In our cohort of patients with severe asthma, the majority receiving very high doses of inhaled or systemic corticosteroids, a blood eosinophil count of greater than 0.45×10^9 cells/L can correctly predict sputum eosinophilia in 9 of 10 cases. We have included only patients with severe asthma in our study, and therefore we believe our results are important because these are the patients who will be considered for novel T_H2-targeting biologic agents.

The search for biomarkers of airway inflammation has been made more urgent by the advance of novel therapies that act specifically by targeting this inflammation. To this end, recent studies that aimed to investigate the potential benefit of mepolizumab in patients with eosinophilic asthma identified patients by using blood eosinophil cutoffs as low as 0.15×10^9 cells/L.[7,8] As demonstrated both here and in the article from the Severe Asthma Research Program group,[4] this cutoff would include many patients who in fact do not have contemporaneous sputum eosinophilia (more than half in this study) and therefore potentially would not be in the group of patients who could benefit from treatment. The consequence of this in a trial of a biologic agent is that the magnitude of benefit could be underestimated or even missed, as happened previously when mepolizumab was trialed in unselected patients.[9] The other potential consequence of using blood eosinophil counts as a surrogate for sputum is the very high risk of false-negative results; from our data, approximately 1 in 5 patients without blood eosinophilia had airway eosinophilia and hence could be missed by using this selection method. Choosing a simple and universally available biomarker (blood eosinophil counts) is attractive, but in this case does not lead to a personalized medicine approach.

In conclusion, we have shown that using a cutoff of 0.45×10^9 cells/L for blood eosinophilia can usefully predict airway eosinophilia in patients with severe asthma receiving high levels of treatment. However, we agree with Hastie et al[3] that lower cutoffs are not useful. We would propose that study design for novel antieosinophil therapies could use this higher cutoff to investigate the effect of these therapies but that patients should also be included with airway but not blood eosinophilia, as previously described.[8] In the meantime, more work needs to be done to develop useful minimally invasive and accurate surrogate markers for airway inflammation, including composite biomarkers comprising, for example, exhaled nitric oxide and serum periostin levels together with blood eosinophil counts.

Stephen J. Fowler, MD[a,b]
Gaël Tavernier, PhD[a]
Robert Niven, MD[a]

From [a]the University of Manchester, Manchester Academic Health Science Centre, and NIHR Translational Research Facility in Respiratory Medicine, University Hospital of South Manchester, Manchester, United Kingdom, and [b]Lancashire Teaching Hospitals NHS Foundation Trust, Preston, United Kingdom. E-mail: stephen.fowler@manchester.ac.uk.
Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

REFERENCES
1. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002;360:1715-21.
2. Nair P, Pizzichini MM, Kjarsgaard M, Inman MD, Efthimiadis A, Pizzichini E, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. N Engl J Med 2009;360:985-93.
3. Hastie AT, Moore WC, Li H, Rector BM, Ortega VE, Pascual RM, et al. Biomarker surrogates do not accurately predict sputum eosinophil and neutrophil percentages in asthmatic subjects. J Allergy Clin Immunol 2013;132:72-80.
4. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, et al. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. Am J Respir Crit Care Med 2009;180:59-99.
5. Fulkerson PC, Rothenberg ME. Targeting eosinophils in allergy, inflammation and beyond. Nat Rev Drug Discov 2013;12:117-29.
6. Pizzichini E, Pizzichini MM, Efthimiadis A, Hargreave FE, Dolovich J. Measurement of inflammatory indices in induced sputum: effects of selection of sputum to minimize salivary contamination. Eur Respir J 1996;9:1174-80.
7. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. Lancet 2012;380:651-9.
8. Ortega HG, Liu MC, Pavord ID, Brusselle GG, FitzGerald JM, Chetta A, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. N Engl J Med 2014;371:1198-207.
9. Flood-Page P, Swenson C, Faiferman I, Matthews J, Williams M, Brannick L, et al. A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. Am J Respir Crit Care Med 2007;176:1062-71.

Vitamin D enhances production of soluble ST2, inhibiting the action of IL-33

To the Editor:

Vitamin D insufficiency is an environmental factor that has been strongly associated with asthma and its severity.[1] The genes *IL33* and *IL1RL1* have been repeatedly identified as predisposing to asthma risk in genome-wide association studies.[2] IL-33 is an alarmin cytokine that acts on multiple pulmonary cell types, including T_H2 lymphocytes, mast cells, and innate lymphoid cells, to promote T_H2-type cytokine secretion and airway inflammatory responses of the kind observed in asthmatic patients.[3,4] The receptor for IL-33 is encoded by *IL1RL1*; differential splicing of the gene can produce a functional membrane-bound receptor (ST2L) or a soluble decoy receptor (sST2).[5] Therefore we investigated whether *IL1RL1* is regulated by vitamin D in cells relevant to asthma. We did this first by measuring gene expression by means of quantitative real-time PCR with 2 different TaqMan probe sets: Hs01073300, which detects mRNA splice variants encoding both the membrane-bound and soluble receptors (the total mRNA for *IL1RL1*), and Hs00249389, which detects mRNA only for the splice variant encoding the membrane-bound receptor ST2L (Fig 1, *A*). Methods are provided in this article's Online Repository at www.jacionline.org.

