

microbiome. These findings suggest that it is important to account for microbial identity when defining healthy indoor environments, and that it may be possible for environmental interventions to alter the indoor microbial environment.

The home and school microbiome may differ because of construction, age of housing, presence of carpeting, or occupancy and composition of inhabitants. Most asthma studies assessing microbial diversity in the home have focused on asthma prevention rather than asthma symptom exacerbation. One recent cross-sectional home-based microbiome study found that higher home bacterial richness was associated with increased asthma severity in children.⁵ While we observed that higher school microbial diversity was associated with increased asthma symptoms, we did not find the same association with home microbial diversity though home microbial diversity was assessed only once and exposure misclassification may have occurred. We assessed the classroom microbiome using vacuumed dust rather than air samples, which may explain why the HEPA filter cleaner intervention was not associated with changes in measured classroom microbial community structure. This was a small pilot study, we were not adequately powered to address the health outcomes of this intervention, and our study cannot be taken to be representative of all schools in the Northeast United States. Our study did have several strengths. (1) It is based in a school setting, where children spend much of their day, and is therefore broadly relevant; future school-based interventions may be more feasible than targeting individual homes to create healthy indoor environments. (2) We used shotgun metagenomics sequencing rather than amplification and sequencing of marker genes such as the 16S rRNA gene, achieving higher taxonomic resolution, avoiding amplification bias,⁶ and allowing for exploratory functional analysis of indoor microbial communities. (3) The study design is longitudinal, with randomized assignment of environmental interventions, allowing for causal interpretations. (4) We found no evidence of contamination in our negative controls.

There are several potential reasons why microbial identity is associated with asthma symptoms. A recent study showed that not all endotoxin is the same; endotoxin from *Escherichia coli* elicited a robust cytokine response in human PBMCs, whereas endotoxin from *Bacteroides dorei* inhibited the ability of *E coli* endotoxin to stimulate a cytokine response.² In our study, different bacterial species contributed to lipid A biosynthesis in homes versus schools. Environmental microbes may produce metabolites that are then inhaled, leading to asthma symptoms.⁷ Alternatively, a recent occupational study of adults working with laboratory mice found that a proportion of their nasal and skin microbiome could be traced to their immediate work environment⁸; it is possible that environmental microbes exert their effect by changing the human microbiome.

While studies to date have focused on manipulating the human microbiome for health, the environmental microbiome may likewise be manipulated for health. Future research should focus on defining what constitutes a healthy indoor microbiome, and the mechanisms by which environmental microbes affect asthma.

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Basophil FcεRI expression is linked to time to omalizumab response in chronic spontaneous urticaria



To the Editor:

Omalizumab has demonstrated excellent efficacy for the treatment of chronic spontaneous urticaria (CSU) in several randomized clinical trials and real-world studies.^{1,2} As a

TABLE I. Baseline characteristics

Characteristic	All responders (n = 44)	FRs (n = 33)	SRs (n = 11)	P value*
Age (y)	49 (14-77)	49 (39-77)	44 (14-77)	.322
Female	30 (68.2)	21 (63.6)	9 (81.8)	.457
Disease duration (mo)	60 (14-360)	60 (14-360)	84 (14-240)	.842
UAS7 at baseline	25 (14-40)	24 (14-40)	26 (21-39)	.285
Blood basophil count ($\times 10^3/\mu\text{L}$)	0.03 (0.01-0.11)	0.03 (0.01-0.09)	0.04 (0.01-0.11)	.271
Total IgE levels at baseline (kU/L)	151 (17-1536)	200 (17-1536)	66 (34-889)	.067
Positive ASST†	21 (56.8)	14 (50.0)	7 (77.8)	.248
Previous treatment with cyclosporine	18 (40.9)	13 (39.4)	5 (45.5)	.738

Values are n (%) or median (range).

UAS7, Weekly Urticaria Activity Score.

*Statistical differences between FRs and SRs were analyzed using Fisher exact test or Mann-Whitney *U* test when appropriate.

†ASST was assessed in only 37 patients (28 FRs and 9 SRs).

