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TLR5 activation exacerbates airway inflammation in asthma

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Abstract

Introduction: Innate immune activation through exposure to indoor and outdoor pollutants is emerging as an important determinant of asthma severity. For example, household levels of the bacterial product lipopolysaccharide (LPS) are associated with increased asthma severity. We hypothesized that activation of the innate immune receptor TLR5 by its bacterial ligand flagellin will exacerbate airway inflammation and asthma symptoms.

Methods: We determined the effect of flagellin co-exposure with ovalbumin in a murine model of allergic asthma. We evaluated the presence of flagellin activity in house dust of asthma patients. Finally, we analyzed the association of a dominant-negative polymorphism in TLR5 (rs5744168) with asthma symptoms in patients with asthma.

Results: We showed that bacterial flagellin can be found in the house dust of patients with asthma and that this bacterial product exacerbates allergic airway inflammation in an allergen-specific mouse model of asthma. Furthermore, a dominant-negative genetic polymorphism in TLR5, the receptor for flagellin, is associated with decreased symptoms in patients with asthma.

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Author contributions:

GSW and SH performed mouse experiments and house dust analysis and tabulated resulting data. RF and CST performed Nanostring analysis. CLI and SHS performed human data analysis. DNC and SG conceived and directed the project, oversaw data analysis and drafted the manuscript. All authors reviewed and approved the manuscript.

The authors have no conflicts of interest regarding this work.

Conclusions: Together, our results reveal a novel genetic protective factor (TLR5 deficiency) and a novel environmental pollutant (microbial flagellin) that influence asthma severity. (Clinical trials NCT01688986 and NCT01087307).

Keywords

asthma; TLR5; flagellin; airway inflammation

Introduction

In the era of personalized medicine, it is critical to understand how gene-by-environment interactions influence asthma onset and activity. Although atopy and adaptive immunity are the most commonly examined factors in asthma pathogenesis, activation of innate immunity by indoor pollutants also plays a significant role. For example, high house dust concentrations of LPS, which activates the innate immune receptor TLR4, are associated with asthma diagnosis and severity (1). We have previously shown that activation of the innate immune receptor TLR5, which recognizes the bacterial protein flagellin (FLA), can promote the development of asthma by priming the allergic response to indoor allergens (2). TLR5 is of particular interest in clinical medicine, because a substantial minority of the population (up to 10% of Caucasians and 3% of African Americans in the US) carry the minor allele of single nucleotide polymorphism (SNP) rs5744168. This allele introduces a STOP codon after the transmembrane domain of TLR5, and creates a dominant-negative decoy receptor, which reduces TLR5 activity by 50–75% (3). Carriage of the minor allele has been associated with protection from systemic inflammation and organ failure in mellioidosis (4) and from inflammation-associated weight loss in cystic fibrosis (5). Based on these observations, we hypothesized that reduced TLR5 activity might protect against inflammation in established asthma. For this report, we adopted a translational research model: we first evaluated house dust from asthma patients' homes for TLR5 activity; we then modeled the effects of TLR5 activation in an allergic inflammation mouse model; finally, we evaluated the role of genetic TLR5 deficiency, which renders individuals unresponsive to environmental TLR5 agonists, in asthma symptomatology (Figure 1). We show that FLA activity is present in house dust extract (HDE) from asthma patients' homes, and that FLA can exacerbate airway inflammation in a mouse model of allergic asthma. FLA predominantly leads to Th1 and Th17 pathway activation after FLA/OVA co-exposure. Furthermore, asthma patients with TLR5 deficiency have decreased asthma symptoms. In aggregate, our results suggest a novel role for bacterial FLA in promoting asthma symptoms and demonstrate how gene-environment interactions modulate asthma activity.

Materials and methods

House dust collection and study design:

To identify whether house dust promotes TLR5 activation, we recruited subjects with physician-diagnosed asthma (N=24). The demographics of these subjects were as follows: 16 Black/African-American, 7 Caucasian, 1 multiracial; 17 female, 7 male; aged 47.6±14.1 years (average ± standard deviation, median 51). All subjects had asthma with a mean FEV1/FVC ratio of 69.3±27.4 (median 71%). We assayed TLR5-dependent bioactivity of

HDE for two reasons: a) because house dust can contain flagellins from many bacterial sources, which are not captured by the monoclonal capture antibodies of conventional ELISAs; b) because a functional assay would better address whether the flagellin in HDE had TLR5-related biological activity, as opposed to being degraded/inactive. Study subjects collected house dust from designated areas in their homes (bed and area next to the bed) according to established methodology from the National Health and Nutrition Examination Survey (6).

HDE Preparation:

House dust was ground, filtered through 425 um coarse metal sieve, weighed, suspended in sterile PBS (100 mg/mL), shaken at 4C overnight, centrifuged at 2000 RPM for 10 minutes at 4 C and filtered overnight at 4 °C through a glass funnel with sterile gauze and further through sterile 100-, 45- and 22-micron filters. The resulting HDE was frozen at −80 °C until used. HDE was added to HEK cultures at a a final concentration of 1 mg/ml culture media.