Both probe sets display similar efficiency of amplification (data not shown). Human primary bronchial epithelial cells (HBECs), CD4 lymphocytes, CD8 lymphocytes, eosinophils, and LUVA mast cells were cultured in the presence or absence of 1α,25-dihydroxyvitamin D3 (1,25[OH]D3), the active form of vitamin D (Fig 1, *B-D*, and see Fig E1, *A* and *B*, in this article's Online Repository at www.jacionline.org). Addition of 1,25(OH)D3 significantly increased the total number of *IL1RL1* mRNA transcripts expressed by HBECs and CD4 and CD8 lymphocytes, as measured by using the Hs01073300 probe set. However, 1,25(OH)D3 did not significantly increase expression of *IL1RL1* mRNA transcripts by primary eosinophils and LUVA mast cells, despite it significantly increasing expression of the
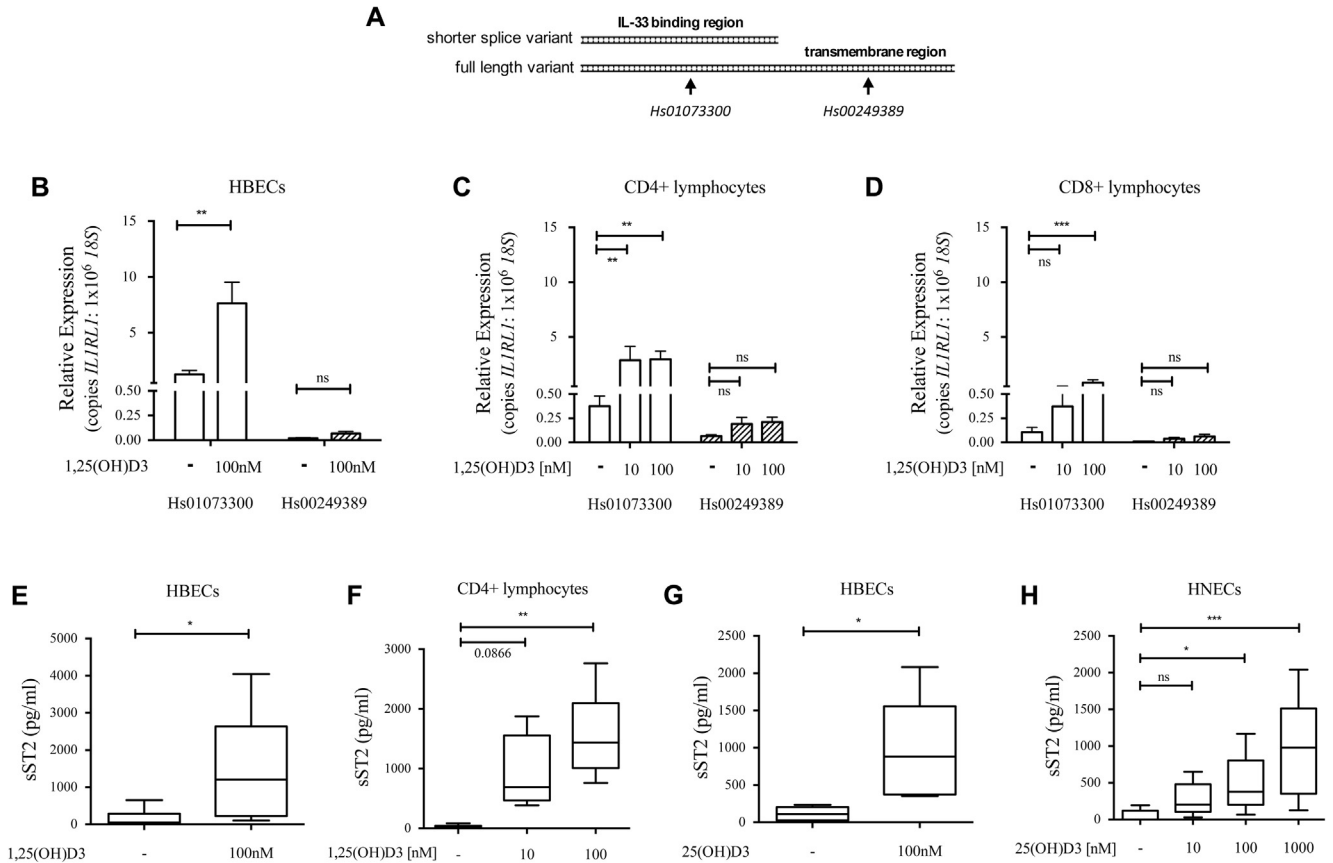
FIG 1. Vitamin D induces expression of sST2. **A,** Schematic for *IL1RL1* mRNA: the 2 splice variants are differentially detected in PCR by using the probe sets Hs01073300 and Hs00249389. **B-D,** *IL1RL1* transcripts detected by each probe set in cultures of HBECs at 24 hours (Fig 1, *B*; n = 7) and CD4 (Fig 1, *C*) and CD8 (Fig 1, *D*) lymphocytes at 7 days (n = 6). **E-H,** sST2 protein in culture supernatants of HBECs at 24 hours (Fig 1, *E*; n = 6), CD4 lymphocytes at 7 days (Fig 1, *F*; n = 6), and HBECs (Fig 1, *G*; n = 6) and HNECs at 24 hours (Fig 1, *H*; n = 5). *ns*, Not significant. *$P \leq .05$, **$P \leq .01$, and ***$P \leq .001$.

gene cathelicidin antimicrobial peptide *(CAMP)*, which is known to be induced by vitamin D (see Fig E1, *A* and *B*).

