

prelingual deafness and goiter.¹ However, hearing threshold and plasma levels of T3 and T4 were not changed after treatment of YS-01 (10 mg/kg/d) for 1 week (see Fig E7, A–C, in this article's Online Repository at www.jacionline.org). In addition, YS-01 did not affect tracheal smooth muscle contraction (see Fig E7, D).

In summary, YS-01 reduced airway hyperresponsiveness and airway inflammation via the inhibition of the SCN[−]/NF-κB pathway. In addition, YS-01 showed protective effects against IL-4- and IL-13-induced goblet cell hyperplasia and ASL depletion (see Fig E8 in this article's Online Repository at www.jacionline.org). These results indicate that pendrin inhibitors are promising candidates for the treatment of allergic asthma.

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Physiologic response to chronic house dust mite exposure in mice is dependent on lot characteristics



To the Editor:

Multiple models of allergic airway inflammation using house dust mite (HDM) extract have been developed to study experimental asthma in mice. Acute exposure models develop airway hyperresponsiveness (AHR), T_H2-mediated airway inflammation, and mucus secretion,^{1,2} but airway remodeling with peribronchial collagen deposition requires chronic exposures.³⁻⁵ Presently there are no standard exposure methodologies, because each model varies with respect to the species, duration, and/or dose of HDM used and different exposure protocols affect phenotypic outcomes.⁴ In addition, phenotypic outcomes depend on the HDM extract source.⁶ Lack of reproducible physiological responses to allergen extracts has long been known and is well documented in mice and humans after cutaneous exposure,^{7,8} but has been less well detailed in models of allergen inhalation.

The National Institutes of Health stresses the importance of scientific rigor and transparency for enhancing reproducibility in research. Among several key areas, they highlight authentication of biological and/or chemical resources. Although most recently published articles (PubMed; 2016-2018) using murine chronic HDM exposure cite the HDM source, fewer than 11% provide the extract lot number or describe its components in detail and almost 40% of articles did not cite the species of HDM extract (see Table E1 in this article's Online Repository at www.jacionline.org). Furthermore, HDM dose normalization in these studies can be based on protein content, antigen content, or dry extract weight. Because HDM extracts are prepared from live mites, and the allergen and endotoxin content can differ between preparations, we hypothesized that the response to extracts may differ among lots even when prepared from the same commercial supplier and used in the same exposure protocol.

As proof of concept, we started with 2 lots of HDM (HDM0 and HDM1) with similar concentrations of total protein, Der P1, and endotoxin units (Greer Laboratories, Lenoir, NC). Respiratory mechanics were evaluated after either acute or chronic administration of HDM. Mechanics parameters were then fit to 2 different mathematical models of the lung—the single-compartment model and the constant-phase model—to determine total respiratory system and compartmentalized responses, respectively (see this article's [Methods](#) section in the Online Repository at www.jacionline.org). When administered acutely (intranasal 100 µg 1×/wk for 3 weeks),