HEK Cell Transfection:

HEK cells were grown in 100 cm culture dishes in DMEM with 10% fetal bovine serum, 1% penicillin/streptomycin and 1% L-glutamine. Cells were transfected with a hemagglutinin-tagged human *TLR5* (Invivogen, San Diego, CA, Cat# puno1-ha-htlr5) or empty vector plasmid (Invivogen, Cat# puno1-mcs) using FuGENE® HD Reagent (Promega, Madison, WI, Cat # E2311). Transfection efficiency was confirmed by western blotting for hemagglutinin using Anti HA-Tag antibody (EMD Millipore, Temecula, CA, Cat#05–904). Cells were treated with HDE for 16 hours before collecting RNA and cell culture supernatant for IL-8 determination.

ELISA Assays:

ELISA assay for human IL-8 was performed using R&D Duoset (R&D Systems, Minneapolis, MN, Cat # D8000C). Cytokines in mouse lavage were analyzed using multiplexed fluorescent bead-based immunoassay (Bio-Rad, Laboratories, Hercules, CA).

Mouse exposures:

Studies were approved by the NIEHS IACUC. Mice were housed in ventilated cages with a 12-hour light-dark cycle and were provided chow and water *ad libitum*. Male C57Bl/6J mice, 6–12 weeks old, were purchased from the Jackson Laboratory and acclimated for at least 7 days. On days 0 and 7, mice were anesthetized with inhaled isoflurane and sensitized by oropharyngeal (o.p.) aspirations of 50 μl PBS containing 50 μg LowEndo OVA (Worthington Biochemical Corporation, Lakewood, NJ, Cat# LS003062) together with 100 ng LPS from *E. coli* (Sigma Chemical, St Louis, MO, Cat# L2630). To elicit asthma-like allergic inflammation, the mice were exposed by o.p. aspirations on day 14 to 50 μl PBS containing either 50 μg OVA alone, 100 μg Recombinant Flagellin (recFla) from *S. typhimurium* (Cat# tlrl-flic-50, InvivoGen), or a combination of these reagents. Control mice were sensitized using OVA/LPS, but not challenged. Mice were euthanized by i.p. overdose

of pentobarbital, and bronchoalveolar lavage was performed 2 days post-challenge for cellular inflammation, or 4 hours post-challenge for analyses of cytokines.

Airway hyperresponsiveness measurements:

4 groups of 8 mice were sensitized to 50 ul PBS containing 50 ug OVA + 100 ng LPS on days 0 and 7, and then challenged on day 14 to either none (control), 50 ug OVA, 100 ug recFla, or OVA + recFla (as above). Two days post challenge, AHR was assessed using the flexiVent mechanical ventilator system (Scireq, Montreal, PQ, Canada) as previously described. Total respiratory system resistance (R) following a baseline measurement and over a 3-minute sampling period following administration of increasing doses of methacholine (MCH) (0 to 100 mg/ml) was recorded using the single compartment model. Individual peak responses following the highest dose of MCH per mouse were used for the analysis. Airway resistant values are represented as R ($cmH_2O.s/mL$).

Tissue processing and NanoString© Analysis:

At 4 hours post-challenge, mice were euthanized, and sterile PBS was perfused through the right ventricle. Apical right lobes were excised and flash-frozen in liquid N_2. Total RNA was isolated using TRIzol (Invitrogen Corporation, Carlsbad, CA) and further processed with RNeasy mini Kit and RNase-free DNase (Qiagen, Valencia, CA, Cat# 74904 and 79254). Gene expression was examined using the NanoString© platform, Mouse Immunology codeset that measures 547 immune genes and 14 housekeeping genes. Gene expression was quantified on the nCounter Digital AnalyzerTM and raw/adjusted counts were generated with nSolver (v4.0)TM software using the geometric mean of the manufacturer's positive and negative controls, and 4 housekeeping genes. nSolver-adjusted data were imported into Partek_v7.0, log2 transformed and quantile normalized for further quality assurance and control. To identify significant differences, a 1-way ANOVA was performed with post-hoc Benjamini-Hochberg FDR corrected p-values and fold-changes generated for each group comparison.

Statistical analysis of EPR samples:

We performed logistic regression analysis using unadjusted and adjusted models for asthma exacerbation. Co-variates in the adjusted model included potential confounders for asthma: sex, race (Caucasians and African Americans), smoking status and household income).

Results

Flagellin is present in house dust and promotes airway inflammation

We first used an established methodology (1) to collect dust from homes of asthma patients and evaluate it for FLA by adding extracts to HEK293 cells stably transfected with human *TLR5* or control empty vector. FLA activity was detected in 13 of the 24 dust samples analyzed (Figure 2).

To test the ability of FLA to exacerbate allergic inflammation in an animal model of established asthma, we sensitized mice with OVA mixed with LPS (OVA/LPS), and challenged them with either OVA alone, or OVA and FLA (OVA/FLA). Challenge with

OVA/FLA caused significant airway hyperresponsiveness, unlike challenges with FLA or OVA alone in C57BL/.6J mice, which are relatively resistant to allergic inflammation-induced airway hyperresponsiveness (Figure 3A). Challenge with OVA/FLA also synergistically increased the number of eosinophils and neutrophils in lung lavage fluid compared to challenge with OVA alone (Figure 3B, $p<0.001$ by 2-way ANOVA). Furthermore, several inflammatory cytokines were synergistically elevated in the combined OVA/FLA exposure compared to OVA alone or FLA alone, including Il-6, the eosinophil-attracting chemokine Ccl11, the lymphocyte-attracting chemokine Ccl20 and the neutrophil-attracting chemokines Cxcl1 and Cxcl2. Other cytokines selectively increased by OVA/FLA included IL-1β, Cxcl9, Cxcl10, Ccl3, and Ccl4 (Figure 4). Interestingly, the type 2 cytokines, Il-4, Il-5 and Il-13, were either unaffected, or in the case of Il-13, suppressed at the protein level in OVA/FLA-challenged mice compared to mice challenged with OVA alone (Supplemental Figure 1).