Higher *IL1RL1* expression levels were detected with the Hs01073300 probe set than with the Hs00249389 probe set in HBEC and lymphocyte cultures. Although 1,25(OH)D3 increased the total copies of *IL1RL1* mRNA measured by using the Hs01073300 probe set in these cell types, it did not increase the number of transcripts encoding ST2L detected with Hs00249389, indicating that vitamin D selectively upregulates the expression of mRNA for the soluble decoy receptor sST2. sST2 concentrations in culture supernatants were measured by means of ELISA to confirm the findings of gene expression studies at the protein level. sST2 concentrations were significantly increased by 1,25(OH)D3 in both HBEC and CD4 lymphocyte cultures (Fig 1, *E* and *F*). In contrast, CD8 lymphocytes produced less than 200 pg/mL sST2 in all conditions, and there was no evidence of upregulation by 1,25(OH)D3 (data not shown).

Vitamin D circulates primarily as the inactive precursor 25-hydroxyvitamin D3 (25[OH]D3); however, epithelial cells constitutively express CYP27B1, the enzyme that converts 25(OH)D3 to 1,25(OH)D3.[6] HBECs cultured with 100 nmol/L 25(OH)D3, a physiologic concentration, increased expression of *IL1RL1* (see Fig E1, *C*) and produced significantly greater amounts of sST2 (Fig 1, *G*). Primary human nasal epithelial cells (HNECs) cultured

with a similar concentration range of 25(OH)D3 also showed a clear concentration-dependent increase in sST2 production (Fig 1, *H*). CD4 lymphocytes did not respond to 25(OH)D3, which is consistent with their lower expression of CYP27B1 (see Fig E1, *D*).

An IL-33 sensitive bioassay was developed using the LUVA mast cell line to test the biological activity of sST2. Twenty-four hours of exposure of LUVA cell cultures to IL-33 resulted in marked homotypic aggregation, which was associated with increased expression of CD54 (intercellular adhesion molecule 1; see Fig E2, *A* and *B*, in this article's Online Repository at www.jacionline.org). IL-33 increased CD54 expression on LUVA cells in a concentration-dependent manner (Fig 2, *A*). Other stimuli can also induce mast cell CD54 expression, and a similar concentration-dependent induction of CD54 expression was achieved with a combination of IFN-γ and IL-4 (Fig 2, *A*). Treatment of LUVA cells with a recombinant sST2-Fc chimera significantly inhibited IL-33–induced CD54 expression ($P = .0076$; Fig 2, *B*, and see Fig E2, *C*) but not IFN-γ plus IL-4–induced CD54 expression (see Fig E2, *D*). Addition of 1,25(OH)D3 itself to LUVA cell cultures did not significantly alter constitutive or induced CD54 expression ($P > .05$; see Fig E2, *E*), as previously reported for another mast cell line.[7]

