

IL-33, but not thymic stromal lymphopoietin or IL-25, is central to mite and peanut allergic sensitization

Derek K. Chu, BHSc,^{a,*} Alba Llop-Guevara, BHSc,^{a,*} Tina D. Walker, BSc,^a Kristin Flader, BSc,^a Susanna Goncharova, MD, PhD,^a Jeanette E. Boudreau, PhD,^{a,†} Cheryl Lynn Moore, BSc,^a Tracy Seunghyun In, BHSc,^a Susan Wasserman, MD, MSc,^b Anthony J. Coyle, PhD,^{a,c} Roland Kolbeck, PhD,^d Alison A. Humbles, PhD,^d and Manel Jordana, MD, PhD^a *Hamilton, Ontario, Canada, Cambridge, Mass and Gaithersburg, Md*

Background: Allergen exposure at lung and gut mucosae can lead to aberrant T_H2 immunity and allergic disease. The epithelium-associated cytokines thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 are suggested to be important for the initiation of these responses.

Objective: We sought to investigate the contributions of TSLP, IL-25, and IL-33 in the development of allergic disease to the common allergens house dust mite (HDM) or peanut.

Methods: Neutralizing antibodies or mice deficient in TSLP, IL-25, or IL-33 signaling were exposed to HDM intranasally or peanut intragastrically, and immune inflammatory and physiologic responses were evaluated. *In vitro* assays were performed to examine specific dendritic cell (DC) functions.

Results: We showed that experimental HDM-induced allergic asthma and food allergy and anaphylaxis to peanut were associated with TSLP production but developed independently of TSLP, likely because these allergens functionally mimicked TSLP inhibition of IL-12 production and induction of OX40 ligand (OX40L) on DCs. Blockade of OX40L significantly lessened allergic responses to HDM or peanut. Although IL-25 and IL-33 induced OX40L on DCs *in vitro*, only IL-33 signaling was necessary for intact allergic immunity, likely because of its superior ability to induce DC OX40L and expand innate lymphoid cells *in vivo*.

Conclusion: These data identify a nonredundant, IL-33–driven mechanism initiating T_H2 responses to the clinically relevant allergens HDM and peanut. Our findings, along with those in infectious and transgenic/surrogate allergen systems, favor a paradigm whereby multiple molecular pathways can initiate T_H2 immunity, which has implications for the conceptualization and manipulation of these responses in health and disease. (*J Allergy Clin Immunol* 2012;■■■:■■■-■■■.)

Key words: T_H2, allergy, asthma, house dust mite, peanut, thymic stromal lymphopoietin, IL-25, IL-33, OX40 ligand, innate lymphoid cells

Allergies are immune-mediated hypersensitivity diseases mainly driven by T_H2 responses to normally tolerated environmental antigens. Allergic asthma is a chronic lung disease characterized by airway infiltration of T_H2 cells and eosinophils, increased mucus production, and reversible airway obstruction. It is estimated that 10% to 20% of the population is allergic to house dust mite (HDM), the most ubiquitous and pervasive aero-allergen worldwide.^{1,2} Food allergy causes symptoms that range from mild erythema and pruritus to acute, life-threatening systemic reactions termed anaphylaxis. Peanut allergy affects approximately 1% to 2% of the general population^{3,4} and is highly associated with anaphylaxis.⁵

Despite substantial advances in understanding the pathophysiology of these diseases, the initial cellular and molecular events that cause susceptible subjects to acquire allergic disease are not well understood. Research on the origin of allergic disease in human subjects is exceedingly difficult because sensitization is often clinically silent. Thus the immunologic basis of allergic sensitization is largely investigated in animal models.

Mechanistically, allergic responses have been proposed to be initiated after epithelial cell contact with antigens that leads to the production of a number of cytokines able to promote T_H2 immunity.⁶ Paramount among these has been thymic stromal lymphopoietin (TSLP), which has been reported to condition dendritic cells (DCs) to favor T_H2 induction through 2 main actions⁷: (1) limitation of IL-12 production⁸⁻¹⁰ and (2) upregulation of the costimulatory molecule OX40 ligand (OX40L).^{11,12} Since the identification of TSLP, additional epithelium-associated cytokines also capable of inducing T_H2 immunity have been discovered, notably IL-25 (IL-17E), which signals through IL-17 receptor B (IL-17RB),^{13,14} and IL-33, which signals through the ST2 receptor.¹⁵ A majority of studies supporting a role for TSLP, IL-25, and/or IL-33 in the generation of allergic responses in the lung and gut have relied on conventional models of nonmucosal intraperitoneal sensitization to the surrogate allergen

From ^athe Department of Pathology and Molecular Medicine, McMaster Immunology Research Centre, and ^bthe Department of Medicine, McMaster University, Hamilton; ^cPfizer, Cambridge; and ^dthe Department of Respiratory, Inflammation and Autoimmunity, MedImmune LLC, Gaithersburg.

*These authors contributed equally to this work.

†Jeanette E. Boudreau, PhD, is currently affiliated with the Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, New York, NY.

Supported by CIHR, Anaphylaxis Canada, and grants from MedImmune LLC. D.K.C. is a CIHR Vanier Scholar. A.L.-G. is supported by a Fundación Caja Madrid doctoral scholarship (Spain). J.E.B. holds an NSERC Doctoral Canada Graduate Scholarship. M.J. holds a Senior Canada Research Chair in Immunobiology of Respiratory Diseases and Allergy.

Disclosure of potential conflict of interest: S. Wasserman has received research support from Anaphylaxis Canada and is employed by McMaster University. A. J. Coyle was an employee of MedImmune LLC and is now an employee of Pfizer. R. Kolbeck is employed by and has stock options in MedImmune LLC. A. A. Humbles is employed by MedImmune LLC and has stock options in MedImmune (AZ). M. Jordana has received research support from MedImmune LLC, Anaphylaxis Canada, and the Canadian Institutes of Health Research and is employed by McMaster University. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication March 13, 2012; revised July 17, 2012; accepted for publication August 2, 2012.

Corresponding author: Manel Jordana, MD, PhD, Department of Pathology and Molecular Medicine, McMaster Immunology Research Centre, McMaster University, MDCL 4013, 1200 Main St W, Hamilton, Ontario L8N 3Z5, Canada. E-mail: jordanam@mcmaster.ca.

0091-6749/\$36.00

© 2012 American Academy of Allergy, Asthma & Immunology

<http://dx.doi.org/10.1016/j.jaci.2012.08.002>

Abbreviations used

BMDC:	Bone marrow–derived dendritic cell
CT:	Cholera toxin
DC:	Dendritic cell
HDM:	House dust mite
IL-17RB:	IL-17 receptor B
ILC:	Innate lymphoid cell
KO:	Knockout
LN:	Lymph node
OVA:	Ovalbumin
OX40L:	OX40 ligand
STAT6:	Signal transducer and activator of transcription 6
TSLP:	Thymic stromal lymphopoietin
TSLPR:	Thymic stromal lymphopoietin receptor
WT:	Wild-type
VSV:	Vesicular stomatitis virus

ovalbumin (OVA) adsorbed to aluminum-based adjuvants, such as aluminum hydroxide (alum). Thus how these cytokines initiate T_H2 sensitization to common allergens at mucosal sites remains to be elucidated.¹⁶ Recent reports showed that intraperitoneal injection of recombinant IL-25, IL-33, or both can expand novel innate lymphoid cell (ILC) populations in the lung and gut that are able to produce T_H2 cytokines and promote host defense¹⁷; however, the presence and contribution of these cells during allergic sensitization remain unknown.

Here we sought to better understand how TSLP, IL-25, and IL-33 influence sensitization to the common allergens HDM and peanut in the lung and gut, respectively. To accomplish this, we used established models of HDM-induced allergic asthma^{18,19} and peanut allergy and anaphylaxis.^{20,21} In stark contrast to the increasingly prevalent opinion that TSLP is a master regulator of T_H2 responses and thus atopic disease,²² we found that TSLP was dispensable for the generation of IL-4–dependent humoral and cellular immunity to HDM or peanut. Similar results were observed with respect to IL-25. In contrast, IL-33 played an essential role. In fact, our findings delineate an IL-33/OX40L/ILC pathway leading to T_H2 immunity in response to 2 clinically relevant allergens at 2 different mucosal sites.

METHODS

Supplemental information can be found in the [Methods](#) section in this article's Online Repository at www.jacionline.org.

Mice

Adult (6–8 weeks old) female BALB/c and C57BL/6 mice, thymic stromal lymphopoietin receptor (TSLPR) knockout (KO; B6 or C.129P2-*Crly*^{tm1Jni}) and IL-17RB KO (B6.129-*Il17rb*^{tm1Bud}), gifts from Amgen, Thousand Oaks, Calif) mice and ST2 KO mice (C.129P2-*Il1rl1*^{tm1Anjm}; gift from MedImmune, Gaithersburg, Md), were obtained from Charles River (San Diego, Wilmington, Saint-Constant, Quebec, Canada). IL-4 KO (B6.129P2-*Il4*^{tm1Cgn}) and wild-type (WT) control animals were from the Jackson Laboratory (Bar Harbor, Me). IL-13 KO (C.129P2-*Il13*^{tm2Anjm}) and signal transducer and activator of transcription 6 (STAT6) KO (B6.129S-*Stat6*^{tm1Jni}) mice were bred in house. All experiments were performed with age-, sex-, and strain-matched control animals and were approved by the Animal Research Ethics Board of McMaster University (Hamilton, Ontario, Canada).

Statistics

Data were analyzed and graphed with GraphPad Prism 5 software (GraphPad Software, San Diego, Calif). Continuous data are expressed as means ± SEMs and were analyzed by using the *t* test (unpaired, 2-tailed) or 1- or 2-way ANOVA with Dunnett or Bonferroni *post hoc* tests. Temperature data were analyzed with repeated measures. Ordinal data are shown as individual mice with medians and were analyzed with Mann-Whitney *U* tests. Differences were considered statistically significant at a *P* value of less than .05.

RESULTS**Airway sensitization to HDM does not require TSLP signaling**

We sought to investigate the requirement of TSLP in experimental allergic asthma to the clinically relevant allergen HDM. This model²³ solely involves mucosal exposure, does not use any exogenous adjuvant, and critically requires the canonical T_H2-inducing molecule IL-4.²⁴ Consistent with a previous report,²⁵ TSLP was detected in the lungs of naive mice, and levels were increased upon 3-day HDM exposure (Fig 1, A), a protocol that induces sensitization without overt inflammation. TSLP upregulation was not detected in sera (data not shown), suggesting that cytokine signaling took place locally. Thus allergic sensitization to HDM is associated with TSLP production in the lung.

To test the functional role of TSLP signaling during sensitization, we used a 10-day intranasal HDM exposure protocol that recapitulates early features of the allergic diathesis.^{18,19} TSLPR KO mice produced levels of T_H2-associated immunoglobulins (IgG₁ and IgE) comparable with those in WT HDM-exposed mice (Fig 1, B and C). Accordingly, the proportion of activated CD69⁺ B cells was similar in both groups (Fig 1, D). Likewise, overall airway inflammation was comparable in HDM-exposed WT and TSLPR KO mice (Fig 1, E), with a similar degree of eosinophil influx (Fig 1, F). Histopathologic evaluation of lung sections showed comparable peribronchial and perivascular inflammatory responses and bronchial goblet cell hyperplasia/hypertrophy with mucus production (Fig 1, G and H). T-cell activation *in vivo* and *ex vivo* cytokine production were intact regardless of the presence or absence of TSLPR (Fig 1, I and J).

Similar results were observed in BALB/c mice treated before and throughout the HDM exposure protocol with a neutralizing anti-TSLP antibody (see Fig E1, A–C, in this article's Online Repository at www.jacionline.org), as well as in C57BL/6 TSLPR KO mice (see Fig E1, D–F). Collectively, these data show that allergic sensitization and airway inflammation in response to HDM occur independently of TSLP and its receptor.

These data, generated by using the common aeroallergen HDM, are in sharp contrast to the results published by 2 different groups reporting TSLP-dependent lung T_H2 responses to the surrogate allergen OVA in mouse models involving intraperitoneal alum-driven sensitization protocols.^{26,27} This discrepancy was not due to colony differences because we observed similar TSLPR dependency in OVA plus alum-sensitized mice (see Fig E1, A–H). Thus although our data confirm a role for TSLP in this OVA model, they do not support the contention that TSLP is pervasively a key factor in the initiation of allergic asthma.