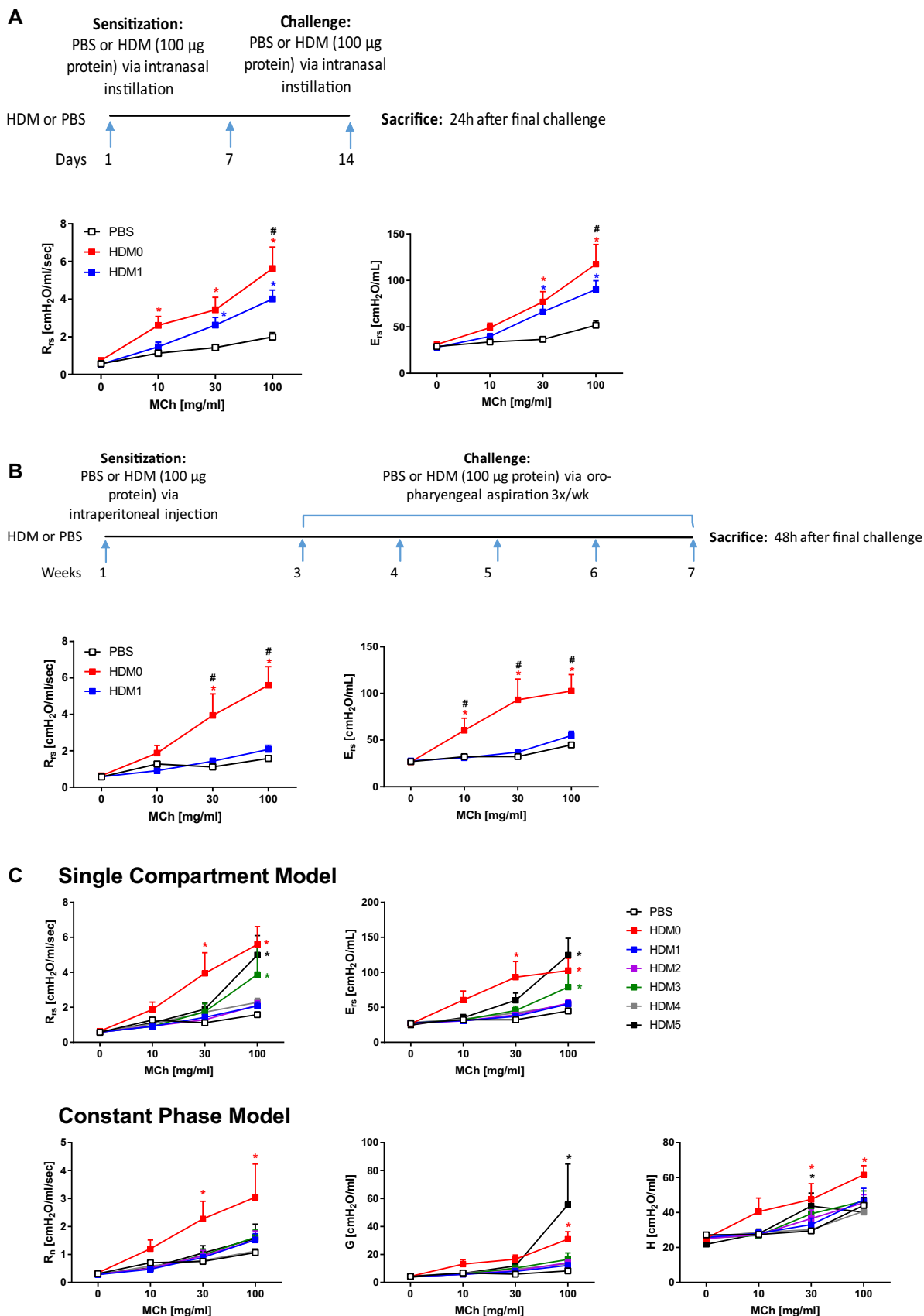


FIG 1. Physiological response to acute and chronic HDM exposure. **A**, Acute HDM challenge induced significant AHR. **B**, Chronic exposure to HDM0 induced significant AHR, whereas HDM1 did not develop AHR. **C**, Different lots of HDM induced variable effects on the development of AHR using 2 models of the lung. *MCh*, Methacholine. **P* < .05 vs PBS controls. #*P* < .05 vs HDM1. *n* = 6-12/group.