To examine the effect of vitamin D–enhanced epithelial sST2 production on the IL-33 bioassay, HNECs were cultured in the
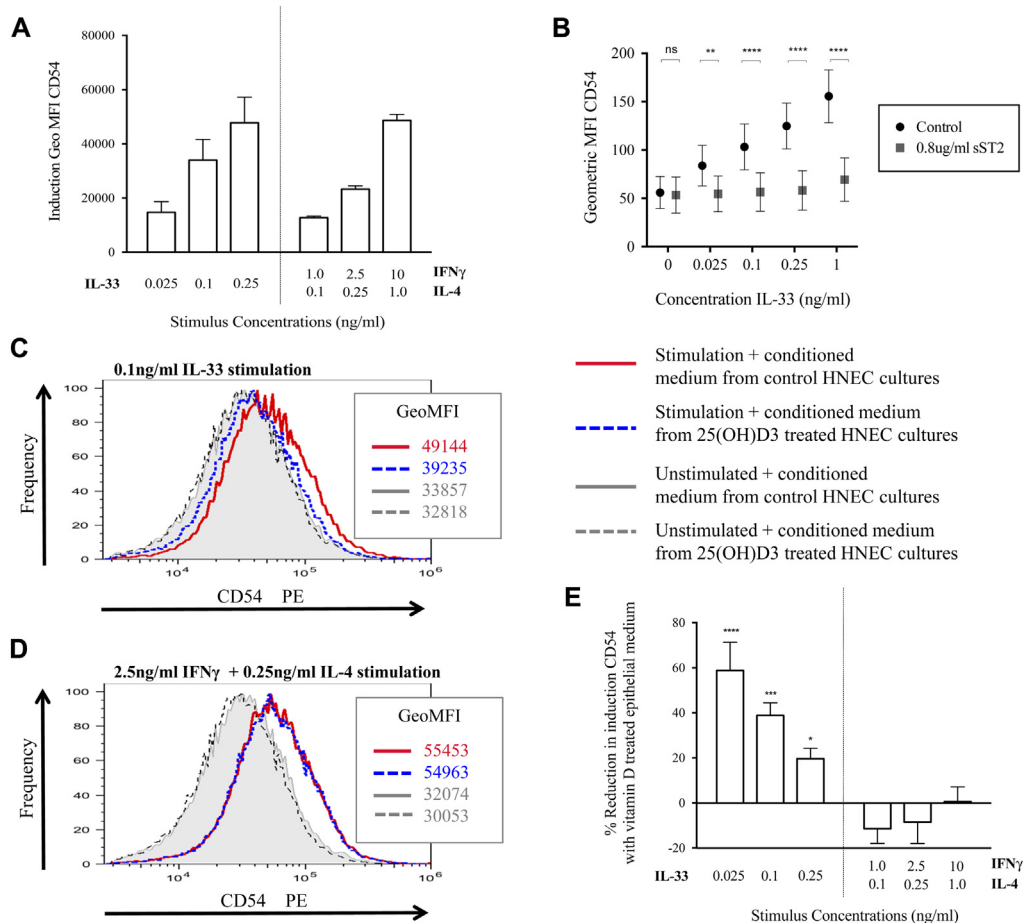
FIG 2. Conditioned medium from vitamin D–treated epithelial cultures inhibits IL-33–induced expression of CD54 by LUVA cells. **A,** Induction of CD54 on LUVA cells above that of unstimulated cells by different stimuli in the presence of conditioned medium from control epithelial cell cultures (n = 7). **B,** Effect of IL-33 on CD54 expression is inhibited by recombinant sST2 (n = 6). **C** and **D,** CD54 expression on LUVA cells cultured with conditioned medium with or without cell stimulation. **E,** Percentage reduction in stimuli-induced CD54 expression on LUVA cells cultured with conditioned medium from 25(OH)D3-treated epithelial cell cultures compared with control epithelial cell cultures (n = 7). *MFI,* Mean fluorescence intensity; *ns,* not significant. *$P \leq .05$, **$P \leq .01$, ***$P \leq .001$, and ****$P \leq .0001$.

presence or absence of 25(OH)D3 for 48 hours, and then the supernatants were added to cultures of LUVA cells stimulated with concentrations of IL-33 or IFN-γ plus IL-4 that induced comparable CD54 expression. Conditioned medium from vitamin D–treated epithelial cells significantly reduced IL-33–induced CD54 expression on LUVA cells compared with conditioned medium from matched epithelial cell cultures not treated with vitamin D ($P = .015$; Fig 2, *C* and *E*). In contrast, there was no significant difference in IFN-γ plus IL-4–induced CD54 expression between LUVA cells cultured with the different epithelial cell–conditioned media ($P > .05$; Fig 2, *D* and *E*). Although mediators in the epithelial cell–conditioned medium other than sST2 might conceivably have affected LUVA cell CD54 expression, the selective effect of vitamin D treatment on IL-33–induced CD54 expression suggests that the sST2 in the vitamin D–treated epithelial cell supernatants is biologically active.

The capacity of IL-33 to induce production of T_H2-type proinflammatory cytokines by multiple cell types likely underpins the reported genetic associations of *IL33* and *IL1RL1* with asthma. Here we report the novel finding that 100 nmol/L vitamin D is

able to augment expression by epithelial cells and lymphocytes of the soluble decoy receptor sST2, which in turn inhibits the actions of IL-33. Importantly, this effect occurs at physiologic concentrations (vitamin D sufficiency is defined as a serum 25[OH]D level of 75 to 150 nmol/L), and similar concentrations of 1,25(OH)D3 have been shown to be able to be generated from 25(OH)D3 in culture.[1] We hypothesize that the capacity of vitamin D to augment the synthesis of an inhibitor of IL-33 *in situ* in the airways mucosa is of potential benefit in the limitation of asthmatic mucosal inflammation. Furthermore, this might in part account for the paradox that epidemiologic studies have repeatedly revealed associations between vitamin D insufficiency and both the risk of more severe asthma[1] and increased serum IgE concentrations,[8] whereas other studies have reported that in culture vitamin D can act directly on T_H2 lymphocytes to promote T_H2 cytokine secretion (eg, Boonstra et al[9]). The apparent beneficial association of vitamin D in asthma *in vivo*, which in many patients is a T_H2-type cytokine pathology, suggests that the direct action of vitamin D in promoting T_H2 lymphocyte responses is less important *in vivo* than other vitamin D–mediated

mechanisms (eg, vitamin D upregulated production of sST2 and the induction of regulatory mechanisms) in inhibiting T_H2-type cytokine responses. Because the enhancement by vitamin D of sST2 production is concentration dependent, this supports therapeutic strategies to boost pulmonary vitamin D levels to reduce asthmatic inflammation.