TLR5 activation promotes Th1-like inflammation in a murine asthma model

To better understand the effects of TLR5 activation in allergic asthma exacerbation, we used the Nanostring platform to study expression of genes with known functions in immunity (Figure 5). We found that combined OVA/FLA exposure induced a distinct signature of gene expression in the lung (Figure 5A,B) with 97 genes being distinct from that of mice challenged with either OVA alone, or FLA alone (Figure 5C). Because the central question of this project was to determine the impact of TLR5 activation on already established allergic asthma, we then focused our analysis on comparisons between the OVA/FLA and OVA groups using Ingenuity pathway analysis. We found that several pathways were differentially activated (Figure 5D): notably pathways such as Th1 activation, B-cell signaling, Neuroinflammation, Th17 and STAT3 were activated after OVA/FLA co-exposure compared to OVA alone, while Th2 and Dendritic Cell Maturation pathways were inhibited after combined exposure. Among the genes that were differentially regulated after OVA/FLA exposure prominent were a number of chemokines and cytokines like Cxcl1, Cxcl3, Ccl3, Ccl20, Il23, Il-1α and Il-1β (Table 1).

TLR5 deficiency is associated with decreased asthma symptoms in patients with asthma

Given our finding that exposure to FLA exacerbates inflammation in a mouse model of asthma, we next investigated whether TLR5 also functions in human asthma. We examined the relationship of the rs5744168 genotype with asthma diagnosis and outcomes, using the Environmental Polymorphisms Registry (EPR), a cohort of subjects who have donated their DNA for genetic analysis and have provided health and exposures information that enables gene-environment associations in disease (7). All subjects signed informed consent and the study was approved by the NIH IRB. We compared 1057 patients with asthma to 667 subjects without lung disease (Supplemental Table 1). Hardy-Weinberg Equilibrium (HWE) was followed for the control and asthma samples (Supplemental Table 2). There was no statistically significant difference in the minor allele frequency between subjects with (5.1%) and without (4.1%) asthma diagnosis, and no difference in the mean age of asthma diagnosis between minor allele carriers and major allele homozygotes (24.9 +/− 19.9 vs. 23.5 +/−18.5 years, $p=0.45$) (Supplemental Table 3). This result was not entirely unexpected, since many factors can lead to asthma development.

We then analyzed the association of *TLR5* genotype with asthma-related outcomes. We evaluated 950 asthma patients who were wildtype for rs5744168, and 107 who were minor allele carriers. Because our mouse model data suggested that FLA exacerbates airway inflammation, we focused on subjects who reported symptoms, and compared symptom burden and asthma activity between minor allele carriers and major allele carriers. We found that minor allele status was associated with a lower symptom burden: less nocturnal awakening due to asthma symptoms (adjusted OR=0.32, CI 0.11–0.94, p=0.038 for 4–14 nights vs 1–3 nights of awakening) and activity limitations due to asthma symptoms (adjusted OR=0.36, CI 0.16–0.82, p=0.01 for 4–14 days vs 1–3 days of activity limitations). There was also a trend for protection from asthma attack in the previous 12 months in minor allele carriers (adjusted OR=0.69, 95% CI 0.44–1.08, p=0.10) (Table 2). In aggregate, these results suggest that functional TLR5 deficiency protects against the development of severe symptoms in asthma.

Discussion

Our current data suggest that TLR5 activation by environmental FLA can exacerbate inflammation and disease activity in established asthma. As FLA is the major structural protein of the bacterial flagella, our findings may also support an adverse role of flagellated microbiota in asthma pathogenesis. Our results therefore expand on previous findings and suggest a complex role of bacterial FLA in asthma development and activity.

We have shown previously that TLR5 activation is sufficient for the development of asthma pathology by priming the allergic response to indoor allergens (2). Our current study did not reveal a statistically significant increase in asthma prevalence among carriers of the dominant-negative mutation. However, it is now clear that “asthma” is not a monolithic disease with a uniform pathobiology, but rather a syndromic assembly of endotypes, with differing and only partially overlapping biological pathways that can be affected in different ways by distinct regional exposure profiles (8). This redundancy in asthma development pathways might mask effects of individual molecular pathways, such as the ones downstream of TLR5. Better identification of asthma endotypes, including exposure profiles might allow future study groups to be stratified to better study the impact of gene-environment interactions on asthma development.

Our analysis of asthma patient questionnaire responses nonetheless suggests that FLA plays a role in asthma exacerbation. Questionnaire-based analyses must be interpreted with caution as they are subject to recall bias. However, our asthma symptom questionnaire was based on the well-validated questionnaire used in the National Health And Nutrition Examination Survey (NHANES) (9). Our human data suggest that FLA and TLR5-mediated effects modulate asthma activity and lead to exacerbation of asthma symptoms. The corollary to this finding is that environmental home remediation by removal of FLA and its likely sources such as house dust mites (10) may improve asthma symptomatology.