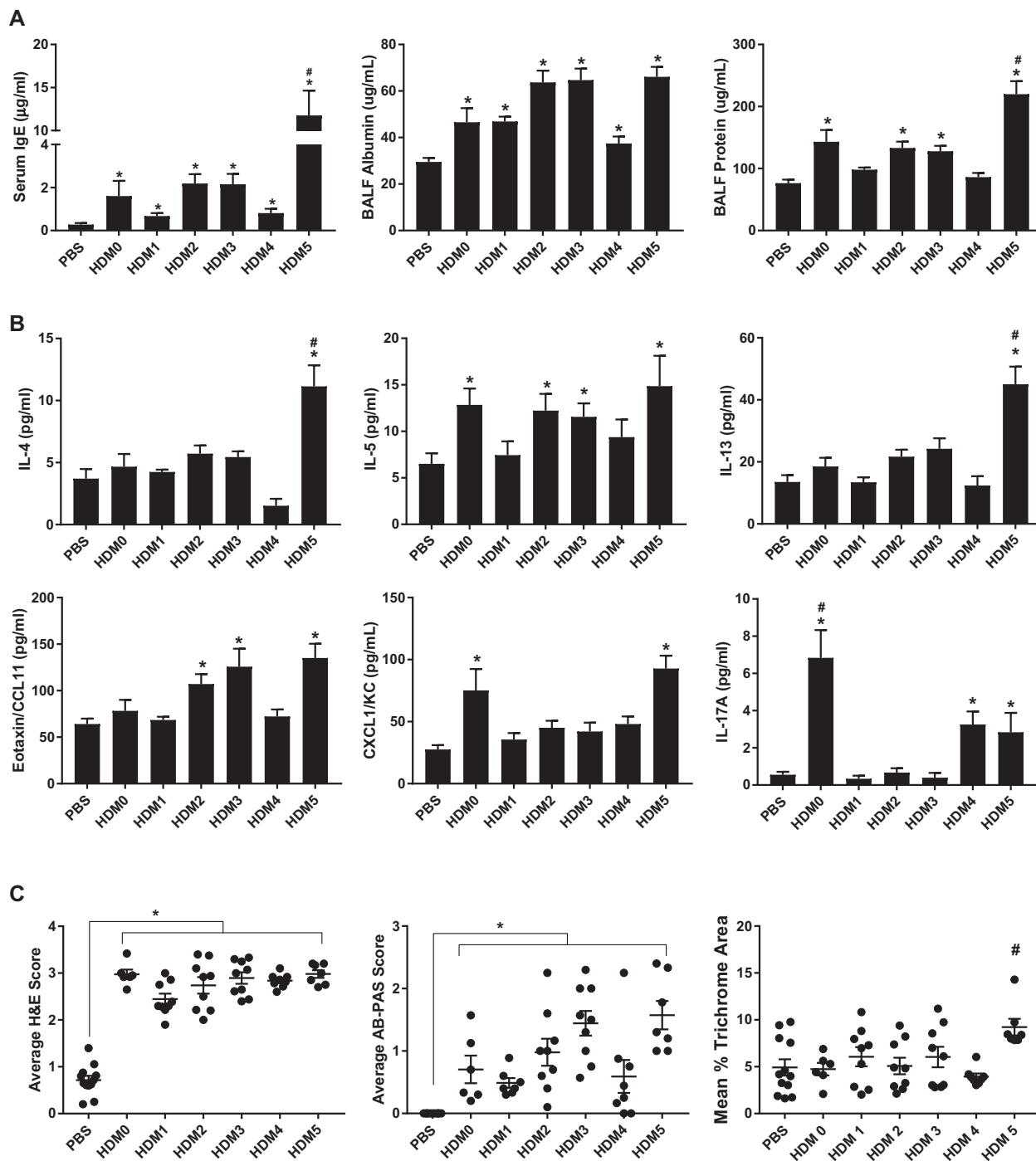


FIG 2. Chronic HDM exposure results in the development of allergic asthma. Markers of allergic asthma and lung injury (**A**) and proinflammatory cytokines/chemokines (**B**) were measured 48 hours after the final HDM challenge. Lung tissue inflammation, mucus secretion, and peribronchial collagen deposition (**C**) were determined from stained lung sections. AB-PAS, Alcian blue-periodic acid-Schiff; H&E, hematoxylin and eosin. * $P < .05$ vs PBS controls. # $P < .05$ vs all other groups. $n = 6$ -12/group.

both lots increased respiratory system resistance (R_{rs}) and elastance (E_{rs}) in response to methacholine challenge (Fig 1, A). Interestingly, after chronic exposure (oropharyngeal aspiration 100 μg 3×/wk for 5 weeks), HDM0 challenge resulted in AHR to methacholine, whereas HDM1 did not (Fig 1, B). Because of the striking differences in AHR, we evaluated respiratory

mechanics after chronic exposure to several HDM lots containing different concentrations of total protein, antigen, and endotoxin (see Table E2 in this article's Online Repository at www.jacionline.org). All lots induced AHR in the acute model (see Fig E1 in this article's Online Repository at www.jacionline.org). However, after chronic exposure, only half of the lots

caused AHR (Fig 1, C); moreover, the response magnitude and type were not equivalent. HDM0 and HDM5 increased total R_{rs} and E_{rs} . However, using the constant-phase model, we found that HDM0 resistance primarily localized to the central airways (R_n), whereas HDM5 localized to the peripheral airways and tissue (G). HDM3 induced significantly increased AHR in the single-compartment model but failed to show AHR in the constant-phase model. Furthermore, the airway response to chronic HDM exposure did not appear to correlate with either Der P1 or endotoxin levels, because the 3 lots that induced AHR contained different ratios of these components, and lots with similar ratios failed to develop AHR. To test whether the combination of antigen and endotoxin concentrations is necessary for AHR, we normalized the dose of HDM1 to contain equal Der P1 and endotoxin content to HDM0 (HDM0→HDM1) (see Table E3 in this article's Online Repository at www.jacionline.org). Chronic exposure to the normalized dose of HDM1 failed to rescue the AHR phenotype (see Fig E2 in this article's Online Repository at www.jacionline.org), suggesting that other components of the extract may be responsible for the development of AHR in this model. Another possible explanation is that the different lots of HDM induced different levels of sensitization. However, to directly address this question we would need to know the levels of HDM-specific IgE before the challenge phase and these lots are no longer available.

We then explored lot-dependent effects on chronic HDM-induced inflammation, allergic antibody production, and lung injury. All lots increased bronchoalveolar lavage fluid (BALF) cellularity compared with controls; however, the cell type varied greatly between groups (see Table E4 in this article's Online Repository at www.jacionline.org). Extracts with "high" Der P1 content increased eosinophils, whereas "high" endotoxin extracts increased neutrophils when compared with "low" Der P1 or endotoxin extracts, respectively. All HDM lots increased serum IgE and BALF albumin. However, HDM5 challenge resulted in the highest serum IgE and total protein in the BALF compared with the other HDM lots (Fig 2, A). Alternatively, chronic exposure to different HDM lots altered inflammatory mediator secretion (Fig 2, B). For example, HDM5 increased all measured cytokines/chemokines, but IL-5 was elevated only in other high Der P1-containing extracts (HDM0, HDM2, and HDM3). In addition, classic T2 immune response-mediated allergic asthma markers (eosinophilic airway inflammation, serum IgE, total protein, and IL-5) correlated with Der P1 concentration in each HDM dose (see Fig E3 in this article's Online Repository at www.jacionline.org). Alternatively, the endotoxin content associated only with the percentage of BALF neutrophils (data not shown). These results are consistent with the development of T_H1 and T_H2 responses after exposure to endotoxin and allergen challenge, respectively.

Histological evaluation of the lung tissue after chronic HDM exposure demonstrated significantly increased peribronchial inflammation and mucus secretion, regardless of the lot number compared with saline-challenged control animals (Fig 2, C). However, most lots did not induce airway fibrosis. Only HDM5 challenge resulted in increased peribronchial collagen-associated deposition, consistent with the elevated inflammation and robust AHR seen in these animals.