Paul E. Pfeffer, MRCP[a]
Yin-Huai Chen, MSc[a]
Grzegorz Woszczek, PhD[a]
Nick C. Matthews, PhD[a]
Elfy Chevretton, FRCS[b]
Atul Gupta, MRCPH[a,c]
Sejal Saglani, MD[c]
Andrew Bush, MD[c]
Chris Corrigan, PhD[a]
David J. Cousins, PhD[a,d]
Catherine M. Hawrylowicz, PhD[a]

From [a]the MRC and Asthma UK Centre for Allergic Mechanisms of Asthma, King's College London, Guy's Hospital, London, United Kingdom; [b]the Department of ENT, Guy's Hospital, Great Maze Pond, London, United Kingdom; [c]the MRC and Asthma UK Centre for Allergic Mechanisms of Asthma, Imperial College London, Department of Paediatric Respiratory Medicine, Royal Brompton Hospital, London, United Kingdom; and [d]the Department of Infection, Immunity and Inflammation, University of Leicester, Glenfield Hospital, Leicester, United Kingdom. E-mail: catherine.hawrylowicz@kcl.ac.uk.

REFERENCES
1. Mann EH, Chambers ES, Pfeffer PE, Hawrylowicz CM. Immunoregulatory mechanisms of vitamin D relevant to respiratory health and asthma. Ann N Y Acad Sci 2014;1317:57-69.
2. Grotenboer NS, Ketelaar ME, Koppelman GH, Nawijn MC. Decoding asthma: translating genetic variation in IL33 and IL1RL1 into disease pathophysiology. J Allergy Clin Immunol 2013;131:856-65.
3. Garlanda C, Dinarello CA, Mantovani A. The interleukin-1 family: back to the future. Immunity 2013;39:1003-18.
4. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family. Nat Rev Immunol 2010;10:103-10.
5. Oboki K, Ohno T, Kajiwara N, Saito H, Nakae S. IL-33 and IL-33 receptors in host defense and diseases. Allergol Int 2010;59:143-60.
6. Hansdottir S, Monick MM, Hinde SL, Lovan N, Look DC, Hunninghake GW. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defence. J Immunol 2008;181:7090-9.
7. Babina M, Krautheim M, Grutzkau A, Henz BM. Human leukemic (HMC-1) mast cells are responsive to 1alpha, 25-dihydroxyvitamin D(3): selective promotion of ICAM-3 expression and constitutive presence of vitamin D(3) receptor. Biochem Biophys Res Commun 2000;273:1104-10.
8. Sharief S, Jariwala S, Kumar J, Muntner P, Melamed ML. Vitamin D levels and food and environmental allergies in the United States: results from the National Health and Nutrition Examination Survey 2005-2006. J Allergy Clin Immunol 2011;127:1195-202.
9. Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HFJ, O'Garra A. 1alpha,25-Dihydroxyvitamin D3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. J Immunol 2001;167:4974-80.

Claudin-1 polymorphism modifies the effect of mold exposure on the development of atopic dermatitis and production of IgE

To the Editor:

Skin barrier dysfunction, which plays a key role in the initiation of atopic dermatitis (AD), may be associated with both genetic susceptibility and environmental risk factors for AD. Importantly, the skin of patients with AD may exhibit not only loss-of-function mutation in filaggrin[1] but also defects in tight junction (TJ) function,[2] all of which can contribute to decreased skin barrier function.

Consistent with these observations, disruption of the epidermal barrier (comprising the stratum corneum and epithelial TJs) is now recognized as a common feature in subjects with AD. A recent study showed that the nonlesional epithelium of patients with AD exhibited marked defects in TJs, possibly due to reduced levels of claudin-1 (CLDN1), a key adhesive protein.[2]

Elevated levels of fungal and bacterial components in house dust,[3] or diversity of the mold components in the environment,[4] may have protective effects to the development of allergic diseases. However, exposure to indoor mold is a risk factor for childhood asthma and allergy,[5] and exposure to fungi in early infancy is associated with an increased risk of AD.[6] The reason for these discrepancies among the effects of various molds on allergy-related health outcomes remains largely unknown.

Several studies have focused on gene-environment interactions in the context of mold exposure. Our group's previous studies showed that mold exposure is a risk factor for the development of AD and allergic rhinitis in children with susceptibility.[6,7] However, no study has yet examined the associations between *CLDN1* polymorphisms and mold exposure, or investigated whether such an association has a functional impact on the development of AD. In this study, we examined the combined effect of the rs9290929 polymorphism, located in the *CLDN1* promoter, and mold exposure during the first year of life on the development of AD symptoms and production of IgE and also investigated the response of cell and mouse skin tissue mediated by mycotoxin (patulin). The definition of AD, the characteristics of the study subjects, the total IgE levels, and the genotyping of the *CLDN1* polymorphism are described in the Methods section in this article's Online Repository at www.jacionline.org. Our results demonstrated that visible mold exposure during the first year of life was an independent risk factor for lifetime AD symptoms (adjusted odds ratio [aOR], 1.60; 95% CI, 1.26-2.03) in the general population-based study (see Table E2 in this article's Online Repository at www.jacionline.org). *CLDN1* polymorphism was associated with susceptibility to AD in the hospital-based case-control study (aOR, 1.48; 95% CI, 1.04-2.12) (see Table E3 in this article's Online Repository at www.jacionline.org). We then analyzed the association between *CLDN1* polymorphism and mold exposure in the general

METHODS
Cell-culture reagents
Ultrahigh-purity 1,25(OH)D3 and 25(OH)D3 (Enzo Life Sciences, Exeter, United Kingdom) were placed in aliquots dissolved in dimethyl sulfoxide (Sigma-Aldrich, Gillingham, United Kingdom). Recombinant proteins were obtained as follows: IL-33, IFN-γ, IL-4, human sST2-Fc chimera, and isotype were all from R&D System (Abingdon, United Kingdom).