Our mouse model data support the human findings. Co-exposure to FLA/OVA (after OVA sensitization) promoted eosinophilic and neutrophilic inflammation of the airways, as well as an increase in a number of inflammatory cytokines previously associated with asthma.

Importantly, co-exposure synergistically promoted eosinophilic inflammation and expression of cytokines and chemokines such as Il-6, Ccl4, Ccl11, Ccl20, Cxcl2, Cxcl9 and Cxcl10, which are associated with difficult-to-treat asthma (11–13) and pronounced eosinophilia (14). This suggests that FLA-induced, TLR5-mediated immune activation does not simply superimpose on OVA-induced inflammation, but rather acts synergistically to alter the type of inflammatory response.

Further gene expression analysis, using Nanostring, confirmed and expanded these insights. The Nanostring platform permits a focused evaluation of immunity- and inflammation related genes, but by its nature does not allow an exhaustive investigation of non-immune pathways. Nevertheless, our analysis of FLA-driven effects in airway inflammation yielded interesting findings. We did not observe an increase in type 2 cytokines after FLA/OVA co-exposure, however we saw increases in eosinophil- and neutrophil-attracting chemokines, suggesting that FLA might promote inflammation through direct activation of the innate immune system. The activation of cytokines downstream the MyD88-NFκB pathway is not surprising, since this is the canonical pathway of TLR5 signaling. However, we also found that mice exposed to both FLA and allergen had increases in IL-6, IL-23, IL-1β and CCL20, which are important molecules in the initiation of Th17 lymphocyte differentiation (15), or the recruitment of these cells to the inflamed lung (16). Indeed, in our mouse model we found that neutrophil counts were increased in the mice exposed to the OVA/FLA combination. In the gene expression analysis, Ccl20 was the highest induced gene by OVA/FLA exposure compared to OVA alone in the mouse model and also was increased at the protein level (Figure 4). Ccl20 is in the IL-17 pathway, being induced by IL-17 in bronchial epithelia (17) but also recruiting IL-17 cells (18) to the lung in allergic inflammation conditions (19). CCL20 is upregulated in severe asthma (20) and may contribute to the pathogenesis of asthma (21). The next-highest-induced gene, Il-23, was recently shown to play a role in the pathogenesis of non-allergic eosinophilic asthma (22) and is also involved in the Th17 pathway of asthma pathogenesis (23, 24), Il-1α, Il-1β and Il-1r2 upregulation suggest a prominent role for the IL-1 pathway downstream of TLR5 activation in allergic asthma , which is not entirely unexpected, since FLA activates the NLRC4 inflammasome as well (25). Interestingly, the IL-1 signaling pathway, which plays a prominent role in asthma pathogenesis (26, 27) also modulates the IL-17 pathway of airway inflammation (28), again suggesting a predominating Th1/Th17 signaling pattern in the after OVA/FLA exposure. Chemokines Ccl3, Ccl4, Cxcl1 were also induced by protein levels in the lavage fluid; they, along with Cxcl3 are found in the lung lavage fluid of steroid-resistant asthma patients; interestingly. another innate immune activator, LPS was also detected in the steroid-resistant lung lavage fluid, supporting that innate immune activation, e.g. through exposure to FLA or LPS, may promote asthma exacerbation (11) In aggregate, these findings support that FLA co-exposure in an allergic asthma model promotes a unique inflammatory profile with Th1/Th17 features. In the bigger picture, this suggests that specific environmental exposures may bias inflammatory responses towards exposure-linked phenotypes and thus specific environmental remediation could be considered as a way to improve asthma symptoms.

FLA effects in inflammation are not uniform. Others have shown that FLA suppresses allergic responses via the generation of regulatory dendritic cells and T-cells (30). However,

in that work the effect of FLA was dose-dependent: high (pharmacological) doses were immunosuppressive, while low doses, like the ones they are likely to be found in house dust or via airway colonization, were immunostimulatory (30). Dose-dependent opposite effects of innate immune activation in asthma have been described before: for example, LPS can enhance or attenuate allergic inflammation depending on timing and dose during exposure (31). Therefore, our data and existing literature support a complex role of innate immunity, as both enhancer and suppressor of allergic airway inflammation dependent on timing and dosing parameters.

Several limitations of our study need to be considered. First, our human subjects were analyzed only cross-sectionally, and we could not obtain information on particulars of treatment, lung function, or disease endotypes. However, the questionnaires we used have been validated through the National Health and Nutrition Examination Survey (32), and thus should accurately capture asthma prevalence and symptom burden. Secondly, house dust flagellin was not directly quantified in house dust but its presence was inferred based on a TLR5 cell culture reporter system. We did attempt to measure flagellin in the house dust extracts. We found, that commercial ELISAs used very specific monoclonal capture antibodies which were not well-suited in capturing the different types of flagellin (from different bacterial sources) that were present in house dust. In addition, presence of ELISA-measurable flagellin may not represent biologically active flagellin, since it is possible that bacterial proteins may decay in the room-temperature environment of a home. For these reasons we used the biological assay as more representative of the true TLR5 activity. Finally, in our mouse model, we used commercially available flagellin and not house dust samples, which may have provided a direct link to the home environment conditions in patients with asthma. However, house dust is not well standardized, so we considered that our findings may not have been generalizable. It is almost certain that different house dust samples would have different effects depending on relative TLR5 activity, activated immune pathways for that particular batch, etc. Therefore, we elected to use commercial flagellin which will allow for replication and comparisons by future research in this area.