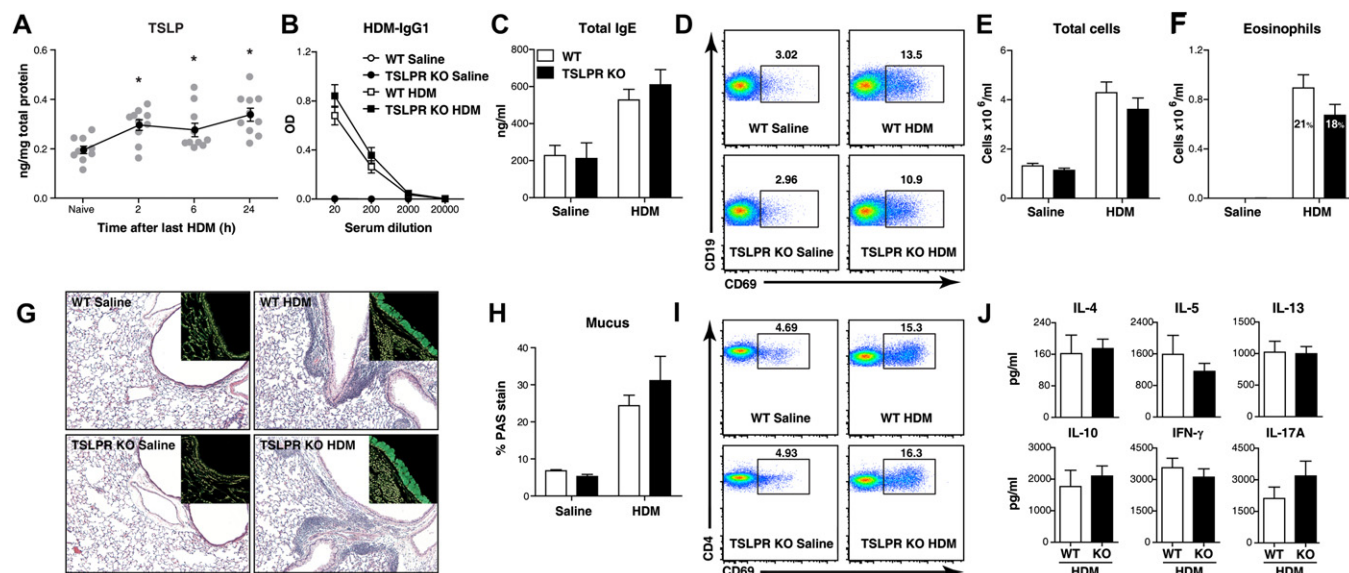


FIG 1. HDM-induced TSLP is not required for allergic sensitization. **A**, TSLP in lung homogenates from naive mice or after 3 days of HDM. **B–J**, BALB/c WT or TSLPR KO mice were exposed to saline or HDM for 10 days. HDM-specific IgG₁ (Fig 1, **B**) and total IgE (Fig 1, **C**) levels in serum. Representative lung CD45⁺CD3⁺CD19⁺ B cells expressing CD69 (Fig 1, **D**). Total (Fig 1, **E**) and eosinophil (Fig 1, **F**) airway infiltrates in bronchoalveolar lavage fluid; mean percentage of eosinophils are shown in bars. Fig 1, **G**, Representative hematoxylin and eosin-stained lung sections (×100 magnification); insets show color-inverted periodic acid-Schiff (PAS)-stained sections (×400 magnification). Fig 1, **H**, Morphometric quantification of periodic acid-Schiff staining. Fig 1, **I**, Representative CD69 expression by lung CD45⁺CD3⁺CD4⁺ T cells. Fig 1, **J**, Cytokine levels in supernatants of splenocytes stimulated with HDM. Shown are 2 to 3 experiments with 3 to 6 mice per group per experiment. **P* < .05 vs naive.

Gastrointestinal sensitization to peanut is IL-4 and STAT6 dependent, but does not require TSLP signaling or basophils

To investigate whether the previous results were idiosyncratic of the site and allergen used, we analyzed the role of TSLP in the induction of T_H2 immunity in the gastrointestinal tract. We have previously shown that intragastric exposure of mice to peanut along with cholera toxin (CT) induces strong gastrointestinal T_H2 responses, such that subsequent peanut challenge elicits systemic anaphylaxis.^{20,28} Previously reported mRNA analysis of steady-state intestinal *tslp* expression showed the highest levels in the caecum and large intestine.¹⁰ After ingestion of peanut plus CT for 3 days, we observed enhanced TSLP protein levels in the duodenum but not in other intestinal segments (Fig 2, **A**). TSLP was not detected in intestinal washes or sera (data not shown). Thus the initiation of peanut allergy is associated with TSLP upregulation in the small intestine.

We next attempted to induce gastrointestinal T_H2 immunity to peanut in the absence of TSLP signaling. Both WT and TSLPR KO mice sensitized to peanut produced similarly increased levels of peanut-specific IgG₁ and IgE (Fig 2, **B** and **C**). Functionally, both groups of mice experienced similar anaphylactic responses upon peanut challenge, as measured by comparable decreases in core body temperature, increases in hematocrit levels (a physiologic measure of systemic vascular leakage), and development of clinical signs ranging from pruritus to seizure or death (Fig 2, **D–F**). Using B cell-independent but CD4⁺ T cell-dependent peritoneal eosinophilic inflammation 72 hours after intraperitoneal challenge as an *in vivo* measure of T-cell function (data not shown),²⁰ we found robust responses in both the peanut-sensitized and challenged groups (Fig 2, **G** and **H**). Furthermore,

ex vivo splenocyte cytokine production was also comparable in both groups (Fig 2, **I**).

Anti-TSLP antibody administration throughout sensitization generated similar results (see Fig E2, A–C, in this article's Online Repository at www.jacionline.org and data not shown). We also considered that the type of antigen, adjuvant, or route of administration might have affected TSLP dependency, but WT and TSLPR KO mice sensitized with OVA plus CT intragastrically, peanut plus alum intraperitoneally, peanut plus CT intraperitoneally, or the conventional peanut plus CT intragastrically produced similarly increased levels of antigen-specific IgG₁ and IgE and, upon challenge, comparable anaphylactic responses (see Fig E2, D–O, and data not shown). Collectively, these results show that intact TSLP signaling is not required for gastrointestinal T_H2 priming and subsequent humoral or cellular food-induced allergic responses.

Basophils, which can respond to and produce TSLP, have been reported to be critical for T_H2 priming in some experimental systems.^{29,30} Anti-CD200R3 antibody (clone Ba103) depletion of basophils during HDM exposure was recently reported to partially decrease eosinophilic airway inflammation but not alter T_H2 priming and cytokine production.³¹ In the case of peanut, depletion of basophils using anti-asialo GM1³² or Ba103²¹ did not impair peanut-specific IgG₁ or IgE production and subsequent anaphylactic responses to peanut (see Fig E2, P–R, and data not shown). Hence basophils are not required for HDM or peanut sensitization.

TSLP has been proposed to be a master regulator of T_H2 immunity and reported to require IL-4.^{33,34} Indeed, canonical T_H2 differentiation requires IL-4-mediated activation of STAT6. We have shown that HDM-induced allergic asthma requires IL-4.²⁴

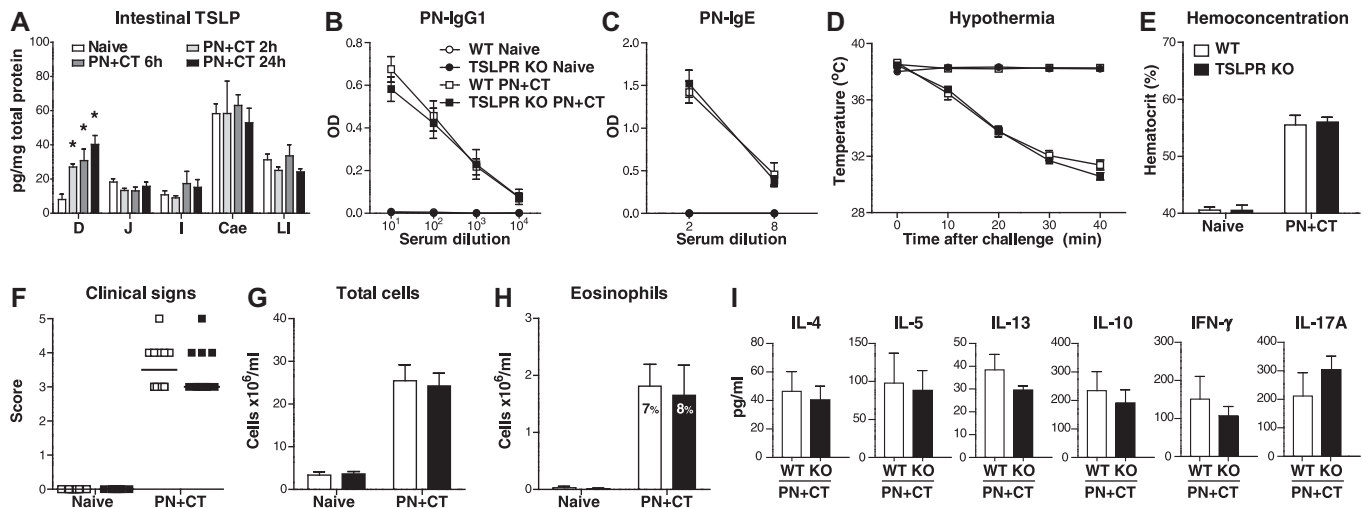


FIG 2. Peanut (PN)-induced TSLP is not required for allergic sensitization. **A**, TSLP in tissues from naive mice or after 3 days of peanut CT. *Cae*, Caecum; *D*, duodenum; *I*, ileum; *J*, jejunum; *LI*, large intestine. **B–I**, C57BL/6 WT or TSLPR KO mice were sensitized to peanut and, 1 week later, challenged intraperitoneally. Serum levels of peanut-specific IgG₁ (Fig 2, **B**) and IgE (Fig 2, **C**) 1 day before challenge. Anaphylactic assessment by core body temperature (Fig 2, **D**), hematocrit levels (Fig 2, **E**), and clinical signs (Fig 2, **F**). Total (Fig 2, **G**) and eosinophil (Fig 2, **H**) infiltrates in peritoneal lavage; mean percentages of eosinophils are shown in bars. Fig 2, **I**, Cytokine production by peanut-stimulated splenocytes. Shown are 2 to 3 experiments with 3 to 8 mice per group per experiment. **P* < .05 versus naive.

We considered that the peanut allergy model did not induce canonical T_H2 induction and hence did not require TSLP. STAT6 or IL-4 KO mice exposed to peanut plus CT did not produce any detectable levels of peanut-specific IgG₁ or IgE (data not shown) or show any clinical or physiologic indication of anaphylaxis upon challenge (see Fig E3, A–F, in this article's Online Repository at www.jacionline.org). In contrast, IL-13 KO mice generated robust IgG₁ and anaphylactic responses (see Fig E3, G–I). Additionally, peanut-stimulated splenocytes from peanut-sensitized WT and IL-13 KO, but not IL-4 and STAT6 KO, mice produced significantly increased amounts of IL-5 compared with levels seen in naive mice (not shown). Therefore this peanut allergy model is dependent on the canonical T_H2-inducing molecules IL-4 and STAT6, yet functions independently of TSLP.

Overall, data from 2 distinct models of allergic disease lead us to conclude, at variance with the recent proposition,^{7,22,35,36} that TSLP does not play a universal role in initiating T_H2 allergic immunity at mucosal surfaces.

HDM and peanut functionally replace TSLP action on DCs

TSLP has been reported to instruct DCs to facilitate T_H2 responses through limiting the production of IL-12p40^{8–10} and inducing OX40L.^{11,12} To better understand why the development of HDM-induced allergic asthma and peanut allergy did not require TSLP, we investigated whether these allergens could influence DC behavior. To achieve this, bone marrow–derived dendritic cells (BMDCs) were conditioned with complete medium, TSLP, HDM, or peanut and subsequently stimulated with medium or LPS. Few (<5%) DCs conditioned with medium alone spontaneously produced IL-12p40, whereas more than 50% of DCs were IL-12p40⁺ upon LPS stimulation (Fig 3, A and B). As expected,^{9,10} TSLP conditioning significantly inhibited this effect. Conditioning with peanut or HDM also limited DC IL-12p40 production to the same extent as TSLP

(Fig 3, A–C). These effects were not due to allergen-elicited TSLP production from DCs³⁷ because culture supernatants did not have detectable levels of TSLP (data not shown). These data indicate that promotion of T_H2 responses by IL-12p40 inhibition can take place after DC contact with either TSLP or the allergens HDM or peanut.

We next investigated whether common allergens could also functionally mimic TSLP induction of OX40L on DCs *in vitro*. We found that vesicular stomatitis virus (VSV) infection of DCs strongly induced OX40L; thus we used this as a positive control. Compared with unstimulated DCs, incubation with HDM or peanut allergens significantly increased the proportion of CD11c⁺OX40L⁺ cells and the median fluorescence intensity of OX40L in a manner comparable with TSLP (Fig 3, D–F). Again, levels of TSLP upon HDM or peanut stimulation were undetectable (data not shown), implying that OX40L induction on DCs was not due to autocrine TSLP signaling. These data indicate that the common allergens HDM and peanut functionally mimic TSLP action on DCs, therefore rendering it redundant during DC conditioning for T_H2 induction.

