The Parasitic 68-mer Peptide FhHDM-1 inhibits mixed granulocytic inflammation and airway hyperreactivity

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Capsule Summary

A peptide derived from the parasitic worm *Fasciola hepatica* inhibits eosinophilic and neutrophilic airway inflammation, mucus production and airway hyperreactivity in a murine model of house dust mite induced asthma.

Key Words:

Helminth, Macrophage, House Dust Mite, Lipopolysaccharide, HMGB1, IL-17, Neutrophils, Eosinophils

Abbreviations:

BALF	Bronchoalveolar lavage fluid
BMDM	Bone marrow derived macrophage
FhHDM-1	*Fasciola hepatica* helminth defense molecule
LPS	Lipopolysaccharide
IPA	Ingenuity Pathway Analysis

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Conflicts of Interest: None

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To the Editor,

During infection of their mammalian hosts, parasitic worms (helminths) secrete molecules which modulate the host immune response towards 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 pre-clinical murine models of type 1 diabetes and multiple sclerosis and therefore represents a new bio-active 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 pro-inflammatory cytokine release in murine and human macrophages *in vitro*[2, 3]. To pin-point 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 h prior to stimulation with bacterial LPS for either 6 or 24 h. Gene expression was profiled on Agilent microarrays 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 h. At 24 h, the expression of 988 LPS-regulated genes was increased and 1,292 genes was decreased by ≥ 2 fold (Fig 1A; Online Repository Table 1). Differentially (-2>fold-change>2, $P < 0.05$ with 5% false discovery rate) expressed genes were analysed using Ingenuity Pathway Analysis (IPA) to identify signalling pathways putatively regulated by FhHDM-1 (Fig 1B). Within the top 20 significantly altered pathways, 16 associated with the

activation of pro-inflammatory 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) signalling and IL-17 mediated allergic inflammation (Fig 1B). The anti-inflammatory effect of the peptide was not specific to Balb/c mice, as BMDMs derived from C57BL6 mice treated with FhHDM-1 showed the same pattern of cytokine suppression in response to LPS (Online Repository Fig E2).

We have previously shown that HMGB1 is an upstream mediator of the allergic asthmatic response in mice[4], and that neutralisation of this protein protects against allergen-induced eosinophilic and neutrophilic inflammation in experimental asthma[4]. IL-17 mediates neutrophilic recruitment to sites of inflammation and is implicated in the neutrophilic asthma phenotype which is relatively resistant to treatment with corticosteroids[5]. Accordingly, we hypothesised 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 (HDM) induced allergic asthma, as this model elicits a mixed granulocytic inflammatory response[4]. C57BL6 mice were sensitized to house dust mite extracts (100μg) 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 2A). Mice received an intravenous injection of FhHDM-1 (5, 10 or 25 μg dose) 30 min 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 dose significantly attenuated allergen induced eosinophil, neutrophil and lymphocyte numbers in the BALF (Fig 2B). 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 dose significantly attenuated allergen-induced airway hyper-reactivity (Fig 2C, Online Repository Fig E3). FhHDM-1 also significantly reduced histological evidence of tissue inflammation and mucus production (Fig 2D). Moreover, and consistent with data from the microarray analysis (Online Repository Table 1) and predictions from the IPA analysis (Fig E1B), treatment with FhHDM-1 significantly inhibited allergen-induced expression of macrophage-derived pro-inflammatory mediators, including IL-6, TNF and CCL2, as well as cytokines/chemokines that mediate eosinophil (IL-5, GM-CSF) and neutrophil (CXCL1, GM-CSF) recruitment (Online Repository Table 2). Furthermore, although not statistically significant, FhHDM-1 reduced the expression of IL-4 and IL-17A (Online Repository Table 2).

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 (OVA) induced asthma, which promotes eosinophilic inflammation, synthetic small molecule analogues 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 h and 30 min prior to intratracheal delivery of LPS (1mg/kg). In this model, treatment with FhHDM-1 also resulted in a significant reduction in the number of neutrophils in BALF 6 h after LPS challenge (Online Repository Fig E4).

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 transcriptional regulator of T-helper 2 cell differentiation[8] which is critical to the development of the eosinophilic inflammation. 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 signalling. Since HMGB1 is released in response to inflammasome activation in macrophages[9], FhHDM-1 may potentially modulate inflammasome-dependent regulation of HMGB1 signalling 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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Figure Legends

Figure 1

FhHDM-1 modulates macrophage gene expression. (A) Clustered profiles for all genes expressed in macrophages that were untreated, treated with LPS (10ng/ml) or treated with LPS (10ng/ml) and FhHDM-1 (15μM). 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 h, as determined by IPA® analysis. The color-code key indicates the extent of activation (orange) or inhibition (blue) of a pathway.

Figure 2

FhHDM-1 protects against allergic 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. (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, x10 original magnification) and PAS (lower panel, x40 original magnification) are shown. Scale bars, 60μm. Data represent mean ± SEM. **P* < .05, ***P* < .01, and ****P* < .001 vs mice treated with PBS. #*P* < .05 and ##*P* < .01 vs mice treated with house dust mite. N = 6 – 8 mice per group.