In conclusion, human and animal studies presented in this work support that environmental FLA and its receptor, TLR5, are important modifiers of asthma. FLA may thereby become another target in environmental remediation of homes of asthma patients, and TLR5 deficiency may be added to the panel of genetic factors affecting asthma activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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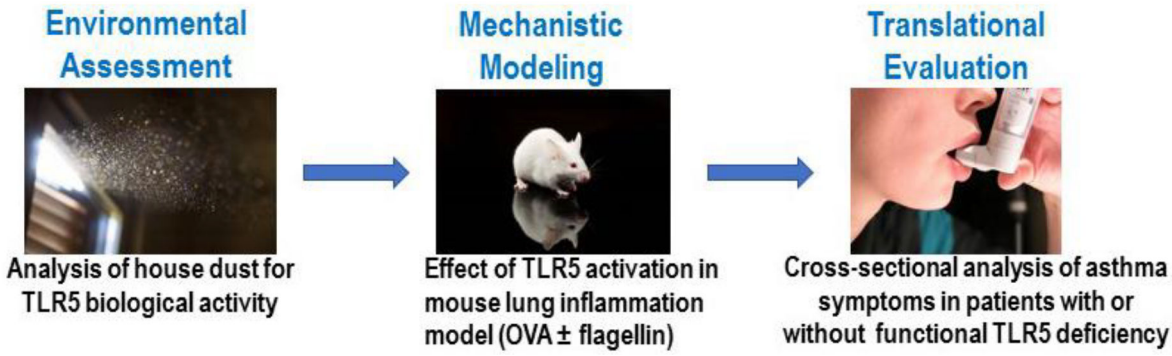

Figure 1. Overview of the research presented in this paper.

Initially we analyzed house dust from homes of asthma patients for TLR5 biological activity. We then used an ovalbumin model of allergic airway inflammation, with or without additional TLR5 activation via flagellin, to evaluate the role of TLR5 activation ion established asthma. Lastly, we translated our findings in human health insights by analyzing the symptoms of asthma patients with or without functional TLR5 (based on self-reported data).

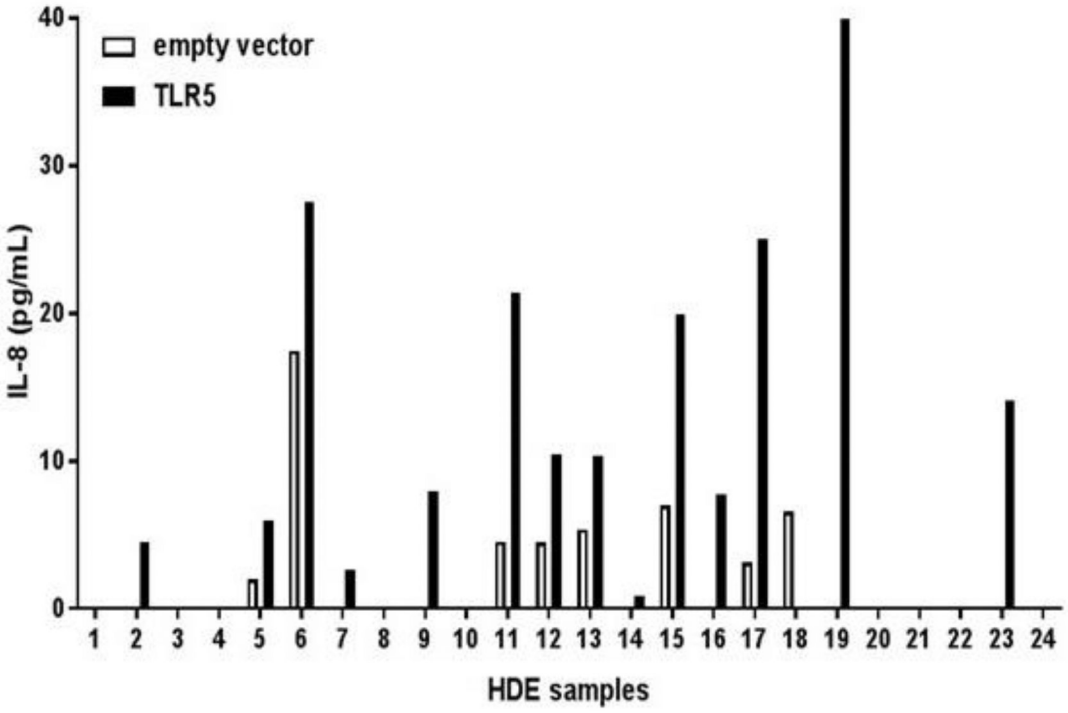

Figure 2. FLA is present in house dust from asthma patients' homes.

FLA activity was assessed by measuring IL-8 in the supernatant of HEK293 cells transfected with either empty vector or human *TLR5* and exposed to house dust extract from 24 homes of asthma patients. Because house dust has several impurities which may induce IL-8, the difference in IL-8 levels between empty vector and TLR5-transfected cells is depicted as indicator of specific FLA activity.