OX40L plays an important role in allergic responses to HDM and peanut

We next asked whether OX40L might be important for T_H2 induction to HDM or peanut *in vivo*. First, we evaluated OX40L protein expression after 3 days of allergen exposure. Lung OX40L expression was increased upon HDM exposure, with induction on CD11c⁺CD11b^{hi}MHCII^{hi} myeloid DCs (Fig 4, A–D). Peanut plus CT administration induced OX40L in the duodenum (Fig 4, E). Accordingly, the proportion of mesenteric lymph node (LN) CD11c⁺CD11b⁺MHCII⁺ DCs expressing OX40L and their median fluorescence intensities were also significantly increased (Fig 4, F–H). The frequency of myeloid DCs in the lung and mesenteric LNs after acute allergen exposure increased 2- to 5-fold (see Fig E4 in this article's Online Repository

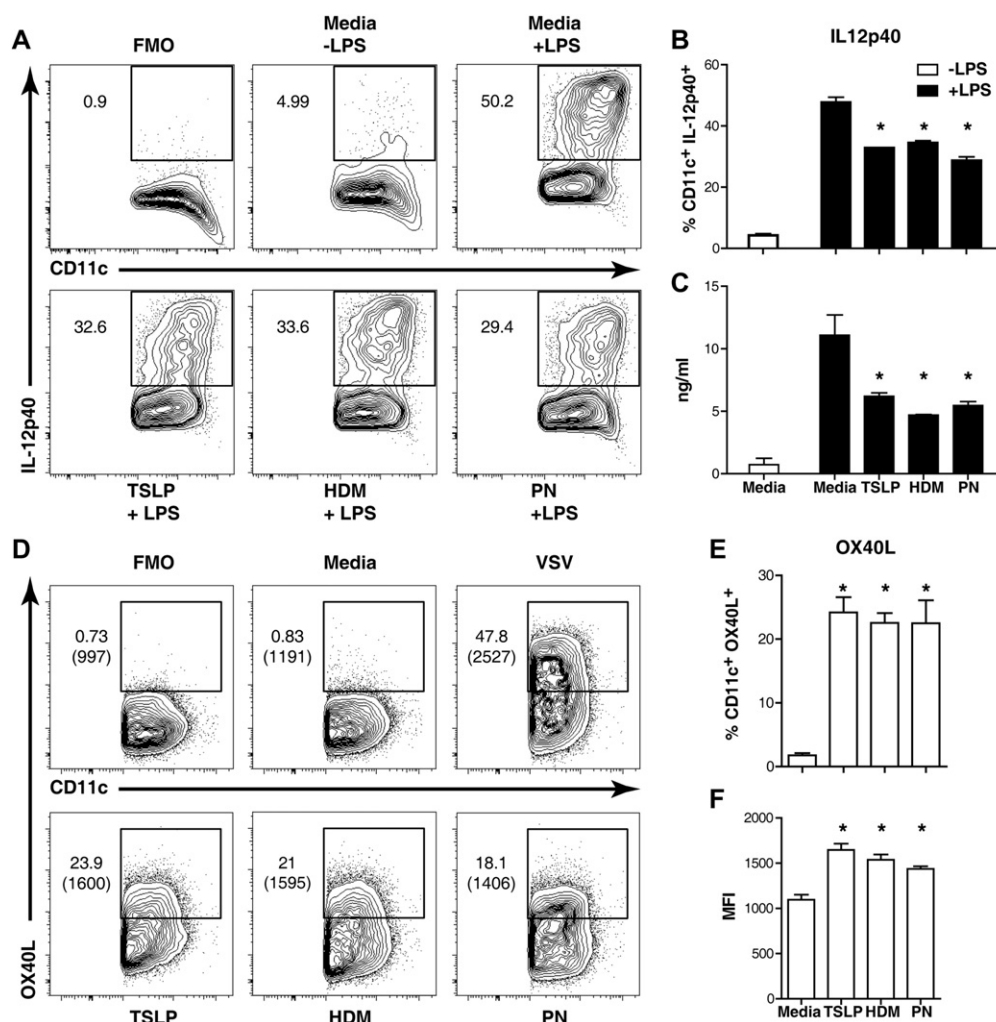


FIG 3. HDM and peanut (PN) mimic TSLP in downregulating IL-12p40 and upregulating OX40L. **A-C**, BMDCs were conditioned with media, TSLP, HDM, or peanut and then stimulated with media or LPS. Representative plots (Fig 3, A) and quantification (Fig 3, B) of CD11c⁺IL-12p40⁺ cells. Fig 3, C, IL-12p40 in culture supernatants. **D-F**, BMDCs were stimulated with media, VSV positive control, TSLP, HDM, or peanut. Representative plots (median fluorescence intensity in brackets; Fig 3, D), quantification of CD11c⁺OX40L⁺ cells (Fig 3, E), and OX40L median fluorescence intensity (Fig 3, F). Shown are 2 to 3 experiments performed in triplicate. **P* < .05 versus media plus LPS (Fig 3, B and C) or media only (Fig 3, E and F). FMO, Fluorescence minus one.

at www.jacionline.org). Thus sensitization to HDM or peanut plus CT is associated with OX40L upregulation, especially on DCs.

Mice were treated with blocking anti-OX40L antibody or control immunoglobulin throughout HDM administration to test the importance of OX40L on HDM sensitization *in vivo*. Compared with control immunoglobulin-treated and HDM-exposed mice, serum IgG₁ and IgE levels and B-cell activation were significantly attenuated in mice treated with anti-OX40L antibody (Fig 5, A-C). The overall inflammatory response was decreased by about 70%, with eosinophil counts being reduced approximately 90% (Fig 5, D and E). Lung tissue histology confirmed these findings and, in addition, demonstrated reduced goblet cell responses (Fig 5, F). CD4⁺ T-cell activation and the expression of IL-4 and IL-5, but not IFN- γ or IL-17A, were significantly decreased in the absence of OX40L signaling, suggesting a selective impairment of T_H2 immune responses (Fig 5, G and H).

Blockade of OX40L during peanut sensitization attenuated peanut-specific IgG₁ and IgE levels (Fig 5, I and J). Upon peanut

challenge, anti-OX40L antibody-treated mice experienced approximately 50% less severe anaphylactic hypothermia and hemoconcentration and had less severe clinical signs compared with control immunoglobulin-treated mice (Fig 5, K-M). In addition to impaired B-cell responses, T-cell immunity was also hindered, with less than half as many inflammatory cells and approximately 90% less eosinophils in anti-OX40L antibody-treated mice (Fig 5, N and O). Production of T_H2, but not T_H1 or T_H17, cytokines was impaired (Fig 5, P). Collectively, these results show that HDM and peanut allergen-driven humoral and cellular responses operate in a TSLP-independent but OX40L-dependent manner.

IL-25 and IL-33 induce OX40L on DCs *in vitro*

Because mucosal allergen exposure results in TSLP production, we next asked whether other epithelium-associated cytokines with T_H2 induction capacity analogous to TSLP, namely IL-25 and IL-33, were produced during HDM or peanut sensitization. After 3

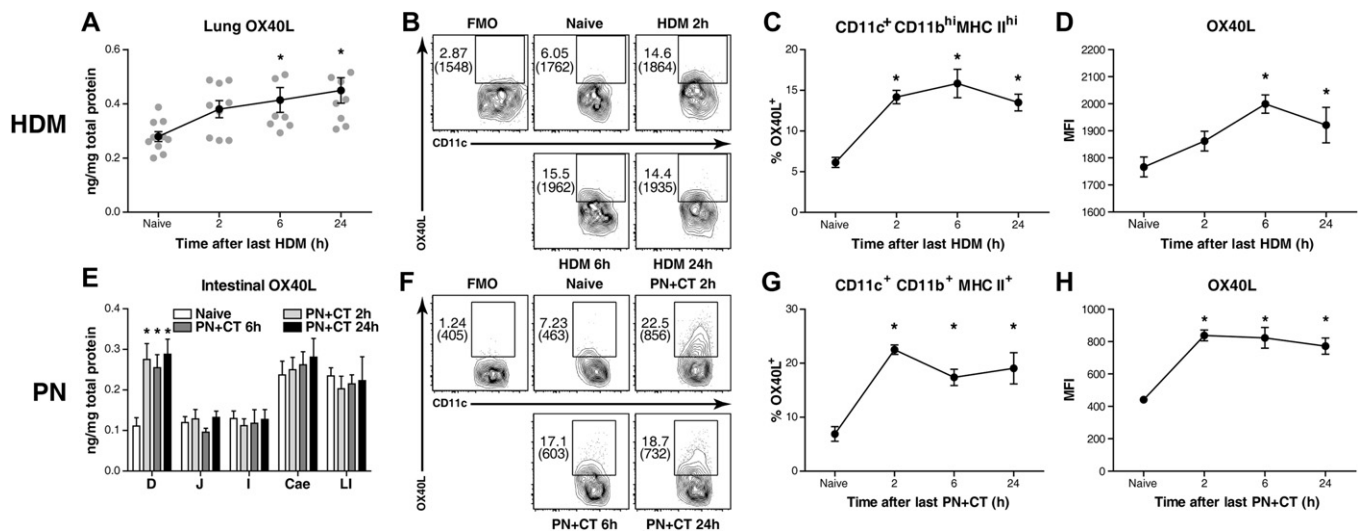


FIG 4. HDM and peanut (PN) sensitization induce OX40L on DCs *in vivo*. Mice were exposed to HDM intranasally (A–D) or to peanut plus CT intragastrically (E–H) for 3 days. Fig 4, A and E, Kinetics of OX40L induction. Representative plots (median fluorescence intensity in brackets) of OX40L on lung CD11c⁺CD11b^{hi}MHCII^{hi} DCs (Fig 4, B) or mesenteric LN CD11c⁺CD11b⁺MHCII⁺ DCs (Fig 4, F). Fig 4, C, D, G, and H, Quantification of OX40L induction on DCs. Shown are 2 to 3 experiments with 5 to 6 mice per group per experiment. **P* < .05 versus naive. Cae, Caecum; D, duodenum; FMO, fluorescence minus one; I, ileum; J, jejunum; LI, large intestine.

days of HDM exposure, lung IL-25 and IL-33 were induced with slightly different kinetics (Fig 6, A and C). Similarly, acute peanut plus CT exposure increased duodenal levels of IL-25 and IL-33 (Fig 6, B and D). These cytokines were not detected in the serum (data not shown), suggesting local rather than systemic influence. They were also not increased in the bronchoalveolar lavage fluid or intestinal washes of allergen-exposed mice (not shown), suggesting that basolateral rather than apical secretion of these cytokines was associated with allergic sensitization.

Because upregulation of IL-25 and IL-33 in allergen-exposed tissues (Fig 6, A–D) preceded or was coincident with increased DC OX40L expression (Fig 4), we next evaluated whether IL-25 and IL-33, like TSLP, also induced DC OX40L *in vitro*. DCs have been previously shown to be responsive to IL-33³⁸ and IL-25.^{39,40} Here we show that these cytokines were equally capable of inducing OX40L on DCs *in vitro* (Fig 6, E and F). TSLP levels were undetectable in all cultures that did not receive TSLP; likewise, IL-25 and IL-33 were undetectable in all cultures that did not receive IL-25 or IL-33 (data not shown). These data suggest that allergen exposure results in the elaboration of multiple epithelium-associated cytokines that converge on DC maturation, including OX40L upregulation. Consequently, we considered whether IL-25, IL-33, or both were important for the induction of allergic disease *in vivo*.

IL-25 and its receptor are dispensable for pulmonary and gastrointestinal allergic sensitization

IL-25 signaling requires the presence of its cytokine-binding receptor subunit, IL-17RB,¹⁴ and exogenous recombinant IL-25 results in severe T_H2 pathology in the lung and gut.¹³ Hence to investigate the requirement of IL-25 signaling on the induction of T_H2 responses to HDM and peanut, we attempted to induce allergic asthma or peanut allergy in IL-17RB KO mice.

Compared with saline-exposed mice, HDM-exposed WT or IL-17RB KO mice had similarly increased HDM-specific IgG₁ and

total IgE levels and, accordingly, B-cell activation, suggesting equivalent sensitization (Fig 7, A–C). Total inflammatory cell numbers, proportions and numbers of eosinophils, and goblet cell responses were comparable between the HDM-exposed WT and IL-17RB KO groups (Fig 7, D–F). In regard to T-cell immunity, CD69⁺CD4⁺ T cells and *ex vivo* HDM-specific cytokine production were equally increased to greater than that seen with saline controls in both WT and IL-17RB KO mice (Fig 7, G and H). Thus T_H2 responses to HDM can fully develop in the absence of IL-25 signaling.

Similar to pulmonary T_H2 responses to HDM, gastrointestinal sensitization of WT and IL-17RB KO mice to peanut resulted in indistinguishably increased peanut-specific IgG₁ and IgE levels above those seen in corresponding naive mice (Fig 7, I and J). Upon peanut challenge, both groups of peanut-sensitized mice had similar anaphylactic responses (Fig 7, K–M). Deficiency of IL-25 signaling also did not impair T-cell priming, as evidenced by peritoneal inflammation and cytokine production (Fig 7, N–P).

Treatment of mice with an anti-IL-25 antibody before and throughout sensitization to HDM or peanut resulted in data similar to those generated by using IL-17RB KO mice (see Fig E5 in this article's Online Repository at www.jacionline.org). Therefore although IL-25 is capable of inducing OX40L *in vitro*, these data comprehensively show that IL-25 signaling is not required to generate B- and T-cell allergic immunity to HDM or peanut *in vivo*.

