, IL-6, IL-9, IL-12 (p40), IL-12 (p70), IL-
65 13, IL-15, IL-17, KC, MCP-1, MIP-1α, RANTES and TNF. Each sample was analyzed in duplicate
66 using the MAGPIX® System. Five parameter logistic regression was performed to predict the
67 concentration of unknown samples. HMGB1 was measured using a specific ELISA (IBL
68 international) according to manufacturer instructions.

70 **Quantification of airway inflammation**

71 Lung sections were stained with hematoxylin and eosin (H&E) and airway inflammation
72 (inflammatory cell infiltrate) semi-quantified by blind scoring the level of inflammatory cell
73 infiltrates surrounding each airway. Scores ranged from 0-4 (0: no inflammatory cell infiltrates
74 around airway, 1: low level cell infiltrates around part of airway, 2: moderate cell infiltrates around
75 part of or entire airway, 3: significant inflammatory cell infiltrates around part of or entire airway,
76 4: airway completely surrounded by inflammatory cell infiltrates). Five airways were scored per
77 mouse. All sections were imaged on Aperio Scanscope XT and Leica DM750 Brightfield
78 microscope.

79

80 **Quantification of mucus**

81 Mucus producing cells were identified by Periodic acid-Schiff (PAS) staining and blind scored from
82 0 to 5 based on percentage of PAS positive airway epithelial cells (AEC) (0: 0% of total AEC, 1: 1-
83 10% of total AEC, 2: 10-30% of total AEC, 3: 30-50% of total AEC, 4: 50-80% of total AEC, 5:
84 >80% of total AEC). Five airways were scored per mouse.

85

86 **Airway hyperresponsiveness (AHR)**

87 AHR was measured 24 h after the last allergen challenge by forced oscillation technique using
88 FlexiVent apparatus (SCIREQ, Montreal, Canada). Briefly, mice were anesthetized using a cocktail
89 of xylazine (0.2mg/10gm) and ketamine (0.4mg/10gm body weight). An 18-gauge blunt needle was
90 inserted into the trachea and mice were kept under mechanical ventilation at 200 breaths/min with a
91 delivered tidal volume of 0.25 mL against a positive end-expiratory pressure (PEEP) of 3 cm H₂O.
92 Total lung resistance (Rrs) and tissue resistance (Rn) were recorded by administering increasing
93 doses of nebulized methacholine (0 to 40 mg/mL) (Sigma-Aldrich, St Louis, MO, USA).

94

95 **Mouse model of LPS induced neutrophilic inflammation**

96 C57BL/6 female mice aged between 10-12 weeks were purchased from Envigo. Housing and
97 experimentation was carried out in accordance with the Animal (Scientific Procedures) Act 1986
98 and current guidelines approved by the Queen's University Ethical Review Committee. FhHDM-1
99 (10µg) was administered via intraperitoneal injection 24 h and 30 min prior to intratracheal delivery
100 of *Pseudomonas* LPS (1mg/kg; Sigma-Aldrich, Serotype 10, source strain ATCC 27316). Control
101 mice were treated with PBS only. After 6 h, BALF was collected and total and differential cell
102 counts were performed.

103

104 **Statistical Analysis**

105 ANOVA for repeated measures was used to determine the levels of difference between groups of
106 mice. Significance levels were set at $P = 0.05$.

107
108 **Online Repository Figure Legends**

109
110 **Figure E1.**

111
112 IPA transcriptional networks of HMGB1 signaling (A) and IL-17 mediated allergic inflammation
113 (B). Signaling components are color coded according to increased (red) or decreased (green)
114 expression in bone-marrow derived macrophages (BMDMs) treated with FhHDM-1 + LPS versus
115 LPS only. The IPA network legend is included in (A).

116
117 **Figure E2**

118 FhHDM-1 inhibits LPS-induced inflammatory mediator release in BMDMs isolated from C57BL6
119 mice. Bars represent mean $\pm$ SEM from six experiments. **** $P < .0001$ vs untreated cells. ##### P
120 $< .0001$ vs cells stimulated with LPS only.

121
122 **Figure E3**

123 FhHDM-1 inhibits allergen-induced airway hyperreactivity. Total lung resistance and tissue
124 resistance were measured by forced oscillation technique using FlexiVent apparatus. Data represent
125 mean $\pm$ SEM. * $P < .05$, and *** $P < .001$ vs mice treated with PBS. ## $P < .01$ vs mice treated with
126 HDM. $N = 6 - 8$ mice per group.

127
128 **Figure E4**

129 FhHDM-1 inhibits LPS-induced increases in total cells and neutrophils in mice challenged with an
130 intratracheal delivery of LPS. Bars represent mean $\pm$ SEM. * $P < .05$, ** $P < .01$, and *** $P < .001$ vs
131 mice treated with HDM or LPS only. $N = 6$ mice per group.

132

133

Online Repository Results

Table 2: Chemokine and cytokine expression in BALF

Cytokine/Chemokine	Vehicle (PBS)	House dust mite	House dust mite + FhHDM-1	<i>P</i>
IL-25/IL-17E	ND	ND	ND	
IL-23	ND	ND	ND	
IL-33	ND	ND	ND	
IFN- γ	ND	ND	ND	
IL-1 β	ND	ND	ND	
IL-12p40	ND	ND	ND	
IL-12p70	ND	ND	ND	
IL-15	ND	ND	ND	
IL-13	ND	ND	ND	
RANTES	0.39 $\pm$ 0.09	0.84 $\pm$ 0.23	0.63 $\pm$ 0.16	NS
IL-9	50.94 $\pm$ 7.05	24.64 $\pm$ 4.10	24.29 $\pm$ 4.66	NS
Eotaxin	4.92 $\pm$ 1.03	15.29 $\pm$ 2.97	13.78 $\pm$ 4.81	NS
IL-17	0.07 $\pm$ 0.06	29.64 $\pm$ 13.11	5.62 $\pm$ 2.39	NS
IL-4	0.21 $\pm$ 0.08	15.64 $\pm$ 3.52	8.91 $\pm$ 3.28	NS
IL-22	0.65 $\pm$ 0.09	4.43 $\pm$ 0.78	2.31 $\pm$ 0.44	*
GM-CSF	0.29 $\pm$ 0.12	2.69 $\pm$ 0.93	0.50 $\pm$ 0.47	*
IL-6	0.16 $\pm$ 0.09	74.20 $\pm$ 21.45	20.69 $\pm$ 7.53	*
CCL2 (MCP-1)	0	4.79 $\pm$ 2.11	0	*
TNF	0.13 $\pm$ 0.04	2.94 $\pm$ 0.7	0.90 $\pm$ 0.30	**
IL-5	1.57 $\pm$ 0.29	61.81 $\pm$ 17.83	11.10 $\pm$ 3.12	**
CXCL1 (KC)	12.05 $\pm$ 1.51	120.71 $\pm$ 17.63	49.19 $\pm$ 11.61	***

Data represent mean (pg/ml) $\pm$ SEM. **P* < .05, ***P* < .01, ****P* < .001 in house dust mite vs house dust mite + FhHDM-1 groups. N = 12 mice per group. ND, not detectable; NS, not significant.