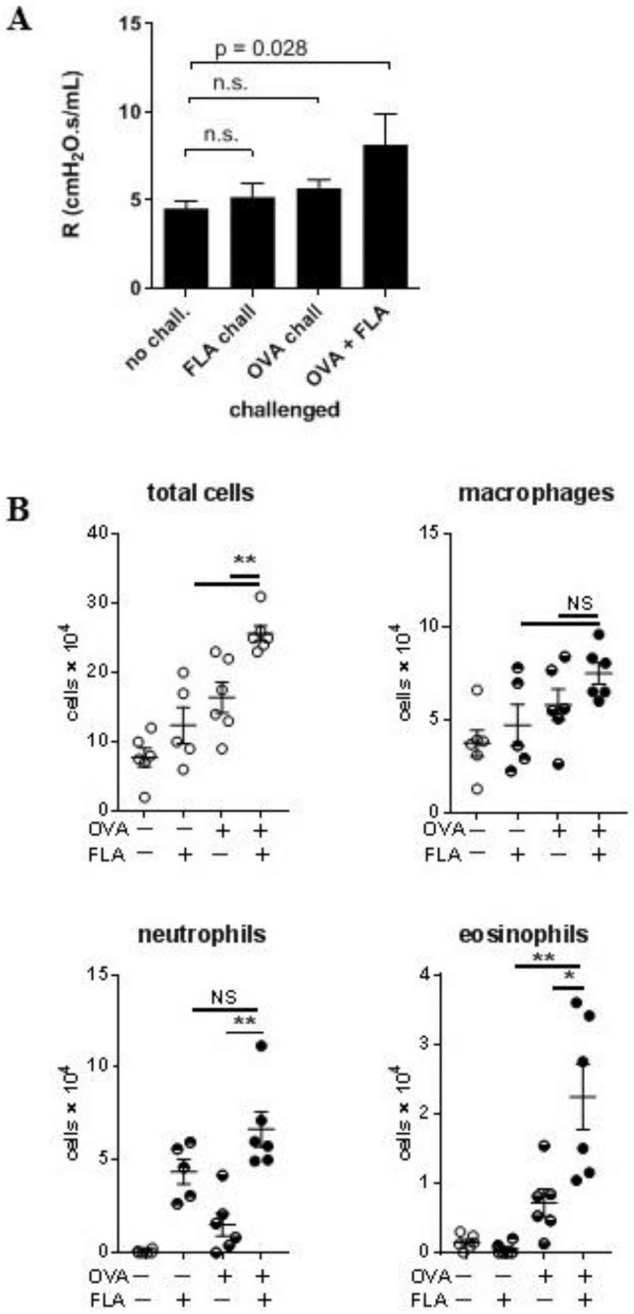

Figure 3. FLA exacerbates allergic airway inflammation in the mouse model.

A). Airway resistance in response to 100 mg/ml aerosolized methacholine, measured by flexiVent, in C57BL/6J mice after sensitization with OVA, and exposure to OVA alone, FLA alone, or OVA+FLA, in comparison to unchallenged mice. Only OVA+FLA exposure leads to significant increase in airway responsiveness in this (relatively resistant) mouse strain. B). FLA + Ovalbumin exposure increases total cells, neutrophils and eosinophils in the lung lavage fluid.