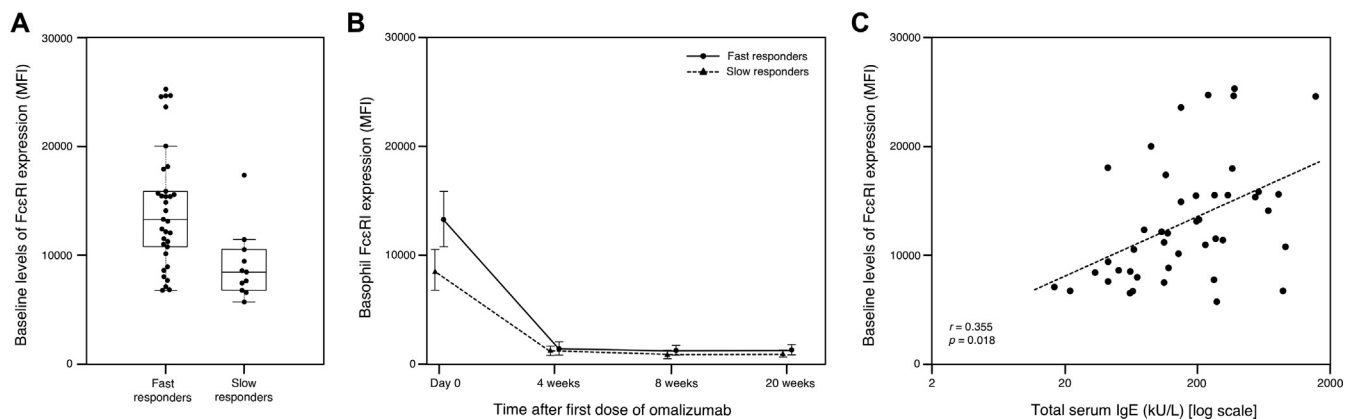


FIG 1. Basophil Fc ϵ RI levels (expressed in MFI) in patients with CSU showing a satisfactory clinical response to omalizumab. **A**, Box-whiskers plots presenting median, interquartile range, maximum and minimum, and individual dots of baseline Fc ϵ RI expression in FRs and SRs ($P = .002$; Mann-Whitney *U* test). **B**, Evolution of the basophil Fc ϵ RI expression (median, interquartile range) in both categories of responders during anti-IgE therapy. **C**, Correlation between baseline Fc ϵ RI expression and baseline total IgE levels (represented in linear and logarithmic [log] scale, respectively) in the 44 patients with CSU included in the study (Pearson correlation coefficient $r = 0.355$; $P = .018$).

monoclonal anti-IgE antibody, omalizumab prevents IgE binding to the high-affinity IgE receptor (Fc ϵ RI) on the surface of basophils and mast cells, resulting in a significant reduction in Fc ϵ RI levels in these cells and, therefore, in their capacity to respond to allergen exposure.³ In this regard, the potential for predicting clinical outcomes during anti-IgE therapy based on basophil allergen response has been investigated in several studies mainly focused on allergic respiratory diseases.⁴ In CSU, meanwhile, it has been recently demonstrated that the modulation of the basophil Fc ϵ RI expression plays a key role in the clinical improvement observed during omalizumab therapy.^{3,5} Thus, a significant drop in the basophil Fc ϵ RI expression is immediately observed after the first dose, which is maintained throughout the duration of the treatment.⁵ Although this phenomenon seems to occur very soon after starting treatment, timing to satisfactory clinical response to omalizumab can be highly variable. The purpose of this study was to evaluate the evolution of the basophil Fc ϵ RI expression in patients with CSU showing a fast and slow clinical response to anti-IgE therapy, with the goal of a better characterization of such individuals and to examine potential associations for these response patterns.

This prospective study included patients with active CSU whose symptoms were not controlled with H1 antihistamines (even up to 4 times the recommended dose). These subjects were treated with omalizumab 300 mg every 4 weeks, and peripheral blood samples were obtained before the first (baseline), second, third, and sixth injections. Basophil Fc ϵ RI expression was measured by flow cytometry from these samples following standard procedures (see this article's Online Repository at www.jacionline.org), and the levels were expressed as mean fluorescence intensity (MFI). Response to treatment was defined as an improvement in the patients' signs and symptoms, achieving a weekly Urticaria Activity Score of 6 or less.^{2,6} The cutoff time chosen for distinguishing fast responders (FRs) and slow responders (SRs) was 4 weeks, which corresponds to the usual first visit after starting treatment. To avoid potential interferences, patients who received anti-IgE therapy before or were diagnosed of a chronic pruritic disease other than CSU (eg, atopic dermatitis) were excluded from the study. It should also be noted that because Fc ϵ RI expression in nonresponders to omalizumab has been extensively studied in a recent article,⁵ these subjects were excluded from the present analysis. The local clinical

research ethics committee granted ethical approval for the study (2012/4913/I).