Commercially available samples of individual *Dermatophagoides pteronyssinus* HDM extracts typically report the total protein, Der P1 allergen, and endotoxin levels. However, extracts can also contain unreported levels of other Der P family allergens, as well as proteases, chitins, and β -glucans, which can stimulate innate immune system responses.⁹ Although markers of allergic inflammation correlated with Der P1 content and percent neutrophilia with endotoxin content, AHR and collagen deposition did not correlate with either Der P1 or endotoxin content. In addition, normalizing the extract dose to contain equal concentrations of Der P1 and endotoxin did not reciprocate inflammatory or AHR responses. These data suggest that components other than Der P1 or endotoxin within the HDM extract lots may contribute to chronic HDM airway responses, and highlight an important area for future mechanistic studies.

In conclusion, although allergen extracts are becoming a powerful tool for studying allergy, asthma, and airway disease in animal models, our data demonstrate that these extracts are complex mixtures that can result in variable phenotypic responses, and studies should proceed with caution. Because of the variability in components within the extracts, it is important to describe both the lot characteristics and the methods used to normalize dosing so that concentrations of specific components can be calculated and compared between studies. In addition, thorough characterization of additional lot components may be needed for mechanistic studies. Rigorous reporting of these parameters will not only provide transparency to the research but also allow for more accurate comparison of results and reproducibility of published work.

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Unconventional ST2- and CD127-negative lung ILC2 populations are induced by the fungal allergen *Alternaria alternata*



To the Editor:

In the last decade, group 2 innate lymphoid cells (ILC2s) have been recognized for their ability to drive type 2 responses in experimental animal models in the absence of T cells.^{1,2} When mice are intranasally challenged with *Alternaria alternata*, a fungal allergen associated with severe asthma and fatal exacerbations in humans,³ ILC2s are potently activated via the IL-33/ST2 (IL-33R) axis to robustly secrete type 2 cytokines.⁴⁻⁶ Animal models, including *A alternata*-induced lung inflammation in mice, have provided critical insight into pathways of ILC2 regulation that appear to apply to ILC2 responses in human disease.⁷ Thus, accurate identification of ILC2s in the lungs of mice is critical to future understanding of their biology and contribution to allergic disease. We assessed whether the conventional ILC2 identification markers ST2 (IL-33R) and CD127 (IL-7R) sufficiently include all type 2 cytokine-producing ILCs in the mouse lung.

Wild-type (WT) mice were challenged intranasally with *A alternata* for 3 consecutive days, and 24 hours after the last challenge, bronchoalveolar lavage (BAL) and lung tissue were harvested. Mice challenged with *A alternata* demonstrated increased peribronchial inflammation (Fig 1, A), robust BAL and lung eosinophil recruitment (Fig 1, B), as well as elevated BAL IL-5 and IL-13 levels (Fig 1, C). Conventional ILC2s were identified as CD45⁺Lineage⁻Thy1.2⁺ST2⁺CD127⁺ lymphocyte-sized cells (Fig 1, D; Fig E1, A; see this article's [Methods section](#) in the Online Repository at www.jacionline.org). As expected, total conventional ILC2s expanded after *A alternata* challenge (Fig 1, E). Unexpectedly, ILC populations lacking either ST2 or CD127 or both proliferated significantly, with reduced apoptosis, and represented approximately 40% of the total ILC population (Fig 1, E; see Fig E1, B, in this article's Online Repository at www.jacionline.org). All ILC subsets varied within 10% before, during, and after *A alternata* (Fig E1, C) challenges and also displayed robust increases in IL-5 (Fig 1, F and G; see Fig E1, D), IL-13 (see Fig E2, A, in this article's Online Repository at www.jacionline.org), and GATA-3 (Fig 1, G; see Fig E2, B) expression. Furthermore, all 4 subpopulations also highly expressed CD25 (IL-2R α), killer lectin like receptor G1 (KLRG1), inducible T-cell costimulator (ICOS), stem cell antigen-1 (Sca-1), c-Kit, CD44, and CD69, and expression of these markers (except c-Kit) was upregulated by *A alternata* (see Fig E3 in this article's Online Repository at www.jacionline.org). RAG2 knockout mice that lack B and T cells but have ILCs showed similar ILC subset activation after *A alternata* challenges (see Fig E4 in this article's Online Repository at www.jacionline.org). Overall, though conventional ILC2s

represented the highest total Th2 cytokine-producing ILCs, the sum of the 3 unconventional populations in WT mice approximated 40% of the total ILC2 burden.

To confirm that processing for ILC cytokines using calcium ionophore (see this article's [Methods section](#)) did not influence the presence of IL-5 production by the different ILC subsets, we performed *A alternata* challenges in heterozygous IL-5 reporter (Red5) mice. CD45⁺Lineage⁻Thy1.2⁺ lymphocytes were gated, and all subsets (based on ST2 and CD127) showed significant increases in IL-5 expression after *A alternata* challenges (Fig 1, H), though the 2 ST2⁻ populations showed the lowest overall % IL-5 production, suggesting a heterogeneous mix of ILCs in these subsets. Using the same mice, we found that unconventional ILC2s represented approximately 25% of all IL-5⁺ CD45⁺Lineage⁻Thy1.2⁺ cells (Fig 1, I). Thus, in WT mice and reporter mice, 25% to 40% of ILC2s are negative for either ST2 or CD127 or both.

