ONLINE METHODS

Animals and allergen models. Five- to eight-week old C57BL/6 and *Tlr5*$^{-/-}$ mice were purchased from Jackson Laboratories. Male mice were used between six and twelve weeks of age, and we conducted our animal experiments in accordance with the NIEHS Institutional Animal Care and Use Committee. We sensitized mice by oro-pharyngeal instillation[10] of 100 µg low-endotoxin OVA (< 1 EU/mg OVA) (Hyglos GmbH) in a 50 µl volume on days 0 and 7. Microbial products tested include *E. coli* LPS (Sigma) and ultra-pure LPS (List Biological Laboratories). Also tested weretriacylated peptide, Pam3CSK4; poly (I:C); sFLA from *S. typhimurium*; endotoxin-free rFLA from *S. typhimurium*, (endotoxin-free FLA from *B. subtilus*; FSL-1 (Pam2CGDPKHKFS), the N-terminal portion of the 44-kDa lipoprotein LP44 of *Mycoplasma salivarium*; ssRNA40; and ODN1826, all from InvivoGen. Except where otherwise indicated, we challenged mice with a 1% OVA (Sigma) aerosol for one h on day 14, and analyzed them on day 16. For multiple exposures, animals were challenged for 30 min daily, and analyzed 1 d after the last exposure. Other mice were sensitized on days 0 and 7 with 10 µg cockroach antigen (Greer Inc.) treated with ProteoSpin endotoxin removal kit (Norgen Biotek), challenged on days 14-17 with 10 µg of the antigen, and harvested three d later.

Histology. Lungs were fixed in 10% formalin, processed, and embedded in paraffin. Left lobe longitudinal sections 5-7 μm thick were stained with Alcian Blue and Periodic acid-Schiff. We scored the airways in a blinded fashion, and considered a single positive mucus-staining cell in an airway diagnostic of a positive result.

Airway Physiology. We assessed responses to aerosolized methacholine using the Flexivent mechanical ventilator system (Scireq), as previously described[40].

Cytokine analysis. We analyzed cytokines in BAL and whole lung homogenates by ELISA or multiplexed bead-based assays as described previously[10]. For analysis of lymph node cytokines, we injected mice with 10^6 naïve, OVA-specific $CD4^+$ T cells from OT-II mice one d prior to allergic sensitization. Mediastinal lymph nodes were harvested 3 d later, and cultured for 3 d in complete medium containing 1 mg ml^{-1} OVA. IL-25, IL-33 and TSLP in BAL were measured by ELISA (R&D Systems).

Flow cytometry. We prepared lymphocytes from the lung, blocked the cells, and stained them for surface antigens and intracellular cytokines as previously described[10]. Stained cells were collected using a BD™ LSR II cytometer (BD Biosciences) and data analyzed using FlowJo 7.2.2 software (Tree Star, Inc.).

HDE preparation and analyses. HDEs were prepared as described previously[41,42]. Endotoxin activity was measured by a Limulus amoebocyte lysate QCL-1000 assay (Lonza). Allergen

concentrations were measured by Multiplex ARray for Indoor Allergens (Indoor Biotechnologies).

Bone marrow chimeric mice. We irradiated mice with 900 rads, injected them 1 d later with 10^7 bone marrow cells, and gave the animals acidified water, supplemented with 500 μg ml^{-1} neomycin. Reconstitution of lung leukocytes was verified to be greater than 94% by flow cytometry, based on staining with antibodies against CD45.2 (clone 104; eBioscience) and CD45.1 (clone A20; eBioscience).

Immunoblotting

HDEs (20 μg/sample) were loaded on a 4-12% Bis-Tris polyacrylamide gel (Bio-Rad laboratories) and resolved by electrophoresis. Proteins were transferred to a PVDF membrane, blocked for 1 h, probed overnight with 0.1 μg ml^{-1} anti-flagellin FliC (clone X5A12; InvivoGen) or (clone FLIC-1; Biolegend), followed by Mouse Trueblot Ultra: anti-mouse Ig HRP (1:1000 dilution; eBioscience). Chemiluminescence by Lumigen PS-3 (GE Healthcare) was used to detect immunoreactive proteins.

ELISA for Flagellin-specific IgG

Microplates were coated with 100 ng/well rFLA (InvivoGen), FLA from *P. aeruginosa*, or *E. coli* LPS and probed with human sera (1:500 dilution), followed by HRP anti-human IgG (BD Pharmingen).

Human studies. The protocol was approved by the Institutional Review Board at NIEHS and Duke University Medical Center. Informed consent was obtained by human subjects. Asthmatics had a physician-diagnosed asthma and AHR (defined as 20% decrease in forced expiratory volume at 1 s (FEV1) after inhalation of < 8 mg/ml of methacholine). Normal subjects had no asthma or allergic rhinitis, and tested negative for methacholine challenge and allergen skin tests. Non-fasting blood samples were drawn from an antecubital vein into a clot-activating container. Serum was separated and stored at -80º until assayed.

Statistics. Data are expressed as mean ± SEM. Statistical differences between groups were generally calculated using the unpaired, two-tailed Student's *t* test. Where multiple groups were compared, we used ANOVA. *P*-values of less than 0.05 were considered statistically significant.