IL-33 signaling mediates allergy to peanut and HDM

Because neither TSLP nor IL-25 played a significant role in initiating HDM or peanut allergic phenotypes, we investigated the relevance of IL-33, which binds the ST2 receptor.¹⁵ Compared with HDM-exposed WT mice, serum HDM-specific IgG₁ levels were significantly decreased and IgE levels were diminished by more than 50% in ST2 KO mice (Fig 8, A and B). Similarly, the extent of airway and lung inflammation and mucus production

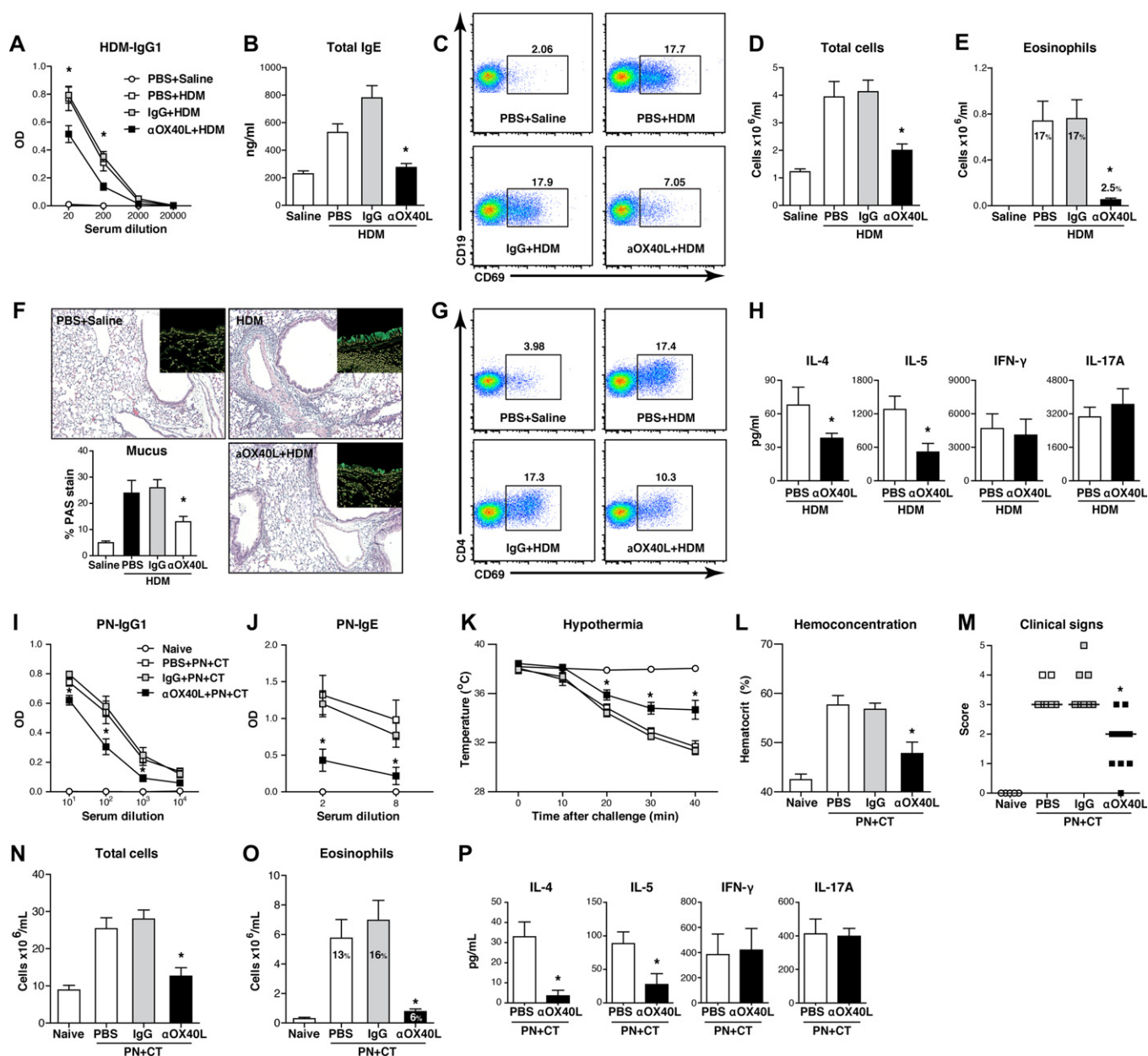


FIG 5. OX40L is required for allergic sensitization to HDM and peanut (PN). **A-H**, Mice received PBS vehicle, IgG, or anti-OX40L before and throughout 10 days of saline or HDM exposure. HDM-specific IgG₁ (Fig 5, A) and total IgE (Fig 5, B) levels in serum. Fig 5, C, Representative CD69 expression by lung CD45⁺CD3⁺CD19⁺ B cells. Total cell (Fig 5, D) and eosinophil (Fig 5, E) counts in bronchoalveolar lavage fluid; mean percentages of eosinophils are shown in bars. Fig 5, F, Representative lung histology stained with hematoxylin and eosin (×100 magnification) or periodic acid–Schiff (PAS) insets (×400 magnification). HDM exemplifies similar sections from PBS plus HDM and IgG plus HDM groups. Periodic acid–Schiff quantification is shown at bottom left. Fig 5, G, Representative CD69 expression by lung CD45⁺CD3⁺CD4⁺ T cells. Fig 5, H, Cytokine production by HDM-stimulated splenocytes. **I-P**, Mice received PBS, IgG, or anti-OX40L during peanut sensitization and were challenged 1 week later. Serum peanut-specific IgG₁ (Fig 5, I) and IgE (Fig 5, J) levels 1 day before challenge. Fig 5, K-M, Anaphylactic assessment. Total (Fig 5, N) and eosinophil (Fig 5, O) cell counts in peritoneal lavage; mean percentages of eosinophils are shown in bars. Fig 5, P, Cytokine production by peanut-stimulated splenocytes. Shown are 3 to 8 mice per group per experiment. **P* < .05 versus PBS or IgG mice exposed to HDM or peanut plus CT.

was lower in HDM-exposed ST2 KO mice, with the infiltration of eosinophils decreased by more than 80% (Fig 8, C-E). The proportion of activated CD4⁺ T cells in the ST2 KO mice was dramatically decreased, as was the *ex vivo* production of IL-4,

IL-5, IFN-γ, and IL-17A (Fig 8, F and G), suggesting an overall impairment in adaptive immune priming.

In response to the peanut sensitization regimen, peanut-specific IgG₁ and IgE levels were diminished by more than 80% in ST2

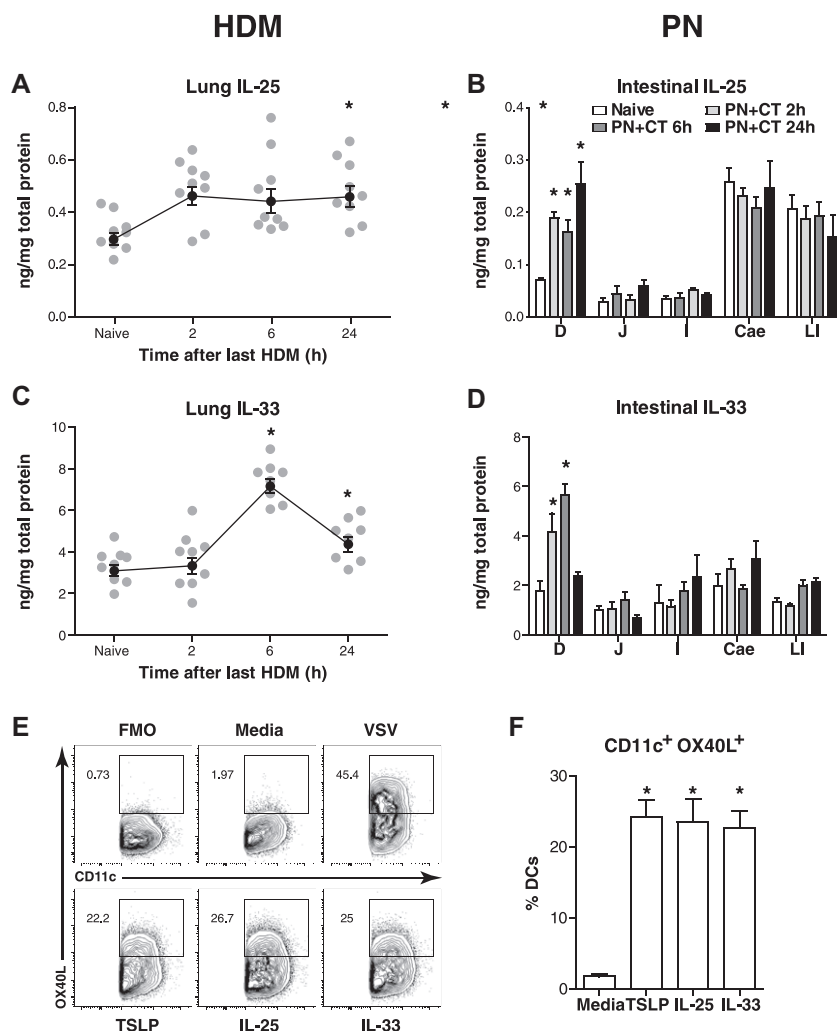


FIG 6. IL-25 and IL-33 induce OX40L on DCs *in vitro*. Kinetics of IL-25 (**A** and **B**) and IL-33 (**C** and **D**) induction after 3 days of HDM or peanut (PN) plus CT. Representative plots (**E**) and quantification (**F**) of CD11c⁺OX40L⁺ BMDCs stimulated with media, VSV, recombinant TSLP, IL-25, or IL-33. Two experiments were performed with 5 to 6 mice per group per experiment (Fig 6, A-D) or replicates of 2 to 4 (Fig 6, E and F). **P* < .05 versus naive or media. Cae, Caecum; D, duodenum; J, jejunum; LI, large intestine.

KO mice compared with those seen in WT control animals (Fig 8, H and I). This translated into protection from anaphylactic shock on peanut challenge because these mice did not exhibit hypothermia and had 50% less hemoconcentration and minor clinical signs of anaphylaxis compared with those seen in sensitized and challenged WT mice (Fig 8, J-L). Eosinophilic inflammation in peanut plus CT-exposed ST2 KO mice was markedly reduced by about 95%, suggesting severely inhibited T_H2 priming (Fig 8, M and N). In agreement, T_H2 cytokine production *ex vivo* was also significantly decreased in ST2 KO mice (Fig 8, O).

Although IL-33 has been reported to play a role during the effector phase of passive anaphylaxis to 2,4-dinitrophenol,⁴¹ we did not find IL-33 to drive the anaphylactic event in our model of active anaphylaxis to peanut. ST2-Fc treatment only during the sensitization phase ameliorated anaphylaxis because of reduced peanut-specific IgE production (see Fig E6, A-D, in this article's Online Repository at www.jacionline.org); treatment before induction of anaphylaxis⁴¹ had no effect on hypothermia or hemoconcentration (see Fig E6, E-H). These data point toward

a central role for IL-33 during sensitization, rather than the effector phase of peanut allergy. Collectively, these data show that unlike TSLP and IL-25, IL-33 plays a nonredundant role in the initiation of respiratory (HDM) and food (peanut) allergy.

Allergen-elicited IL-33 potently induces DC OX40L and ILCs *in vivo*

We observed that TSLP, IL-25, and IL-33 could induce OX40L *in vitro*, yet only IL-33 was required for the generation of T_H2 immunity to HDM or peanut *in vivo*. To better understand how DCs responded to these cytokines *in vivo*, mice received 0.5 μg of recombinant TSLP, IL-25, or IL-33 intranasally for 3 days, and 24 hours later, tissues were analyzed.^{12,14,42} In the lung the levels of total and DC OX40L were increased in response to IL-33 but not TSLP or IL-25 (Fig 9, A and B), suggesting that OX40L induction *in vivo* is exquisitely sensitive to IL-33.