In light of recent reports of ILC plasticity,^{8,9} we investigated whether *A alternata* also induced ILC1 and ILC3 responses. Surprisingly, IFN- γ was detected in the BAL and increased significantly after *A alternata* challenge (see Fig E5, A, in this article's Online Repository at www.jacionline.org) though IL-17A was not detected (not shown). IFN- γ ⁺ ILCs were increased in percent and total numbers (Fig E5, B), whereas IL-17A⁺ ILCs were largely increased in total numbers only in challenged mice (Fig E5, C). We found that conventional and unconventional ILC2s expressed similar levels of the ILC1 surface marker IL-18R,⁹ and expression was nearly doubled after *A alternata* challenge (Fig E5, D). All 4 populations also expressed low levels of ROR γ t (Fig E5, E) and T-bet (Fig E5, F) relative to GATA-3, and expression was not significantly different after *A alternata* challenge. To elucidate whether the mixed ILC phenotypes were due to hybrid cells or heterogeneous populations, we investigated the simultaneous expression of ILC1, ILC2, and ILC3 cytokine production (see Fig E6, A and B, in this article's Online Repository at www.jacionline.org). We detected low levels of IL-5⁺IFN- γ ⁺ ILC2s and IL-5⁺IL-17A⁺ ILC2s in each population, and the frequency of double-positive cells within each ILC2 subpopulation increased with *A alternata* challenge. IL-17⁺ cells largely appeared to be distinct from IL-5⁺ cells though overall IL-5⁺IFN- γ ⁺ and IL-5⁺IL-17A⁺ ILC2s were increased after *A alternata* challenge (Fig E6, A and B). In aggregate, these results suggest that the ILC populations contain a heterogeneous mixture of hybrid ILCs and distinct ILC1s and ILC3s.

We next compared these ILC populations using RNA sequencing and quantitative PCR with fluorescence-activated cell-sorted lung ILC subsets (see Fig E7, A, in this article's Online Repository at www.jacionline.org; see this article's [Methods section](#)) from mice challenged with *A alternata*. In agreement with our flow cytometry protein-level data, we found high levels of ILC2-defining surface markers, transcription factors, and cytokine transcripts expressed by all 4 subpopulations, relative to ILC1 and ILC3 signatures (Fig 2, A and B). Importantly, RNA-Seq and quantitative PCR confirmatory studies demonstrated that the ST2⁻ populations express reduced levels of Il1rl1 transcript (encodes ST2), and the CD127⁻ populations express less Il7ra transcript (Fig E7, B and C). Consistent with the protein data, the ST2⁺ populations expressed more ILC2-related transcripts (Gata3, Il5, Il9, Il13, and Rora) compared with the ST2⁻ counterparts. The ST2⁺CD127⁻ population overall expressed the greatest number of ILC2-related transcripts, whereas the

METHODS

Experimental animals

Male C57BL/6J mice, 6- to 8-week old, were purchased from Jackson Laboratory (Bar Harbor, Me). All animal experiments were conducted in accordance with the American Association for the Accreditation of Laboratory Animal Care guidelines and approved by the Duke University Institutional Review Board and Institutional Animal Care and Use Committee.

Development of allergic airway disease

Allergic airway disease was induced using either an acute or a chronic HDM exposure model. For the acute model, mice were challenged with 50 μ L of HDM (100 μ g total protein) or saline intranasally on days 1, 7, and 14 under light isoflurane anesthesia. Airway physiology measurements and tissue harvests were conducted on day 15. For the chronic model, mice were sensitized with an intraperitoneal injection of HDM (100 μ g total protein) or saline on day 1 in a volume of 200 μ L. Starting on day 14, mice were challenged with HDM (100 μ g total protein) or saline in a 50- μ L volume by oropharyngeal aspiration under light isoflurane anesthesia 3 times a week for a total of 5 weeks as previously described.^{E1} Airway physiology measurements and tissue harvests were conducted 48 hours after the last challenge. Different lots of *Dermatophagoides pteronyssinus* extract (XPB70D3A2.5; Greer Laboratories) were compared.

Airway physiology measurements

Airway responsiveness to acetyl- β -methylcholine (Sigma, St Louis, Mo) was measured 24 to 48 hours after the final HDM exposure using a computer-controlled small animal ventilator (FlexiVent FX, Scireq, Montreal, Quebec, Canada). Mice were anesthetized with urethane (1-2 g/kg; Sigma), tracheotomized and intubated with a metal endotracheal tube (18G blunt needle), placed on a 37°C water-heated pad, and mechanically ventilated at a rate of 150 breaths/min with a tidal volume of 10 mL/kg and a positive end-expiratory pressure of 3 cm H₂O. To block spontaneous respiratory effort, mice were given pancuronium bromide intraperitoneally (0.8 mg/kg; Sigma) 5 minutes before assessment of airway responses. To measure respiratory mechanics, both 1-second single-frequency and 3-second multifrequency forced oscillation waveforms were applied using the Flexiware 7.6 software default mouse inhaled dose-response script. The resulting pressure, volume, and flow signals were fit to 2 different mathematical models of the lung (single compartment and constant phase) to determine total respiratory system resistance (R_{rs}) and elastance (E_{rs}) or Newtonian resistance (R_n), tissue damping (G), and tissue elastance (H), respectively (as previously described^{E2,E3}) using the Scireq Flexiware 7.6 software (SCIREQ, Montreal, Quebec, Canada). Methacholine (10-100 mg/mL) was aerosolized using the FlexiVent FX aeroneb nebulizer attachment. The peak response at each dose was averaged and graphed along with the average peak baseline measurement for each group.