Primary human epithelial cell culture
Primary HBECs were acquired from Lonza (Basel, Switzerland) and locally from endobronchial brushings obtained at fiberoptic bronchoscopy from adult volunteers (Guy's Research Ethics Committee, South London REC Office 3, REC approval no. 09/H0804/108, March 16, 2010). HNECs were obtained from resected nasal turbinates from adult patients undergoing nasal turbinectomy for clinical indications (City Road & Hampstead NRES Committee London, REC approval no. 12/LO/1931, January 14, 2013). Excised tissue was dissected and then treated with Liberase TL (Roche Diagnostics, Indianapolis, Ind) before isolation of epithelial cells with CD326 magnetic microbeads (magnetic cell sorting [MACS]; Miltenyi Biotech, Bisley, United Kingdom). Cells were expanded in flasks of Bronchial Epithelial Cell Growth Medium, which was constituted by supplementing Bronchial Epithelial Basal Medium (Lonza) with SingleQuot Supplements (Lonza). Epithelial cells were used for experiments at passages 3 to 5.

Cell purity after passage checked by means of flow cytometry with an allophycocyanin-labeled anti-human CD326 antibody (Miltenyi Biotech) and appropriate isotype control was greater than 99%.

Monolayers for cell-culture experiments were inspected daily by using light microscopy, and once approaching confluence, medium was changed to Bronchial Epithelial Cell Growth Medium containing all SingleQuot Supplements except bovine pituitary extract, retinoic acid, and hydrocortisone.

Cell-culture experiments with HBECs were conducted with triplicate wells for each condition in each experiment. For conditioned medium, culture supernatant was collected and centrifuged at 250g for 7 minutes before aliquots of the supernatant were used as conditioned medium.

Peripheral blood lymphocyte culture
PBMCs were obtained by means of density centrifugation with Lymphoprep density gradient medium (Axis-Shield, Dundee, United Kingdom). CD4+ and CD8+ lymphocytes were isolated by using Dynabeads (Invitrogen, Life Technologies, Paisley, United Kingdom). Cell purity was assessed by means of flow cytometry with appropriate isotype controls and was 95% to 98% for all experiments. Lymphocytes were cultured at 1×10^6 cells/mL in RPMI 1640 (Gibco, Life Technologies, Paisley, United Kingdom) supplemented with 50 U/mL IL-2 (Eurocetus, Harefield, United Kingdom), 10% FBS (PAA, Yeovil, United Kingdom), 2 mmol/L L-glutamine (Gibco, Life Technologies), and 0.1% 50 mg/mL gentamicin (Gibco, Life Technologies) on cell-culture plates coated with anti-CD3 (1 μg/mL; OKT-3) for 7 days.

Peripheral blood eosinophil culture
PBMCs were obtained, as described previously. Eosinophils were isolated with a MACS negative selection kit (MACS, Miltenyi Biotech), according to an adapted manufacturer's protocol. Cell purity was 96% to 99% for all experiments. Eosinophils were cultured at 1×10^6 cells/mL in RPMI 1640 supplemented with 10% FBS, 2 mmol/L L-glutamine, and 0.1% 50 mg/mL gentamicin for 20 hours.

Quantitative real-time PCR
Epithelial cell monolayers were lysed with Qiazol reagent (Qiagen, Manchester, United Kingdom) before storage at −80°C pending extraction of mRNA with an miRNeasy Mini Kit (Qiagen), according to an adapted manufacturer's protocol with an off-column DNA digest with TurboDNase (Ambion, Life Technologies, Paisley, United Kingdom). mRNA was extracted from lymphocyte culture cell pellets by using an RNeasy Mini Kit (Qiagen), according to the manufacturer's protocol. mRNA was quantified with a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Waltham, Mass) and converted to cDNA by using RevertAid Reverse Transcriptase and complementary reagents (Fermentas, Thermo Scientific). Relative quantification of *IL1RL1* relative to the *18S* housekeeping gene was conducted in triplicate by means of real-time quantitative PCR with the TaqMan primers Hs01073300 and Hs00249389 (Applied Biosystems, Life Technologies, Paisley, United Kingdom).

sST2 ELISA
Concentrations of sST2 protein in cell-culture supernatants were quantified by using a Human ST2 ELISA DuoSet (R&D Systems), according to the manufacturer's protocol.