Correlating with OX40L induction, we also observed the expansion of a lineage-negative population (CD45⁺Lin⁻;

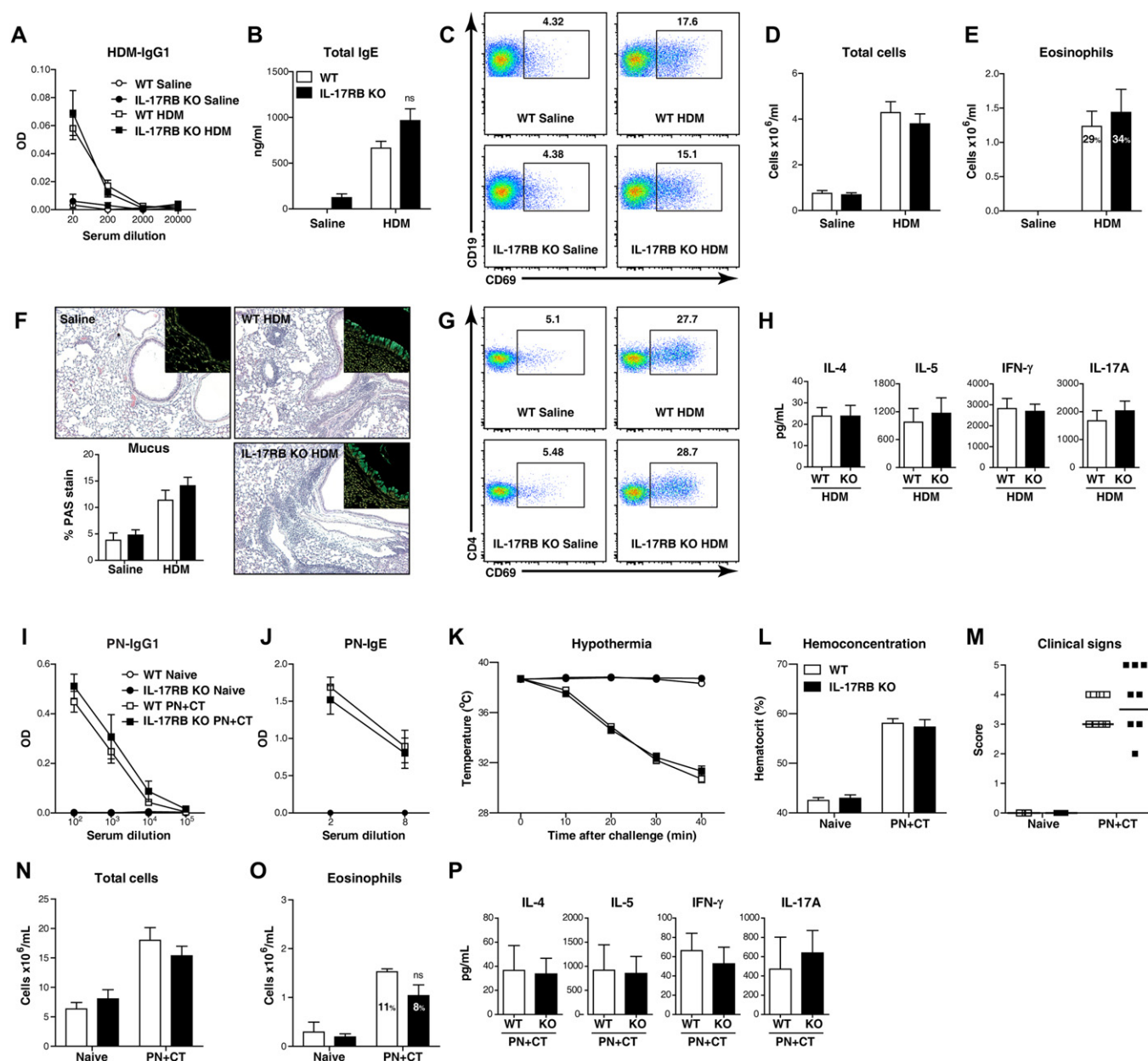


FIG 7. IL-17RB is dispensable for allergic sensitization to HDM and peanut (PN). **A-H**, WT and IL-17RB KO mice were exposed to 10 days of HDM. Serum HDM-specific IgG₁ (Fig 7, A) and total IgE (Fig 7, B) levels. Fig 7, C, Representative lung CD45⁺CD3⁺CD19⁺ B-cell expression of CD69. Total (Fig 7, D) and eosinophil (Fig 7, E) counts in bronchoalveolar lavage fluid; mean percentages are shown in bars. Fig 7, F, Representative lung histology stained with hematoxylin and eosin ($\times 100$ magnification) or periodic acid-Schiff (PAS; $\times 400$ magnification) insets. Saline exemplifies similar sections from the WT saline and IL-17RB KO saline groups. Periodic acid-Schiff analysis is shown at bottom left. Fig 7, G, Expression of CD69 by lung CD45⁺CD3⁺CD4⁺ T cells. Fig 7, H, Cytokine production by HDM-stimulated splenocytes. **I-P**, WT or IL-17RB KO mice were sensitized to peanut and challenged intraperitoneally 1 week later. Serum peanut-specific IgG₁ (Fig 7, I) and IgE (Fig 7, J) levels 1 day before challenge. Fig 7, K-M, Anaphylactic assessment. Total (Fig 7, N) and eosinophil (Fig 7, O) cell counts in peritoneal lavage fluid; mean percentages are shown in bars. Fig 7, P, Cytokine production by peanut-stimulated splenocytes. Shown are 2 experiments with 2 to 8 mice per group per experiment.

CD3⁺CD19/B220⁺CD11c⁺CD11b⁺ expressing c-kit, Sca-1, and Thy1.2 (CD90.2) after administration of IL-33 but not TSLP or IL-25 (Fig 9, C and D). Expression of ST2 and inducible costimulator was present on 30% to 50% of these cells (data not shown). Recent reports describe these novel cells as ILCs, which can respond to IL-25, IL-33, or both and expand on airway viral

or gastrointestinal helminth infection.¹⁷ Increased OX40L expression and expansion of ILCs was observed not only in the lung but also in the thoracic LNs, small intestine, and mesenteric LNs upon intraperitoneal IL-33 (data not shown). These data suggest that T_H2 induction to HDM or peanut relies on the ability of IL-33 to upregulate OX40L and expand ILCs. Accordingly, the

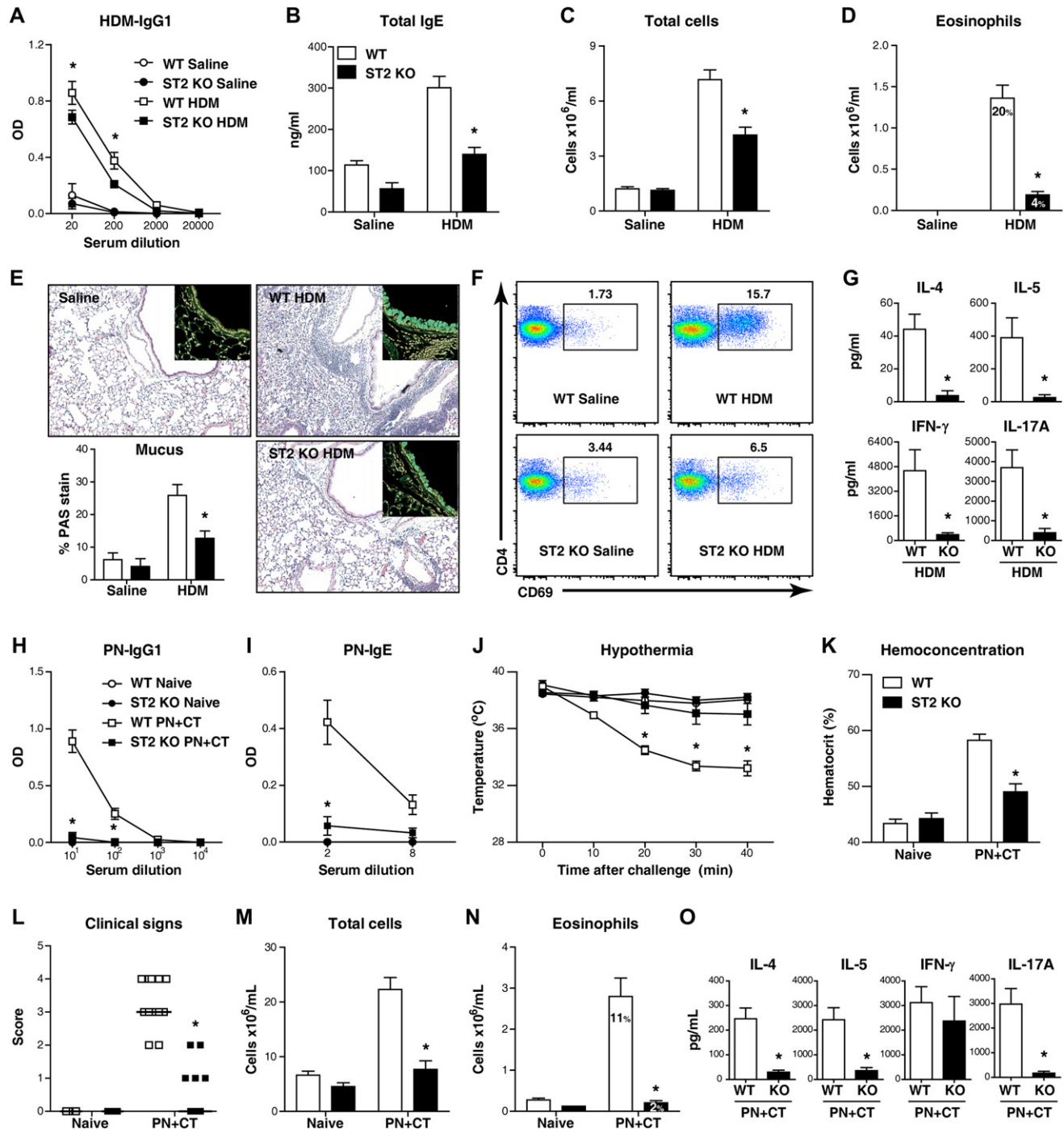


FIG 8. The IL-33 receptor ST2 is required for allergic sensitization to HDM and peanut (PN). **A-G**, WT and ST2 KO mice were exposed to 10 days of HDM. Serum HDM-specific IgG₁ (Fig 8, A) and total IgE (Fig 8, B) levels. Total (Fig 8, C) and eosinophil (Fig 8, D) counts in bronchoalveolar lavage fluid; mean percentages are shown in bars. Fig 8, E, Representative lung histology stained with hematoxylin and eosin ($\times 10$ magnification) or periodic acid-Schiff (PAS; $\times 40$ magnification) insets. Saline exemplifies similar sections from the WT saline and ST2 KO saline groups. Periodic acid-Schiff analysis is shown at bottom left. Fig 8, F, Expression of CD69 by lung CD45⁺CD3⁺CD4⁺ T cells. Fig 8, G, Cytokine production from HDM-stimulated splenocytes. **H-O**, WT or ST2 KO mice were sensitized to peanut and then challenged intraperitoneally 1 week later. Serum peanut-specific IgG₁ (Fig 8, H) and IgE (Fig 8, I) levels 1 day before challenge. Fig 8, J-L, Anaphylactic assessment. Total (Fig 8, M) and eosinophil (Fig 8, N) cell counts in peritoneal lavage fluid; mean percentages are shown in bars. Fig 8, O, Cytokine production by peanut-stimulated splenocytes. Shown are 2 experiments with 2 to 8 mice per group per experiment. * $P < .05$ versus WT mice exposed to HDM or peanut plus CT.

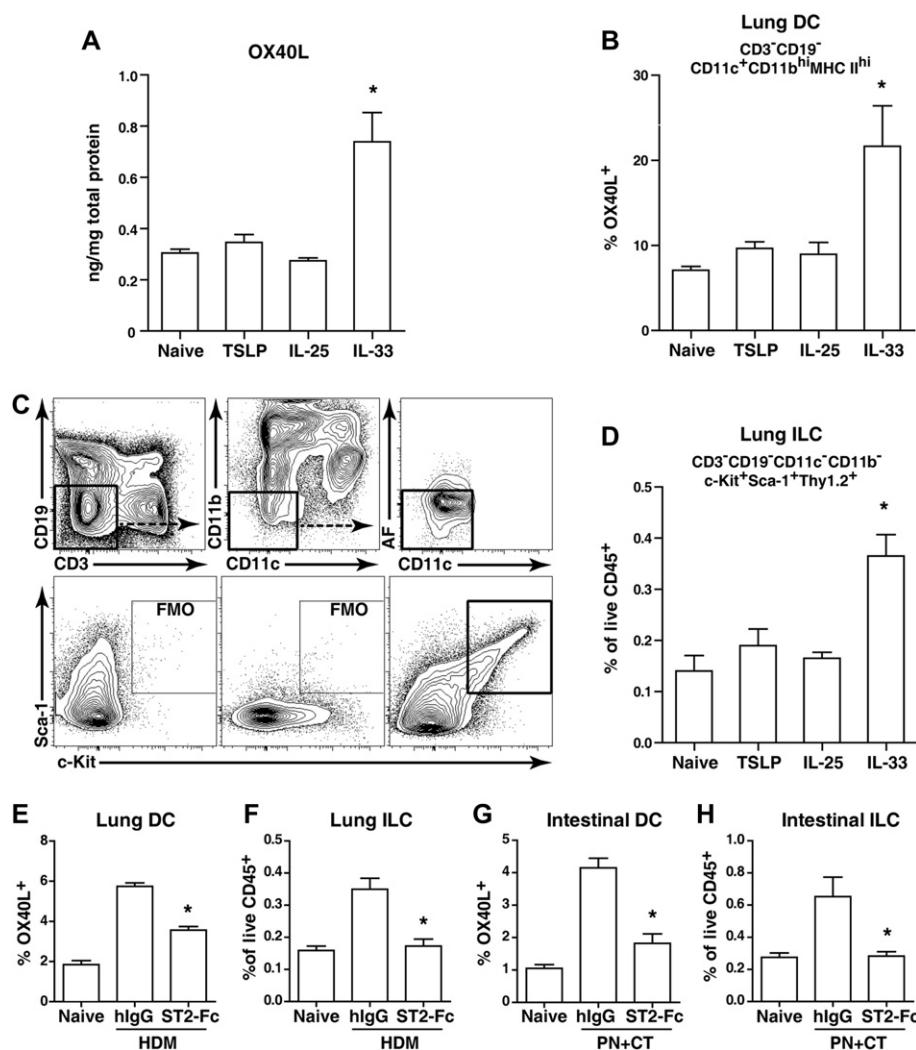


FIG 9. HDM and peanut (PN) induce DC OX40L and ILCs *in vivo* through IL-33. Recombinant TSLP, IL-25, or IL-33 (A-D) or HDM or peanut plus CT (E-H) was administered intranasally or intragastrically for 3 days. Fig 9, A, OX40L normalized to total protein in lung homogenates. Fig 9, B, Quantification of DC OX40L induction. Fig 9, C, Gating strategy for ILCs. Fig 9, D, Quantification of ILC expansion. Fig 9, E-H, Effect of ST2-Fc or IgG on DC OX40L and ILC induction. Shown are 2 to 3 experiments with 2 to 5 mice per group per experiment. **P* < .05 versus naive, TSLP, or IL-25 groups (Fig 9, A-D) or allergen-exposed immunoglobulin-treated mice (Fig 9, E-H). AF, Autofluorescence; FMO, fluorescence minus one.

proportion of lung and small intestinal OX40L⁺ DCs and ILCs was abrogated in allergen-exposed mice treated with ST2-Fc compared with those treated with control immunoglobulin (Fig 9, E-H). IL-33, more so than TSLP or IL-25, is therefore central to HDM- or peanut-mediated upregulation of OX40L and expansion of ILCs during allergic sensitization.