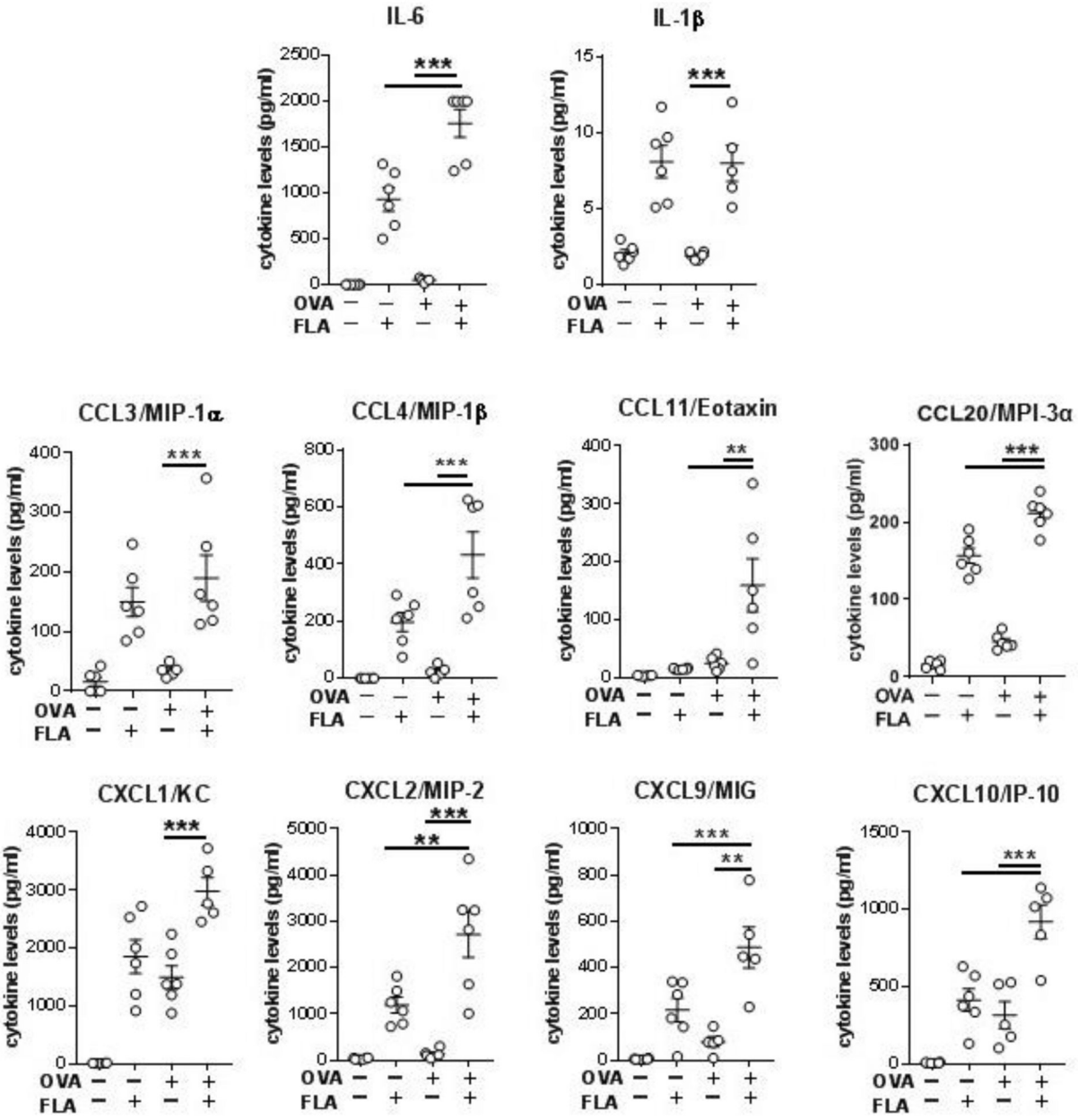

Figure 4. FLA/OVA co-exposure increases cytokine and chemokine levels in mouse lung compared to OVA or FLA exposure alone.

*p<0.5, **p<0.01, ***p<0.001, ANOVA with Holm-Sidak post-hoc correction.

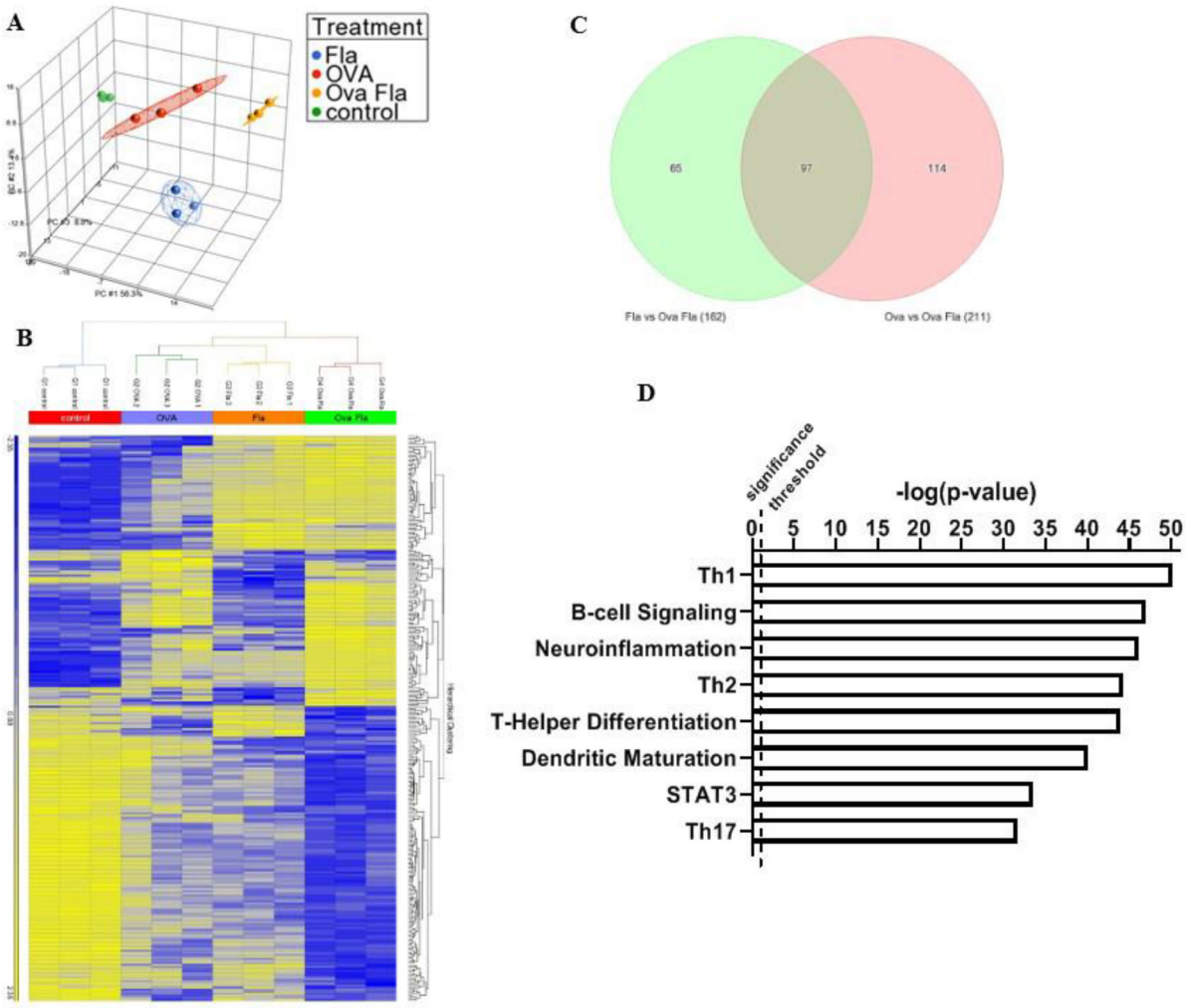

Figure 5. FLA/OVA co-exposure generates a unique gene expression signature.