LUVA cell culture and IL-33 bioassay
The LUVA mast cell line was a kind gift from Dr J. Steinke, University of Virginia.[E1] LUVA cells were expanded in flasks in StemPro-34 serum free medium with associated nutrient supplement (Gibco, Life Technologies), L-glutamine (Gibco, Life Technologies), and 1% penicillin-streptomycin (10,000 U/mL, Gibco, Life Technologies). For the IL-33 bioassay, LUVA cells were cultured for 24 hours at 0.5 million cells/mL in RPMI 1640 supplemented with 10% FBS, 2 mmol/L L-glutamine, and gentamicin. The cell cultures were stimulated with a concentration series of recombinant IL-33 or IFN-γ plus IL-4 in the presence/absence of recombinant sST2-Fc chimera, isotype-Fc chimera, 100 nmol/L 1,25(OH)D3, or 40% HNEC conditioned medium. Cell-surface expression of CD54 (intercellular adhesion molecule 1) was measured by means of flow cytometry with a phycoerythrin-labeled anti-human CD54 antibody (BioLegend, London, United Kingdom) and appropriate isotype control on a FACSCalibur flow cytometer (BD Biosciences, San Jose, Calif) for initial experiments (Figs 2, *B*, and E2, *B*) and an Attune flow cytometer (Life Technologies, Paisley, United Kingdom) for later experiments (Fig 2, *A* and *C-E*, and Fig E2, *C-E*). Surface expression of CD54 was quantified as the geometric mean fluorescence intensity (GeoMFI). For stimulated LUVA cell cultures, induction of CD54 was calculated as the CD54 GeoMFI for unstimulated cell cultures subtracted from the GeoMFI for stimulated cell cultures in that experiment. The percentage reduction in stimulus-induced CD54 expression on LUVA cells cultured with vitamin D–treated epithelial cell–conditioned medium compared with induction of CD54 with control conditioned medium for each stimulus concentration was calculated to analyze the effect of vitamin D–treated epithelial cell–conditioned medium on stimulus-induced CD54 expression by LUVA cells.

Statistical analysis
Data were analyzed with GraphPad Prism 6 software (GraphPad Software, La Jolla, Calif). For analysis of parametric data, *t* tests, repeated-measures ANOVA, and 2-way repeated-measures ANOVA were used with posttest-corrected multiple comparisons where the ANOVA was significant. For nonparametric data, Wilcoxon matched-pairs signed-rank tests or Friedman tests (with Dunn multiple comparison tests for comparison with control conditions) were used. Unless otherwise stated, parametric data are shown as means ± SEMs, and nonparametric data are shown as box and whisker plots with medians, 25th to 75th percentiles, and minimum to maximum ranges.