DISCUSSION

It has been increasingly recognized that T_H2 immunity results from adaptive immune responses that are shaped by initial innate signals. Among these, epithelium-associated cytokines, such as TSLP, IL-25, and IL-33, have been the subject of intense investigation. Here we investigated the initiation of allergic asthma and food allergy using models involving 2 clinically relevant allergens and different mucosal sites and sensitization strategies. We show that the development of HDM and peanut allergy, both of which evolve through canonical T_H2 pathways (see Fig E3),²⁴ is TSLP

and IL-25 independent and, conversely, requires IL-33 for OX40L induction, ILC expansion, and, ultimately, disease manifestation. We used established KO mice, with their proper strain-matched control animals, as well as neutralizing antibody strategies to validate our conclusions.

Studies in models of allergic inflammation^{26,27} have led to the proposition that TSLP is a master regulator of allergic responses.^{22,35} Notably, sensitization in these studies was achieved through exposure of a nonmucosal site (eg, peritoneal cavity) to the model antigen OVA adsorbed to aluminum-based adjuvants. At variance, our data in the HDM model demonstrate that the extent of allergic sensitization, airway eosinophilic inflammation, and lung pathology was comparable in the presence or absence of TSLP signaling. Furthermore, peanut sensitization, systemic anaphylactic responses, and eosinophilic inflammation were intact in the absence of TSLP, its receptor or basophils. Similarly, anaphylaxis was not prevented when using OVA instead of peanut, which is in accordance with a recent report showing intact

OVA plus CT-induced anaphylaxis in TSLPR-deficient mice.⁴³ Thus the requirement for TSLP in generating T_H2 immunity to allergens is not as pervasive as originally proposed. To our knowledge, TSLP-independent T_H2 responses have only been reported in helminth infection systems.^{8,44}

The ability of TSLP to induce T_H2 responses has been mainly attributed to inhibition of IL-12 production and upregulation of OX40L on DCs.^{8,10,11} Here we show that HDM and peanut inhibited DC IL-12 production similarly to TSLP, thus making it redundant. This concept is in agreement with data suggesting that TSLP-independent antihelminth responses are due to helminth excretory-secretory products that supplant TSLP inhibition of DC IL-12.⁸ Furthermore, HDM and peanut also mimicked TSLP induction of OX40L on DCs. Thus our data identify common allergens (HDM and peanut) that mimic TSLP actions on DCs, resulting in the induction of T_H2 responses independently of TSLP.

In OVA peptide-pulsed DC/T-cell cocultures, Blázquez and Berin⁴⁵ suggested a critical role for OX40L in T_H2 cytokine production. Here we found that allergen exposure induced OX40L on DCs *in vitro*, as well as *in vivo*, in the lung and duodenum. Neutralization of OX40L significantly impaired HDM or peanut sensitization, with effects similar to those reported recently in HDM-exposed OX40 KO mice.⁴⁶ Thus our work shows that these models of HDM and peanut allergy originate from a TSLP-independent but OX40L-dependent pathway.

We (Fig 6) and others^{25,47} have shown increased levels of IL-25 and IL-33 in the lung after HDM exposure. Here we also report that these cytokines are produced in the duodenum after peanut plus CT exposure. TSLP and IL-33 have been shown to induce OX40L on DCs *in vitro*.^{11,48} We confirm these data and further demonstrate that IL-25 can upregulate OX40L to a similar extent as TSLP and IL-33. Consequently, these data indicate that multiple epithelium-associated cytokines are elaborated upon allergen exposure to the respiratory and gastrointestinal mucosae, all converging on, at least, OX40L upregulation on DCs for T_H2 induction.

Although exogenous IL-25 is capable of inducing T_H2 responses,¹³ we found no significant effect on immunoglobulin, cytokine, and inflammatory parameters when we blocked IL-25 during HDM or peanut sensitization or examined IL-17RB KO mice. These findings are at variance with previous reports using experimental models of intraperitoneal OVA plus alum-induced asthma in which blockade of IL-25 significantly reduced T_H2 cytokine production and eosinophilic inflammation.⁴⁹ IL-25 has also been implicated in promoting T_H2 immunity in host defense against some helminths that infect the gut.^{50,51} Thus although IL-25 is increasingly considered key in the regulation of T_H2 immunity,⁵² our data clearly indicate that IL-25 is not necessary for T_H2 priming and subsequent effector responses to allergens, such as HDM and peanut.

With respect to IL-33, Coyle et al⁵³ initially showed a critical role for ST2, which was later identified as the IL-33 receptor, in both T_H2 cellular and humoral responses in an OVA model.⁵³ However, subsequent studies yielded variable conclusions on the importance of IL-33 for T_H2 responses.^{54,55} Our data demonstrate that IL-33 signaling is required for T_H2 priming to both HDM and peanut. These effects translated into significantly less lung pathology and anaphylaxis and were associated with a superior ability of IL-33, over TSLP or IL-25, to increase OX40L levels, particularly on DCs, *in vivo*. In addition, IL-33 markedly

expanded a population of ILCs, which have recently been identified and shown to be capable of producing T_H2 cytokines.¹⁷ Although ILCs were reported to be expanded during secondary responses to OVA,⁵⁶ we report, for the first time, that allergic sensitization to HDM and peanut are associated with ILC expansion and that this is IL-33 dependent.

TSLP, IL-25, and IL-33 are typically recognized as epithelium-derived cytokines, but other cell types are able to produce these cytokines, particularly in chronic inflammatory states. The specific cellular sources, targets, and functional effects of these cytokines on chronic allergic disease severity, progression, and/or exacerbation are only beginning to be investigated. For example, IL-25 has recently been shown to be associated with collagen deposition, lung dysfunction, or both in atopic patients and mice,^{57,58} and Oboki et al⁴² have generated IL-33 KO mice and suggested IL-33 to be important in the maintenance of airway inflammation in a model of chronic HDM exposure.

Our data, along with those of previous reports using other experimental systems, reveal that TSLP, IL-25, and IL-33 are differentially required to generate T_H2 responses. For example, antihelminth host defense to *Trichuris muris* is dependent on TSLP, IL-25, and IL-33, whereas *Nippostrongylus brasiliensis* requires IL-25 but not TSLP or IL-33.^{8,10,50,54,59} In the case of allergic responses, OVA plus alum-driven T_H2 initiation is dependent on all of these cytokines (Fig E1, G-K),^{26,27,49,53} whereas HDM- and peanut plus CT-induced responses rely on IL-33 but not TSLP or IL-25. Clearly, diverse antigen-host interactions are able to launch distinct molecular programs highlighting a diversity of pathways leading to T_H2 immunity. This might provide a mechanistic basis for the growing clinical recognition that allergic diseases are heterogeneous. Whether these clinicopathologic variants/endotypes are a result of different subtypes of T_H2 cells (eg, TSLP driven, IL-25 driven, and/or IL-33 driven), each with their own molecular signature and effector armamentarium and, ultimately, distinct therapeutic susceptibilities, remains to be explored.

We thank Drs Jack Gaudie, Jonathan Bramson, and Kathy McCoy for insightful comments. We thank Michael R. Comeau and Dr Alison L. Budelsky (Amgen) for providing TSLPR KO and IL-17RB KO mice and antibodies, Dr Andrew N. J. McKenzie for the ST2 KO and IL-13 KO mice, and Dr Waliul Khan for the STAT6 KO mice. We also thank Mary Jo Smith, Mary Bruni, Joshua Kong, Samuel Legge, and Melissa Provenza for technical help; Marie Bailey for administrative assistance; and Dr Christine Keng and Marcel Serra-Julià for additional support.

Key messages

- TSLP and IL-25 are redundant but IL-33 is central in the initiation of T_H2 responses to the common allergens HDM and peanut.
- IL-33-mediated allergic sensitization to these airway and food allergens requires DC OX40L and is associated with expansion of ILCs.

REFERENCES

1. Arshad SH, Tariq SM, Matthews S, Hakim E. Sensitization to common allergens and its association with allergic disorders at age 4 years: a whole population birth cohort study. *Pediatrics* 2001;108:E33.