A total of 44 patients (31 women) responded to treatment with omalizumab. Thirty-three (75%) of 44 patients responded within 4 weeks and were classified as FRs, whereas the remaining 11 (25%) required more administrations to respond (SRs; median time to response, 20 weeks; range, 8-36 weeks). No significant differences regarding clinical and demographic features were found between both groups (Table I). For clinical reasons, the autologous serum skin test (ASST) was performed in only 37 patients, with the result being positive in 50% (14 of 28) and 77.8% (7 of 9) of the FRs and SRs, respectively ($P = .248$; Fisher exact test). Regarding the basophil FcεRI expression, the median value at baseline was significantly higher in FRs (median, 13,247 of MFI; range, 6,753-25,281) than in SRs (median, 8,428 of MFI; range, 5,720-17,375) ($P = .002$; Mann-Whitney U test; Fig 1, A). However, no significant differences were observed regarding the evolution of the FcεRI expression during anti-IgE therapy between FRs and SRs, with both showing a sharp reduction at 4 weeks that was maintained throughout the duration of the treatment ($P > .05$ at 4, 8, and 20 weeks; Test for the equality of medians; Fig 1, B). Finally, a positive correlation was found between levels of total serum IgE and baseline FcεRI expression (Pearson correlation coefficient $r = 0.355$; $P = .018$; Fig 1, C).

Few data are available regarding the patterns of response to omalizumab in CSU. Data from phase III clinical trials suggest that there might be 2 categories of responders: those who respond after just 1 dose of omalizumab (within 4 weeks), and those who required 2 or more doses to achieve the disease control.⁶ However, the exact mechanisms responsible for these responses remain unknown and, more importantly, immunological markers predicting this phenomenon are currently scarce. In this sense, Gericke et al⁷ postulated that serum autoreactivity may be associated with the time to symptom relief with anti-IgE therapy.⁷ Thus, the authors found that a slow response could probably be predicted by a positive basophil histamine release assay and ASST. In other words, a slow response to omalizumab might occur mainly in patients with autoantibodies directed against either the cell-bound IgE or FcεRI. Although no significant differences were found between ASST and time to response in our population, a positive ASST result was more frequently observed in SRs. Moreover, according to our analysis, baseline FcεRI expression may also be associated with the time to response to omalizumab. Thus, patients with very high baseline levels might be more likely to achieve an early therapeutic response. A possible explanation is that these autoantibodies against FcεRI, which are presumably present in SRs, could interfere in the measurement of basophil FcεRI expression by flow cytometry, causing lower levels than in FRs. Apart from this difference, the FcεRI expression seems to exhibit a similar evolution during anti-IgE therapy in both categories of responders, showing a sharp drop immediately after the initial dose, which is maintained during the subsequent administrations. This finding suggests that the FcεRI downregulation may not be a definitive mechanism of action in some patients and therefore, the combination of more than 1 pharmacological mechanism seems necessary to fully explain the pattern of symptoms improvement seen with omalizumab therapy in CSU.

Regarding total IgE levels, previous studies have demonstrated a positive correlation between basophil FcεRI expression and

total serum IgE levels in various disease states, such as asthma, atopic dermatitis, and hyper-IgE syndrome.⁸ Similarly, this association could also be observed in patients with CSU. However, in this condition, which is not considered a classic allergic disease, the correlation seems to be weaker, suggesting that other factors may also have a significant influence on FcεRI expression. In addition, it has been recently postulated that total IgE levels could have an important role in predicting the clinical response to omalizumab in CSU.⁹ In our study, although not significant on the 5% level, a trend toward lower IgE levels in SRs was also noted. The value of total IgE as a useful biomarker of time to omalizumab response in CSU may be somewhat controversial, given the wide range and overlapping of values (therefore, not a clear distinction) observed in both categories of responders.

In summary, although further investigations are needed to confirm these preliminary observations, our study suggests the existence of a link between baseline levels of basophil FcεRI expression and time to response to omalizumab in CSU, with higher levels shown in FRs. The knowledge of the patterns of therapeutic response would have an important impact in clinical practice, helping physicians to improve their approach on the patients' follow-up.