BALF and analysis

Lungs were washed 3 times with PBS + 100 μ M EDTA + 40 μ M DTPA (Diethylenetriaminepentaacetic acid) via gravity inflation and withdraw with a syringe inserted into a side port of the tracheal tubing. BALF was spun to remove cells and then albumin and total protein were measured using a Mouse Albumin ELISA kit (Immunology Consultants Laboratory, Inc, Portland, Ore) and a Pierce BCA protein Assay kit (Thermo Scientific, Hercules, Calif), respectively. The remaining BALF was concentrated to 0.25 mL using a centrifugal filter (Millipore) and cytokines were measured using a custom Procartaplex (Invitrogen, Carlsbad, Calif). Cell pellets were treated with RBC lysis buffer (BioLegend, San Diego, Calif) and then resuspended in 1 mL HyClone (Thermo Scientific). Total cells were counted with a Scepter Handheld

Cell Counter (Millipore, Burlington, Mass). Differential cell counts were obtained under light microscopy after staining with Hema 3 solution (Protocol, Kalamazoo, Mich).

Histological analysis

Left lung lobes were isolated and fixed by gravity inflation (20 cm H₂O) and immersion in 10% formalin. Lungs were paraffin-embedded, cut into 5- μ m sections, and stained by AML Laboratories (Saint Augustine, Fla). After individual staining, 10 images of randomly chosen variable size airways were photographed at 20 $\times$ magnification and analyzed in a blinded examination. Lung tissue inflammation was semiquantitatively determined from hematoxylin and eosin and Alcian blue-periodic acid-Schiff-stained sections using a 4-tiered scoring system as previously described.^{E4,E5} For hematoxylin and eosin-stained slides, normal lung was given a score of grade 0 (no inflammation). Grade 1 was given to those fields that displayed slight inflammation, with cells scattered in the peribronchiolar region but were scattered and not so numerous that they created any depth. Grade 2 (mild) was given when there was a mild inflammation, with inflammatory cells appearing in the peribronchiolar region to such a degree that they formed a layer of cells around the bronchiole but were not so thick that they engulfed the blood vessels. A score of grade 3 (moderate) was assigned to those sections that had more disseminated peribronchiolar/perivascular inflammatory infiltrates no more than 3 cells deep and formed a contiguous layer of cells that surrounded the bronchiole and the blood supply. The grade was advanced to 4 (severe) when inflammatory infiltrates 4 or more cells deep created a thick layer surrounding the bronchiole and continued around the vasculature. For Alcian blue-periodic acid-Schiff-stained slides, mucin production was semiquantitatively determined using a scale ranging from 0 to 4, where 0 indicated no staining within the airway epithelium, 4 indicated greater than 75% airway epithelium staining, and scores 1, 2, and 3 represented up to 25%, 50%, and 75% staining, respectively.

To visualize collagen deposition, sections were stained with Masson's trichrome stain as previously described.^{E4} Briefly, images of 10 stained airway tissue cross sections per mouse were imported into ImageJ software (National Institutes of Health, Bethesda, Md) and relative quantities of Masson's trichrome staining were determined by calculating the percentage of peribronchial staining within the total tissue area, after subtracting the area of the lumen of the airway.

Statistics

Data were analyzed using GraphPad Prism 7 software (GraphPad Software Inc, San Diego, Calif). Complex data sets were analyzed using either 1-way or 2-way ANOVA, for comparing column or grouped data, respectively, followed by multiple comparison analysis. Correlations were determined using a linear regression model. The level of significance for all tests was set at P less than .05.

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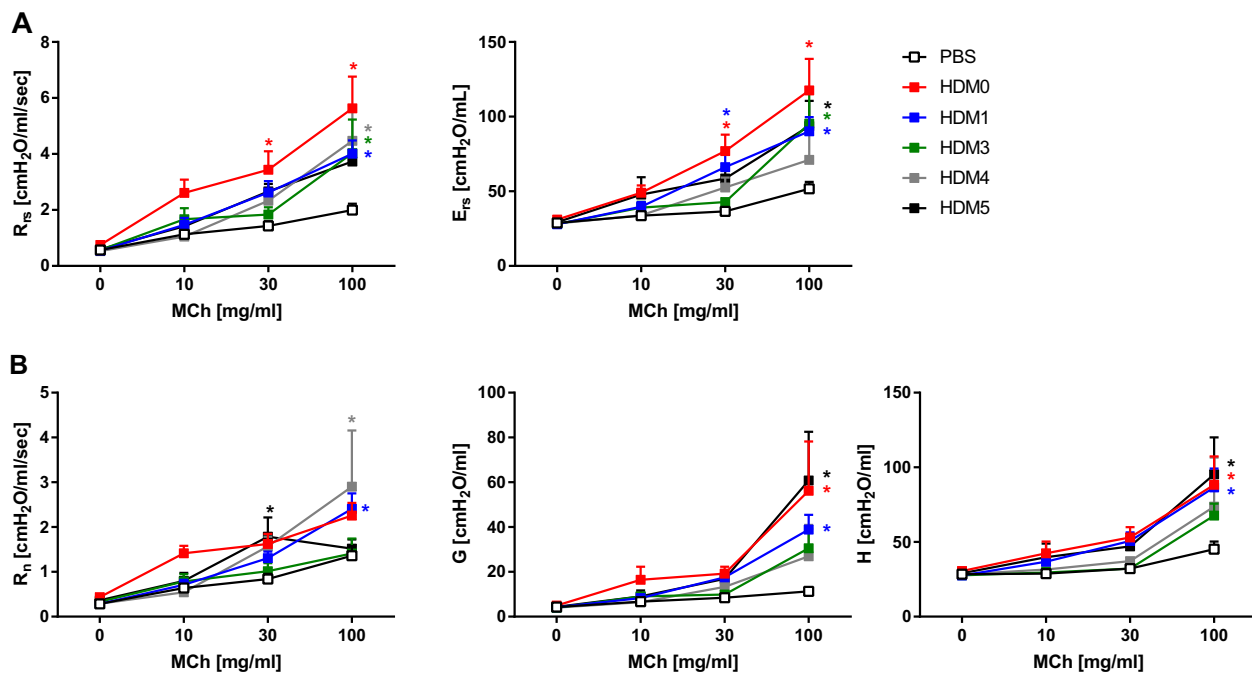