2. Sunyer J, Jarvis D, Pekkanen J, Chinn S, Janson C, Leynaert B, et al. Geographic variations in the effect of atopy on asthma in the European Community Respiratory Health Study. *J Allergy Clin Immunol* 2004;114:1033-9.
3. Chafen JJ, Newberry SJ, Riedl MA, Bravata DM, Maglione M, Suttrop MJ, et al. Diagnosing and managing common food allergies: a systematic review. *JAMA* 2010;303:1848-56.
4. Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, et al. The prevalence of food allergy: a meta-analysis. *J Allergy Clin Immunol* 2007;120:638-46.
5. Burks AW. Peanut allergy. *Lancet* 2008;371:1538-46.
6. Bulek K, Swaidani S, Aronica M, Li X. Epithelium: the interplay between innate and Th2 immunity. *Immunol Cell Biol* 2010;88:257-68.
7. Ziegler SF, Artis D. Sensing the outside world: TSLP regulates barrier immunity. *Nat Immunol* 2010;11:289-93.
8. Massacand JC, Stettler RC, Meier R, Humphreys NE, Grecis RK, Marsland BJ, et al. Helminth products bypass the need for TSLP in Th2 immune responses by directly modulating dendritic cell function. *Proc Natl Acad Sci U S A* 2009;106:13968-73.
9. Zaph C, Troy AE, Taylor BC, Berman-Booty LD, Guild KJ, Du Y, et al. Epithelial-cell-intrinsic IKK-beta expression regulates intestinal immune homeostasis. *Nature* 2007;446:552-6.
10. Taylor BC, Zaph C, Troy AE, Du Y, Guild KJ, Comeau MR, et al. TSLP regulates intestinal immunity and inflammation in mouse models of helminth infection and colitis. *J Exp Med* 2009;206:655-67.
11. Ito T, Wang YH, Duramad O, Hori T, Delespesse GJ, Watanabe N, et al. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med* 2005;202:1213-23.
12. Seshasayee D, Lee WP, Zhou M, Shu J, Suto E, Zhang J, et al. In vivo blockade of OX40 ligand inhibits thymic stromal lymphopoietin driven atopic inflammation. *J Clin Invest* 2007;117:3868-78.
13. Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity* 2001;15:985-95.
14. Rickel EA, Siegel LA, Yoon BR, Rottman JB, Kugler DG, Swart DA, et al. Identification of functional roles for both IL-17RB and IL-17RA in mediating IL-25-induced activities. *J Immunol* 2008;181:4299-310.
15. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005;23:479-90.
16. Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? *Nat Rev Immunol* 2010;10:225-35.
17. Spits H, Cupedo T. Innate lymphoid cells: emerging insights in development, lineage relationships, and function. *Annu Rev Immunol* 2012;30:647-75.
18. Cates EC, Fattouh R, Wattie J, Inman MD, Goncharova S, Coyle AJ, et al. Intranasal exposure of mice to house dust mite elicits allergic airway inflammation via a GM-CSF-mediated mechanism. *J Immunol* 2004;173:6384-92.
19. Llop-Guevara A, Colangelo M, Chu DK, Moore CL, Stieber NA, Walker TD, et al. In vivo-to-in silico iterations to investigate aeroallergen-host interactions. *PLoS One* 2008;3:e2426.
20. Sun J, Arias K, Alvarez D, Fattouh R, Walker T, Goncharova S, et al. Impact of CD40 ligand, B cells, and mast cells in peanut-induced anaphylactic responses. *J Immunol* 2007;179:6696-703.
21. Arias K, Chu DK, Flader K, Botelho F, Walker T, Arias N, et al. Distinct immune effector pathways contribute to the full expression of peanut-induced anaphylactic reactions in mice. *J Allergy Clin Immunol* 2011;127:1552-61.e1.
22. Liu YJ. Thymic stromal lymphopoietin: master switch for allergic inflammation. *J Exp Med* 2006;203:269-73.
23. Cates EC, Fattouh R, Johnson JR, Llop-Guevara A, Jordana M. Modeling responses to respiratory house dust mite exposure. *Contrib Microbiol* 2007;14:42-67.
24. Johnson JR, Swirski FK, Gajewska BU, Wiley RE, Fattouh R, Pacitto SR, et al. Divergent immune responses to house dust mite lead to distinct structural-functional phenotypes. *Am J Physiol Lung Cell Mol Physiol* 2007;293:L730-9.
25. Kool M, Willart MA, van Nimwegen M, Bergen I, Pouliot P, Virchow JC, et al. An unexpected role for uric acid as an inducer of T helper 2 cell immunity to inhaled antigens and inflammatory mediator of allergic asthma. *Immunity* 2011;34:527-40.
26. Al-Shami A, Spolski R, Kelly J, Keane-Myers A, Leonard WJ. A role for TSLP in the development of inflammation in an asthma model. *J Exp Med* 2005;202:829-39.
27. Zhou B, Comeau MR, De Smedt T, Liggitt HD, Dahl ME, Lewis DB, et al. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immunol* 2005;6:1047-53.
28. Arias K, Baig M, Colangelo M, Chu D, Walker T, Goncharova S, et al. Concurrent blockade of platelet-activating factor and histamine prevents life-threatening peanut-induced anaphylactic reactions. *J Allergy Clin Immunol* 2009;124:307-14, e1-2.
29. Siracusa MC, Saenz SA, Hill DA, Kim BS, Headley MB, Doering TA, et al. TSLP promotes interleukin-3-independent basophil haematopoiesis and type 2 inflammation. *Nature* 2011;477:229-33.
30. Nakanishi K. Basophils as APC in Th2 response in allergic inflammation and parasite infection. *Curr Opin Immunol* 2010;22:814-20.
31. Hammad H, Plantinga M, Deswarte K, Pouliot P, Willart MA, Kool M, et al. Inflammatory dendritic cells—not basophils—are necessary and sufficient for induction of Th2 immunity to inhaled house dust mite allergen. *J Exp Med* 2010;207:2097-111.
32. Nishikado H, Mukai K, Kawano Y, Minegishi Y, Karasuyama H. NK cell-depleting anti-asialo GM1 antibody exhibits a lethal off-target effect on basophils in vivo. *J Immunol* 2011;186:5766-71.
33. Zhou B, Headley MB, Aye T, Tocker J, Comeau MR, Ziegler SF. Reversal of thymic stromal lymphopoietin-induced airway inflammation through inhibition of Th2 responses. *J Immunol* 2008;181:6557-62.
34. Jessup HK, Brewer AW, Omori M, Rickel EA, Budelsky AL, Yoon BR, et al. Intradermal administration of thymic stromal lymphopoietin induces a T cell- and eosinophil-dependent systemic Th2 inflammatory response. *J Immunol* 2008;181:4311-9.
35. Ziegler SF. The role of thymic stromal lymphopoietin (TSLP) in allergic disorders. *Curr Opin Immunol* 2010;22:795-9.
36. Ito T, Liu YJ, Arima K. Cellular and molecular mechanisms of TSLP function in human allergic disorders—TSLP programs the “Th2 code” in dendritic cells. *Allergol Int* 2012;61:35-43.
37. Kashyap M, Rochman Y, Spolski R, Samsel L, Leonard WJ. Thymic stromal lymphopoietin is produced by dendritic cells. *J Immunol* 2011;187:1207-11.
38. Rank MA, Kobayashi T, Kozaki H, Bartemes KR, Squillace DL, Kita H. IL-33-activated dendritic cells induce an atypical TH2-type response. *J Allergy Clin Immunol* 2009;123:1047-54.
39. Claudio E, Sonder SU, Saret S, Carvalho G, Ramalingam TR, Wynn TA, et al. The adaptor protein CIKS/Act1 is essential for IL-25-mediated allergic airway inflammation. *J Immunol* 2009;182:1617-30.
40. Gratchev A, Kzhyshkowska J, Duperrier K, Utikal J, Velten FW, Goerdts S. The receptor for interleukin-17E is induced by Th2 cytokines in antigen-presenting cells. *Scand J Immunol* 2004;60:233-7.
41. Pushparaj PN, Tay HK, H'Ng SC, Pitman N, Xu D, McKenzie A, et al. The cytokine interleukin-33 mediates anaphylactic shock. *Proc Natl Acad Sci U S A* 2009;106:9773-8.
42. Oboki K, Ohno T, Kajiwara N, Arae K, Morita H, Ishii A, et al. IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc Natl Acad Sci U S A* 2010;107:18581-6.
43. Blázquez AB, Mayer L, Berin MC. Thymic stromal lymphopoietin is required for gastrointestinal allergy but not oral tolerance. *Gastroenterology* 2010;139:1301-9.
44. Ramalingam TR, Pesce JT, Mentink-Kane MM, Madala S, Cheever AW, Comeau MR, et al. Regulation of helminth-induced Th2 responses by thymic stromal lymphopoietin. *J Immunol* 2009;182:6452-9.
45. Blázquez AB, Berin MC. Gastrointestinal dendritic cells promote Th2 skewing via OX40L. *J Immunol* 2008;180:4441-50.
46. Damayanti T, Kikuchi T, Zaini J, Daito H, Kanehira M, Kohu K, et al. Serial OX40 engagement on CD4+ T cells and natural killer T cells causes allergic airway inflammation. *Am J Respir Crit Care Med* 2010;181:688-98.
47. Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med* 2009;15:410-6.
48. Besnard AG, Togbe D, Guillou N, Erard F, Quesniaux V, Ryffel B. IL-33-activated dendritic cells are critical for allergic airway inflammation. *Eur J Immunol* 2011;41:1675-86.
49. Ballantyne SJ, Barlow JL, Jolin HE, Nath P, Williams AS, Chung KF, et al. Blocking IL-25 prevents airway hyperresponsiveness in allergic asthma. *J Allergy Clin Immunol* 2007;120:1324-31.
50. Fallon PG, Ballantyne SJ, Mangan NE, Barlow JL, Dasvarma A, Hewett DR, et al. Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *J Exp Med* 2006;203:1105-16.
51. Owyang AM, Zaph C, Wilson EH, Guild KJ, McClanahan T, Miller HR, et al. Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. *J Exp Med* 2006;203:843-9.
52. Barlow JL, McKenzie AN. IL-25: a key requirement for the regulation of type-2 immunity. *Biofactors* 2009;35:178-82.
53. Coyle AJ, Lloyd C, Tian J, Nguyen T, Eriksson C, Wang L, et al. Crucial role of the interleukin 1 receptor family member T1/ST2 in T helper cell type 2-mediated lung mucosal immune responses. *J Exp Med* 1999;190:895-902.
54. Hoshino K, Kashiwamura S, Kuribayashi K, Kodama T, Tsujimura T, Nakanishi K, et al. The absence of interleukin 1 receptor-related T1/ST2 does not affect

- T helper cell type 2 development and its effector function. *J Exp Med* 1999; 190:1541-8.
55. Townsend MJ, Fallon PG, Matthews DJ, Jolin HE, McKenzie AN. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. *J Exp Med* 2000;191:1069-76.
56. Barlow JL, Bellosi A, Hardman CS, Drynan LF, Wong SH, Cruickshank JP, et al. Innate IL-13-producing nuocytes arise during allergic lung inflammation and contribute to airways hyperreactivity. *J Allergy Clin Immunol* 2012;129: 191-8.e4.
57. Corrigan CJ, Wang W, Meng Q, Fang C, Wu H, Reay V, et al. T-helper cell type 2 (Th2) memory T cell-potentiating cytokine IL-25 has the potential to promote angiogenesis in asthma. *Proc Natl Acad Sci U S A* 2011;108:1579-84.
58. Gregory LG, Mathie SA, Walker SA, Pegorier S, Jones CP, Lloyd CM. Overexpression of Smad2 drives house dust mite-mediated airway remodeling and airway hyperresponsiveness via activin and IL-25. *Am J Respir Crit Care Med* 2010;182:143-54.
59. Senn KA, McCoy KD, Maloy KJ, Stark G, Frohli E, Rulicke T, et al. T1-deficient and T1-Fc-transgenic mice develop a normal protective Th2-type immune response following infection with *Nippostrongylus brasiliensis*. *Eur J Immunol* 2000;30:1929-38.

METHODS

Sensitization and treatment protocols

HDM administration. Twenty-five micrograms of HDM extract (Greer Laboratories, Lenoir, NC) in 10 μ L of saline was administered intranasally to isoflurane-anesthetized mice for 3 or 10 consecutive days.^{E1,E2} Mice were killed 24 hours after the last exposure, unless otherwise specified.

Peanut plus CT administration. One milligram of peanut (Kraft, Northfield, Ill) with 10 μ g of CT (List Biological Laboratories, Campbell, Calif) in PBS was gavaged intragastrically by using 12-gauge, 2.5-inch intragastric feeding needles (DELVO SA, Biel/Bienne, Switzerland) daily for 3 consecutive days or weekly for 4 weeks.^{E3-E5} In some experiments 1 mg of OVA was gavaged instead of peanut, or the route of administration was intraperitoneal instead of intragastric.

OVA or peanut and alum administration. Fifty micrograms of OVA (grade V; Sigma, St Louis, Mo) emulsified in 1.3 mg of aluminum hydroxide (Sigma) was administered intraperitoneally on days 1 and 14.^{E6} Anesthetized mice were challenged intranasally with 150 μ g of OVA in 40 μ L of PBS for 4 days, starting from day 21. Mice were analyzed 24 hours after the last challenge. In some experiments peanut was used instead of OVA, and mice were challenged intraperitoneally with 500 μ g of peanut instead of intranasally to induce anaphylaxis and inflammation.

Antibody administration. The following antibodies and corresponding controls (rat, rabbit, or human IgG; Sigma) in PBS were administered intraperitoneally either 24 hours before the first HDM exposure and every 4 to 5 days of the HDM protocol or 24 hours before each peanut sensitization: 1 mg of anti-TSLP or anti-IL-25 (Amgen, with neutralizing activity^{E7,E8}), 250 μ g of ST2-Fc (R&D Systems, Minneapolis, Minn), 200 or 400 μ g of anti-OX40L (R&D Systems), 100 μ L anti-asialo GM1 (Wako, Richmond, Va), or 100 μ g of intravenous Ba103 (Hycult Biotech, Uden The Netherlands). In some experiments 25 μ g of ST2-Fc was administered intranasally.

Cytokine administration. Recombinant mouse TSLP, IL-25, or IL-33 (0.5 μ g, R&D systems or eBioscience [San Diego, Calif]) was administered intranasally or intraperitoneally daily for 3 days, and tissues were analyzed 24 hours later.

Induction and grading of peanut-induced anaphylaxis

One to 2 weeks after the last sensitization, 500 μ g of peanut (Greer Laboratories) or OVA in PBS was injected intraperitoneally. Mice were monitored for 40 minutes after challenge for changes in rectal (core) body temperature (VWR International, West Chester, Pa). Forty minutes after challenge, heparin-anticoagulated blood was centrifuged at 6000 to 6200 rpm for 1 minute to measure hematocrit. Clinical signs were evaluated as follows: 0, no clinical signs; 1, pruritis (repetitive hind leg ear canal scratching); 2, periorbital/periauricular edema, piloerection; 3, lethargy/decreased activity, lying prone; 4, no response to whisker provocation; and 5, end point (seizure or death).

Collection of specimens

Peripheral blood was collected, and serum was stored at -20°C . Lungs underwent lavage with PBS containing complete protease inhibitor (Roche, Mannheim, Germany). Total cell counts were determined by using a hemocytometer. Cytospin preparations were stained with the Protocol Hema 3 stain set (Fisher Scientific, Hampton, NH), and differential cell counts were determined by using standard hemocytologic criteria. In some cases the left lung was slowly inflated and fixed with 10% formalin for histology.

Seventy-two hours after anaphylaxis, peritoneal lavage was performed with 10 mmol/L ice-cold EDTA in PBS.

Tissue homogenization

Samples were collected in 500 μ L of PBS containing complete protease inhibitor (Roche), rotor-stator homogenized (Polytron; Kinematica, Lucerne, Switzerland), incubated with 1% Triton-X100 for 40 minutes at 4°C , and spun,

and supernatants were spun again (12,000 rpm for 10 minutes at 4°C). Supernatants were collected, and protein content was quantified by using the Bradford assay (Bio-Rad Laboratories, Hercules, Calif) and stored at -80°C .

Cytokine and immunoglobulin analysis

Cytokines were measured by using ELISA (kits from eBioscience and R&D Systems) or Luminex assay (multiplex kits from Millipore, Temecula, Calif). Total or antigen-specific immunoglobulin levels were measured by using sandwich ELISA.^{E1,E4,E5,E9} Antigen-specific immunoglobulin ELISAs are depicted as OD – background, where background = $2 \times (\text{SD} + \text{Average of at least 20 blank wells})$.