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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;

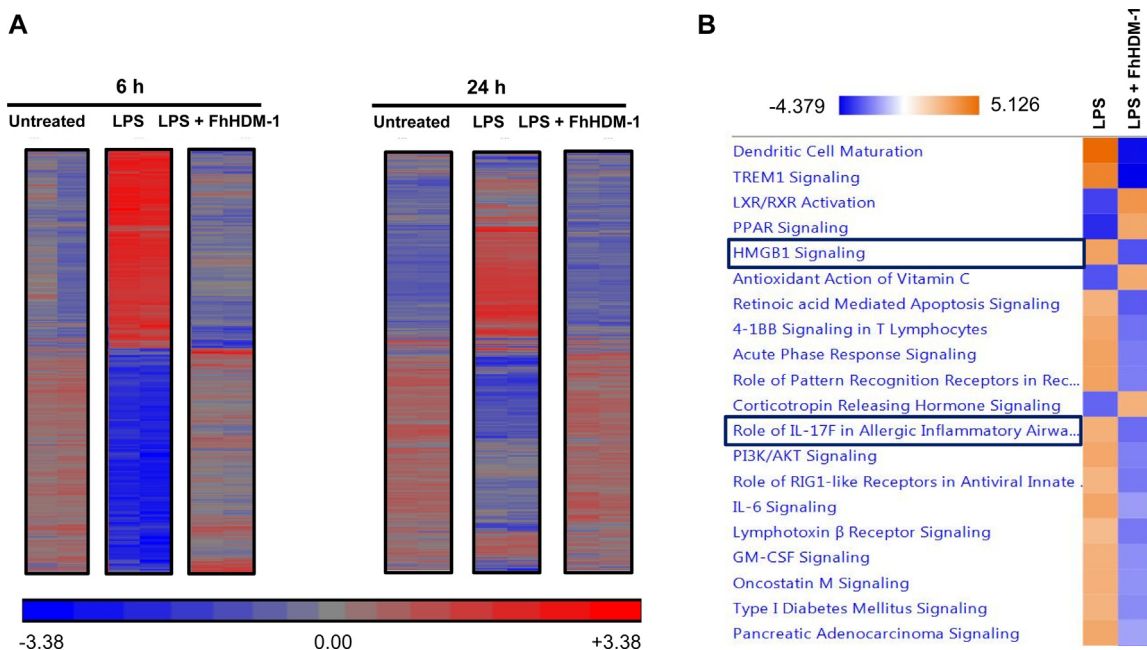


FIG 1. FhHDM-1 modulates macrophage gene expression. **A**, Clustered profiles for all genes expressed in macrophages that were untreated, treated with LPS (10 ng/mL) or treated with LPS (10 ng/mL) and FhHDM-1 (15 μ mol/L). Changes in fold expression are depicted for 2 experimental replicates. The color-code 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.

METHODS

Basophil cell preparation and flow cytometry

Flow cytometry analysis was performed following standard procedures. Briefly, 150 μ L anticoagulated blood was incubated on the same day of collection for 20 minutes at 4°C with an excess of human immunoglobulins to block unspecific binding. Afterwards, blood was stained with either anti-CD123-PE (BD Biosciences, San Jose, Calif) or anti-CD193-APC (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) to identify basophils and with anti-Fc ϵ R1a-FITC (clone CRA1, eBioscience, San Diego, Calif) or an isotype control to establish the expression of Fc ϵ R1 on the surface of blood basophils. The samples were then lysed and fixed using the FACS Lysing Solution (BD Biosciences) and analyzed by flow cytometry in a FACSCanto using the FACS-Diva software. At least 2×10^5 events were acquired. Instrument settings (eg, scatter and voltage settings and compensation matrix) and experimental conditions (eg, antibody clones and dilution) remained strictly constant for all samples throughout the study. To ensure consistency in the analysis, the same investigator processed and analyzed all samples and 2 independent researchers correlated the levels of basophil Fc ϵ R1 expression and the clinical scores.

Autologous serum skin test

The ASST was performed as previously described.^{E1} Briefly, venous blood was taken before the first administration of omalizumab. Samples were centrifuged at 2500 rpm for 10 minutes and the serum separated. Afterwards, 50 mL of fresh undiluted autologous serum was injected intradermally into the patient's volar forearm. Similar volumes of 0.9% NaCl saline and 100 mg/mL histamine were used as negative and positive controls, respectively. A positive ASST result was considered when the diameter of serum-induced wheal was more than 1.5 mm compared with the saline-induced response at 30 minutes.

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