FIG E1. Lung mechanics response to acute HDM exposure. Using both the single-compartment (**A**) and constant-phase (**B**) models, lung function assessment demonstrated that 6 different lots of HDM induced similar effects on the development of AHR. All lots induced significant AHR compared with PBS-challenged controls, although the degree of responsiveness was not equal. * $P < .05$ compared with PBS controls; $n = 4-16$ /group.

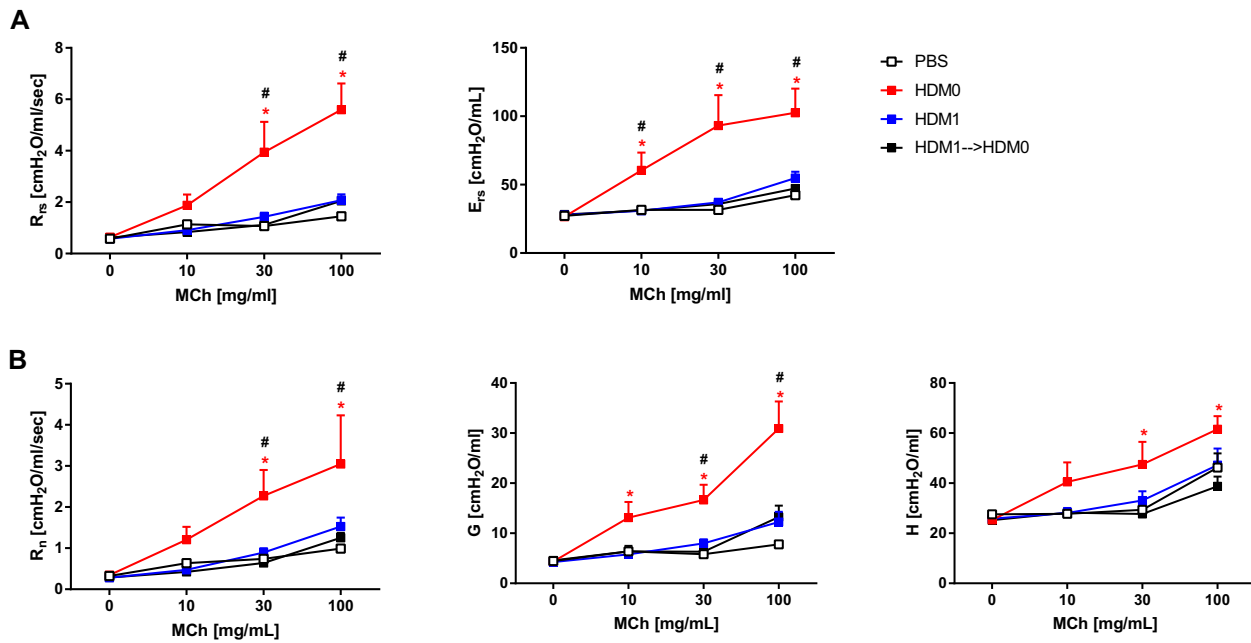


FIG E2. Normalization of HDM1 to HDM0 does not rescue AHR phenotype. Normalizing the dose of HDM1 to contain the same amount of Der P1 antigen and endotoxin content as HDM0 did not affect the development of AHR in either the single-compartment model (**A**) or the constant-phase model (**B**) of the lung. * $P < .05$ compared with PBS controls. # $P < .05$ compared with all other groups; $n = 5$ -9/group.