A). Principal component analysis. B). Supervised Hierarchical Clustering of group expression means using the 276 genes significantly elevated in the (FLA vs FLA/OVA) and (OVA vs FLA/OVA) comparisons. Various shades of yellow indicate higher, and blue lower, relative expression. C)Venn diagram of gene activation groups, comparing unique genes between FLA/OVA to FLA and FLA/OVA to OVA comparisons. . D) Gene enrichment score for relevant pathways comparing OVA vs. OVA/FLA exposure (the pattern was very similar in the FLA vs OVA/FLA comparison).

Table 1

Gene expression comparison in mouse lung tissue after OVA or FLA/OVA exposure. Significant genes that were more than 2-fold up- or down-regulated are entered. P-values are adjusted after ANOVA.

Gene Symbol	p-value(OVA/FLA vs OVA)	FDR(OVA/FLA vs OVA)	Fold-Change(OVA/FLA vs OVA)
Ccl20	5.72E-05	0.00156927	7.76796
ll23a	1.26E-07	6.86E-05	7.45
Il1r2	9.48E-05	0.00213512	6.50668
Cxcl3	0.00110474	0.0071808	6.48361
Cd14	9.56E-07	0.00017397	5.48166
Ccl3	0.000344283	0.0036817	5.11353
Il1m	2.10E-05	0.000763894	4.10273
Fkbp5	0.0042383	0.0165294	4.06113
Il1a	6.26E-05	0.00162754	4.02917
Trem1	0.000289177	0.00336805	3.98792
Il1b	0.000315129	0.00351144	3.87289
Clec4e	1.35E-06	0.000184031	3.59719
Tnfrsf9	0.00352473	0.0148039	2.84872
Ccl4	0.00263185	0.0125843	2.84333
Tnfaip3	0.000383349	0.00387608	2.78183
Il18rap	4.82E-06	0.000292312	2.75738
S100a9	0.0108444	0.0323555	2.68214
S100a8	0.00977506	0.0294872	2.52318
Cd69	0.000462905	0.00428383	2.40266
Cxcl1	0.00563854	0.0202243	2.37838
Cd80	0.000454642	0.00427991	2.36071
Csf2	0.00946382	0.0289377	2.36006
Marco	4.57E-05	0.00146702	2.30461
Nfkbia	0.00086232	0.00595983	2.29296
Ptafr	0.000157212	0.00256279	2.15858
Ebi3	8.31 E-05	0.00206299	2.09236
Ltf	0.0032733	0.0139626	2.06389
T nfsf15	0.000170527	0.00258633	2.03272
Nox4	0.00288848	0.013034	−2.03463
Ciita	1.23E-05	0.000651218	−2.03548
Ahr	0.000152843	0.00256279	−2.04796
Il17re	0.000701463	0.00536415	−2.11183
Cx3cr1	0.00393919	0.0160251	−2.15134
Gata3	0.000101672	0.00213512	−2.15973
Ms4a1	0.000808271	0.00565789	−2.25903
Ccrl1	0.000443766	0.00425082	−2.37796

Gene Symbol	p-value(OVA/FLA vs OVA)	FDR(OVA/FLA vs OVA)	Fold-Change(OVA/FLA vs OVA)
T nfsf10	0.000717185	0.00536415	−2.37852
Plau	0.00010991	0.00217245	−2.61768

FDR= false discovery rate

Table 2

Asthma symptom severity and health care use by TLR5 rs5744168 carrier status

Asthma Symptoms and Health Care Use in All Participants with Asthma by TLR5 status										
		Major			Het/Minor		Logistic Regression			
	N[1]	n[2]	%	N[1]	n[2]	%	Unadjusted Odds Ratio	95% CI	Adjusted Odds Ratio[3]	95% CI
Asthma-related health care use										
Episode/attack in past 12 months	938	430	45.8%	106	42	39.6%	0.78	0.51 to 1.17	0.69	0.44 to 1.08
Asthma-related ER visit in past 12 months	940	122	13.0%	105	13	12.4%	0.95	0.51 to 1.75	0.99	0.49 to 1.98
Asthma-related prescription meds in past 12 months	939	607	64.6%	104	68	65.4%	1.03	0.68 to 1.58	1.00	0.63 to 1.59
Asthma-related symptoms (no. of days / last 2 wks)										
Days Awakened at Night	899			102						
1 to 3 vs 4 to 14							0.35	0.23 to 0.97	0.32	0.11 to 0.94
Days with Activity Limitations	898			103						
1 to 3 vs 4 to 14							0.48	0.23 to 1.01	0.36	0.16 to 0.82
Days with Wheeze	903			103						
1 to 3 vs 4 to 14							0.84	0.45 to 1.59	0.70	0.34 to 1.42

[1] The total number of respondents to asthma outcomes differs between categories due to item non-response.

[2] Total number of respondents answering yes for each category

[3] Analyses were adjusted for race, gender, smoking status, and household income.