REFERENCE
E1. Laidlaw TM, Steinke JW, Tinana AM, Feng C, Xing W, Lam BK, et al. Characterization of a novel human mast cell line that responds to stem cell factor and expresses functional FceRI. J Allergy Clin Immunol 2011;127:815-22.
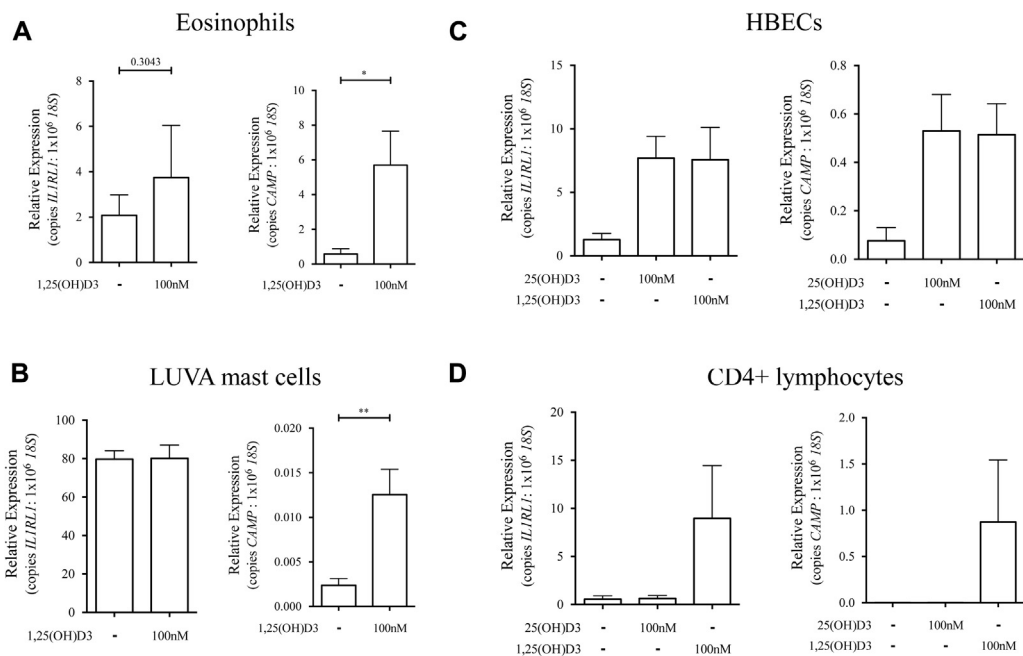
FIG E1. Further data on the effect of vitamin D on *IL1RL1* expression. **A** and **B,** 1,25(OH)D3 (100 nmol/L) increases the expression of mRNA for *CAMP* in primary human eosinophils (Fig E1, *A*) and LUVA mast cells (Fig E1, *B*) but not mRNA for *IL1RL1*, as measured by using the Hs01073300 probe set in either cell type (n = 5 for both cell types). **C** and **D,** 25(OH)D3 (100 nmol/L) increases the expression of mRNA for both *IL1RL1* and *CAMP* in HBECs (Fig E1, *C*) but not in CD4^{+} lymphocytes (Fig E1, *D*; Fig E1, *C*: n = 5; Fig E1, *D*: n = 3). *$P \leq$ .05 and **$P \leq$ .01.
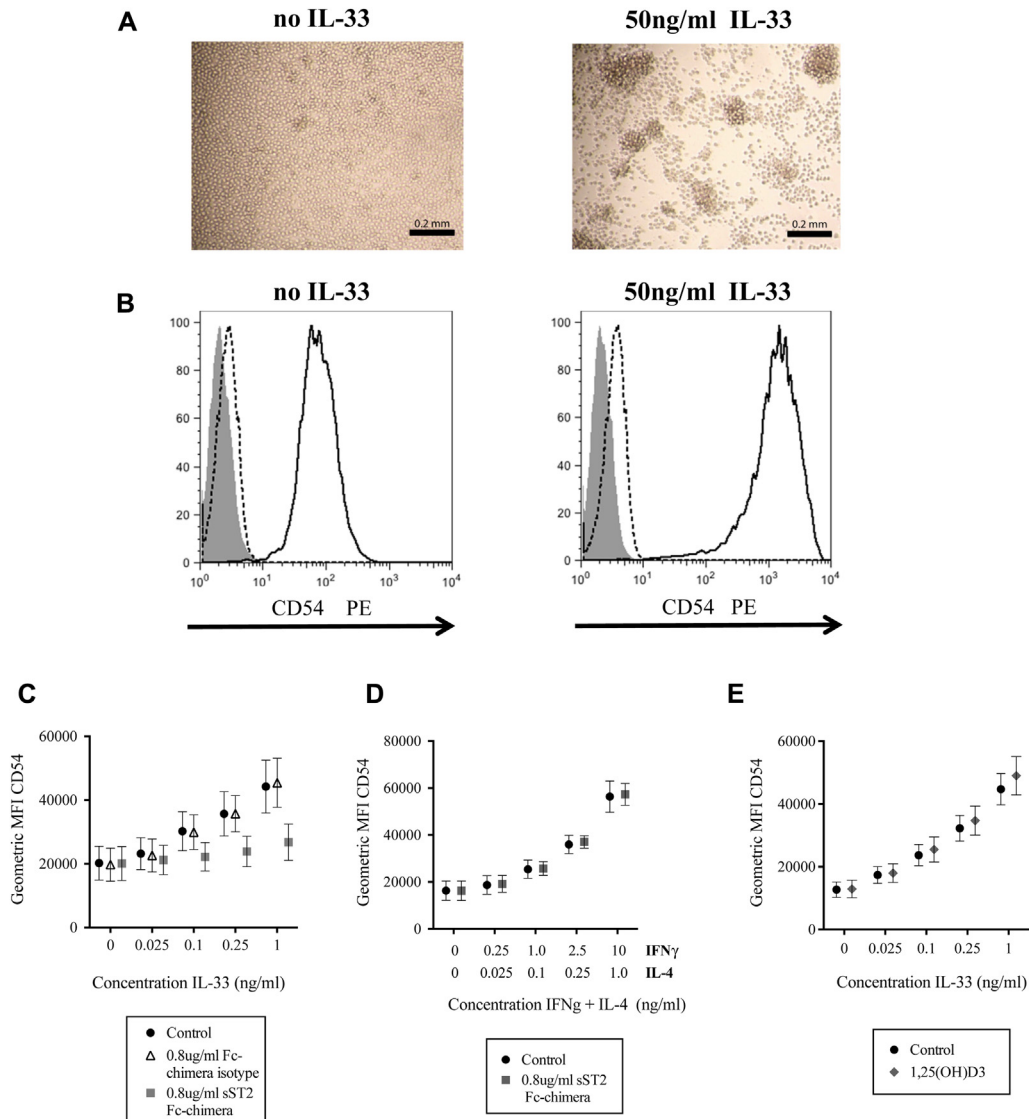
FIG E2. Stimulation with IL-33 induces LUVA cell expression of CD54. **A** and **B,** IL-33 causes homotypic aggregation of LUVA cells (×10 magnification; Fig E2, *A*) associated with increased expression of CD54 (*filled gray histogram*, unstained; *dashed line*, isotype control; *solid line*, anti-CD54; Fig E2, *B*). **C,** Inhibition of IL-33–induced LUVA cell CD54 expression by the recombinant sST2-Fc chimera is not observed with an isotype Fc chimera (n = 3). **D,** IFN-γ plus IL-4–induced LUVA cell CD54 expression is not inhibited by addition of recombinant sST2-Fc chimera (n = 4). **E,** Effect of IL-33 on LUVA cell CD54 expression is not affected by addition of active vitamin D (100 nmol/L 1,25[OH]D3) directly to the LUVA cells (n = 4).