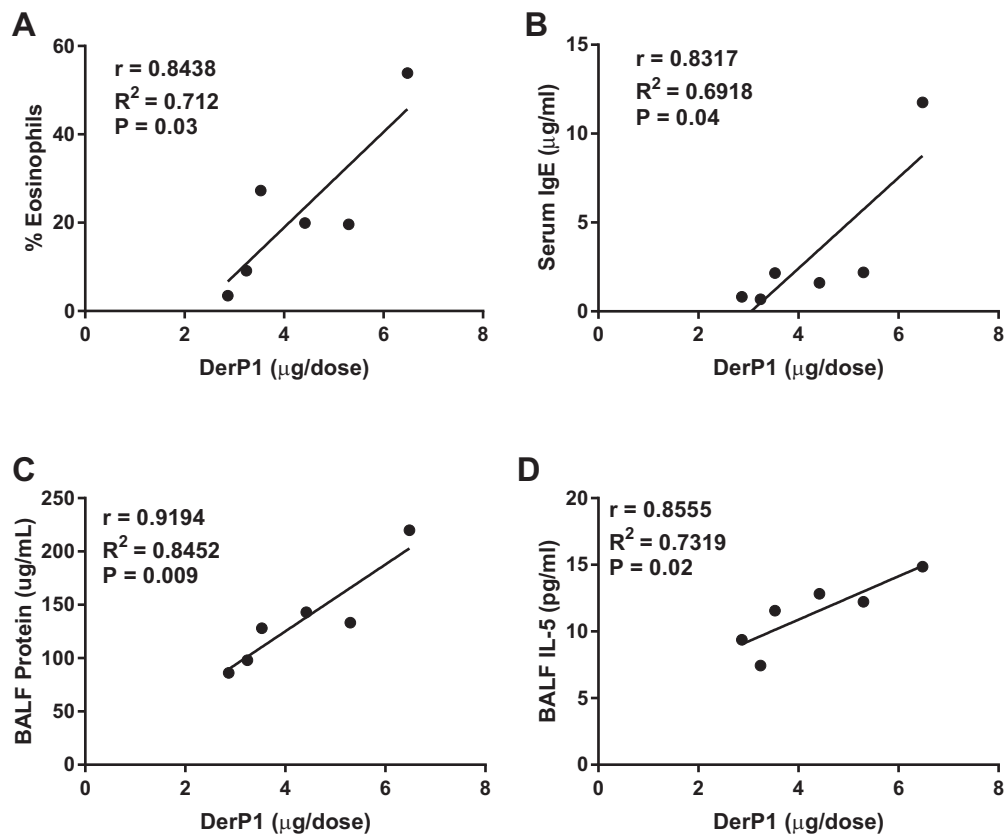


FIG E3. Increase in type 2 immune response–mediated allergic asthma markers is significantly correlated with antigen content of HDM extract. Percent eosinophilic inflammation (**A**), serum IgE (**B**), BALF protein (**C**), and BALF IL-5 (**D**) increase as the amount of Der P1 present in the HDM extract is increased.

TABLE E1. PubMed articles from 2016 to 2018 reporting murine chronic HDM protocols

Articles	HDM species			Lot no. reported	Normalization of dose		
	Der P	Der F	Unreported		Protein	Antigen	Weight
Articles (140)	56	31	55	15	15	5	118
%	40.0	22.14	39.28	10.71	10.71	3.57	84.29

TABLE E2. Comparison of Greer HDM lot numbers

HDM reference no.	HDM lot no.	Total protein (mg/vial)	Der P1 (μg/vial)	Endotoxin (EU/vial)	@2 mg/mL total protein		In 50-μL dose @ 2 mg/mL	
					Der P1 (μg/mL)	Endotoxin (EU/mL)	Der P1 (μg)	Endotoxin (EU)
HDM0	253983	3.22	142.31	36250	88.39	22515.5	4.42	1125.78
HDM1	296771	3.49	113.21	28750	64.88	16475.64	3.24	823.78
HDM2	305470	2.16	114.57	805	106.08	745.4	5.30	37.72
HDM3	317430	3.75	132.57	880	70.7	469.3	3.53	23.46
HDM4	280245	3.51	100.89	25750	57.49	14672.36	2.87	733.62
HDM5	326779	2.26	146.45	812.5	129.60	719.03	6.48	35.95

TABLE E3. Normalization of HDM1 to HDM0

HDM reference no. (lot no.)	Total protein (mg/vial)	Der P1 (μ g/vial)	Endotoxin (EU/vial)	@2 mg/mL total protein		In 50- μ L dose @ 2 mg/mL	
				Der P1 (μ g/mL)	Endotoxin (EU/mL)	Der P1 (μ g)	Endotoxin (EU)
HDM0 (253983)	3.22	142.31	36250	88.39	22515.5	4.42	1125.78
HDM1 (296771)	3.49	113.21	28750	64.88	16475.64	3.24	823.78
@2.73 mg/mL total protein							
HDM1 $\rightarrow$ HDM0				88.44	22460.9	4.42	1123.05

TABLE E4. BALF percent cell type

	PBS	HDM0	HDM1	HDM2	HDM3	HDM4	HDM5
%Macrophage	96.25*	48.52	66.89 ^{†‡§}	55.21 [§]	48.42	62.75 ^{‡§}	35.25
%Neutrophil	1.61	23.38 ^{‡§ ¶#}	7.45	2.35	1.16	19.90 ^{‡§ #}	2.52
%Eosinophil	0.37	19.92 ^{**}	9.13	19.65 ^{**}	27.28 ^{¶**}	3.47	53.86*
%Lymphocyte	1.77	8.19	16.53	22.77 [§]	23.14 ^{†§}	13.88	8.36

*All other groups.

[†]HDM0.

[‡]HDM3.

[§]HDM5.

^{||}Significantly greater than PBS.

[¶]HDM1.

[#]HDM2.

^{**}HDM4.