BMDC generation and stimulation

GM-CSF–derived BMDCs were generated^{E10} and then incubated with media, HDM, crude peanut extract, 100 ng/mL recombinant mouse (rm) TSLP (R&D Systems), rmIL-25 (R&D Systems), and rmIL-33 (R&D Systems) for 16 to 24 hours and analyzed directly or then stimulated with 10 ng/mL *Escherichia coli* 0111:B4 LPS (cell culture tested; Sigma). BMDC viability was greater than 80%. For intracellular cytokine staining, BMDCs were stimulated with or without LPS for 12 hours and brefeldin A (eBioscience) for the last 6 hours. Some BMDCs were infected with 25 multiplicity of infection VSV- Δ M51, which transduces DCs without significant progeny virus production or effect on viability.^{E11}

Cell isolation and culture

Lungs. Lungs were chopped into small pieces and then digested with Collagenase type I (Gibco, Invitrogen, Carlsbad, Calif) in HBSS for 1 hour at 37°C before being pressed through 40- μ m cell strainers, and RBCs were lysed with ACK buffer. Cells were washed and filtered again before analysis.

Intestines. After flushing intestinal contents with cold PBS, the intestines were opened longitudinally, fat and mucus were removed manually, and the intestines were cut into approximately 1-cm pieces before epithelium and mucus were removed by means of repeated 15-minute 37°C incubations in PBS with 10 mmol/L EDTA, 10 mmol/L HEPES, 1 mmol/L dithiothreitol, and vortexing. Epithelium-free lamina propria was then incubated with 0.25 Wünsch units/mL Liberase (Roche) for 60 minutes with 20 U/mL DNase I (Roche), pressed through a 40- μ m strainer, and washed before analysis.

Spleens. Spleens were pressed through 40- μ m strainers and incubated with ACK before being resuspended in complete RPMI (RPMI supplemented with 10% FBS, 1% L-glutamine, 1% penicillin/streptomycin, and 0.1% β -mercaptoethanol [Gibco, Invitrogen]). Viable cells (8×10^5) were cultured in triplicate in medium alone or with 15.625 μ g/mL HDM or 250 μ g/mL crude peanut extract in flat-bottom, 96-well plates (BD, San Jose, Calif). After 5 days, triplicates were pooled, and cell-free supernatants were stored at -20°C .

LN. LNs were triturated between frosted slides in HBSS, washed, and filtered (40 μ m).

Flow cytometry

To minimize nonspecific and Fc receptor–mediated binding, cells were first incubated with anti-CD16/CD32, (2.4G2; eBioscience) in 0.5% to 1% BSA and 10 mmol/L EDTA-supplemented PBS for at least 15 minutes at 4°C . Antibodies were from BD Biosciences, eBioscience, BioLegend (San Diego, Calif), or MD Biosciences (Saint Paul, Minn) and were titrated before use. ST2–fluorescein isothiocyanate (FITC), CD11c–Alexa Fluor (AF) 488, peridinin-chlorophyll-protein complex (PerCP)–Cy5.5, phycoerythrin (PE)–Cy7, or allophycocyanin (APC); OX40L–PE or biotin (eBioscience and Cedarlane [Burlington, NC]); IL-12p40–PE or APC; CD3–PE–Cy5, PerCP–Cy5.5, PE–eFluor 710, PE–Cy7, AF700, or eFluor 450 or V500; CD19–PerCP–Cy5.5, PE–Cy7, or APC–Cy7; c-Kit–PE–Cy5, PE–Cy7, or APC; MHC II (I^A/I^E)–AF700, eFluor 450, or eFluor 650; Sca-1–AF700; CD86–FITC, AF700, or biotin; CD45–APC–Cy7 or eFluor 605; Thy1.2–APC–eFluor 780; CD8–eFluor 450 or PerCP; CD11b–PE–Cy7, AF700, or Brilliant Violet 421; B220–APC–eFluor 780 or V500; CD4–APC, PerCP, eFluor 650, or V500; CD127–PE–Cy7; NK1.1–APC or PE–Cy7; CD49b (DX5)–PE, PE–Cy7 or biotin; CD69–PE or PE–Cy7; IgE–FITC or PE; or inducible

costimulator–FITC were used. Propidium iodide (eBioscience) and forward scatter/side scatter were used to exclude dead cells and doublets. BD Cytfix/Cytoperm was used for intracellular staining. BD CompBeads were used for compensation, except for V500-conjugated antibodies, where cells were stained instead. Data were acquired on a BD LSR II and analyzed with FlowJo software (Tree Star, Ashland, Ore), including software compensation. Fluorescence minus one controls were used for gating.

Histology and morphometric analysis

Lung tissue was embedded in paraffin and cut at a thickness of 4 μm . Sections were stained with hematoxylin and eosin or periodic acid–Schiff. Images of the main airways were analyzed by using a custom system (Northern Eclipse software; Empix Imaging, Mississauga, Ontario, Canada) that calculates the percentage of tissue area that was positively stained within a 30- μm -wide band from the basement membrane extending into the airway lumen.^{E9}

REFERENCES

- E1. Cates EC, Fattouh R, Wattie J, Inman MD, Goncharova S, Coyle AJ, et al. Intranasal exposure of mice to house dust mite elicits allergic airway inflammation via a GM-CSF-mediated mechanism. *J Immunol* 2004;173:6384-92.
- E2. Llop-Guevara A, Colangelo M, Chu DK, Moore CL, Stieber NA, Walker TD, et al. In vivo-to-in silico iterations to investigate aeroallergen-host interactions. *PLoS One* 2008;3:e2426.
- E3. Arias K, Baig M, Colangelo M, Chu D, Walker T, Goncharova S, et al. Concurrent blockade of platelet-activating factor and histamine prevents life-threatening peanut-induced anaphylactic reactions. *J Allergy Clin Immunol* 2009;124:307-14, e1-2.
- E4. Arias K, Chu DK, Flader K, Botelho F, Walker T, Arias N, et al. Distinct immune effector pathways contribute to the full expression of peanut-induced anaphylactic reactions in mice. *J Allergy Clin Immunol* 2011;127:1552-61.e1.
- E5. Sun J, Arias K, Alvarez D, Fattouh R, Walker T, Goncharova S, et al. Impact of CD40 ligand, B cells, and mast cells in peanut-induced anaphylactic responses. *J Immunol* 2007;179:6696-703.
- E6. Zhou B, Comeau MR, De Smedt T, Liggitt HD, Dahl ME, Lewis DB, et al. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immunol* 2005;6:1047-53.
- E7. Taylor BC, Zaph C, Troy AE, Du Y, Guild KJ, Comeau MR, et al. TSLP regulates intestinal immunity and inflammation in mouse models of helminth infection and colitis. *J Exp Med* 2009;206:655-67.
- E8. Rickel EA, Siegel LA, Yoon BR, Rottman JB, Kugler DG, Swart DA, et al. Identification of functional roles for both IL-17RB and IL-17RA in mediating IL-25-induced activities. *J Immunol* 2008;181:4299-310.
- E9. Fattouh R, Midence NG, Arias K, Johnson JR, Walker TD, Goncharova S, et al. Transforming growth factor-beta regulates house dust mite-induced allergic airway inflammation but not airway remodeling. *Am J Respir Crit Care Med* 2008;177:593-603.
- E10. Boudreau J, Koshy S, Cummings D, Wan Y. Culture of myeloid dendritic cells from bone marrow precursors. *J Vis Exp* 2008;17:769.
- E11. Boudreau JE, Bridle BW, Stephenson KB, Jenkins KM, Brunelliere J, Bramson JL, et al. Recombinant vesicular stomatitis virus transduction of dendritic cells enhances their ability to prime innate and adaptive antitumor immunity. *Mol Ther* 2009;17:1465-72.

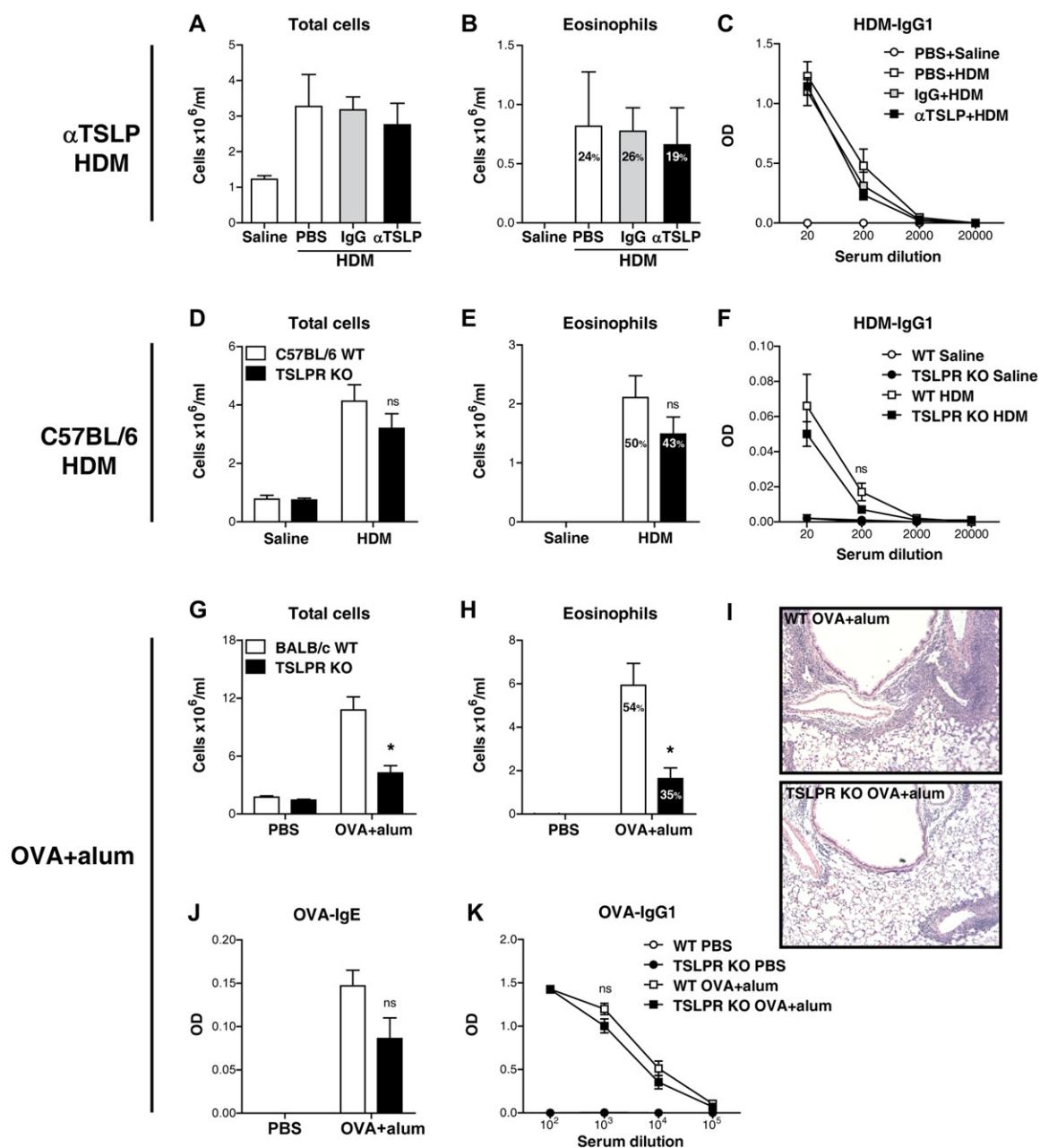


FIG E1. The TSLP-TSLPR axis is differentially required in OVA- and HDM-induced immune-inflammatory responses. **A-C**, BALB/c mice received PBS vehicle, rat IgG, or anti-TSLP blocking antibody 1 day before and throughout the HDM exposure protocol. **D-F**, C57BL/6 WT and TSLPR KO mice were exposed daily to HDM intranasally for 10 days. **G-K**, As previously reported,^{E6} BALB/c WT and TSLPR KO mice were sensitized with OVA plus alum intraperitoneally on days 1 and 14 and then challenged with OVA intranasally for 4 days starting on day 21. Total cells (Fig E1, A, D, and G) and eosinophil counts (Fig E1, B, E, and H) in bronchoalveolar lavage fluid. Serum HDM-specific IgG₁ (Fig E1, C and F) and OVA-specific IgE (Fig E1, J) or IgG₁ (Fig E1, K) levels. Fig E1, I, Representative lung sections stained with hematoxylin and eosin to assess inflammation. One experiment with 2 to 7 mice per group (Fig E1, A-C), 2 experiments with 3 to 7 mice per group per experiment (Fig E1, D-F), or 2 experiments with 4 to 5 mice per group per experiment (Fig E1, G-K). **P* < .05 versus PBS plus HDM and IgG plus HDM (Fig E1, A-C), WT HDM (Fig E1, D-F), or WT OVA plus alum (Fig E1, G-K). PN, Peanut.

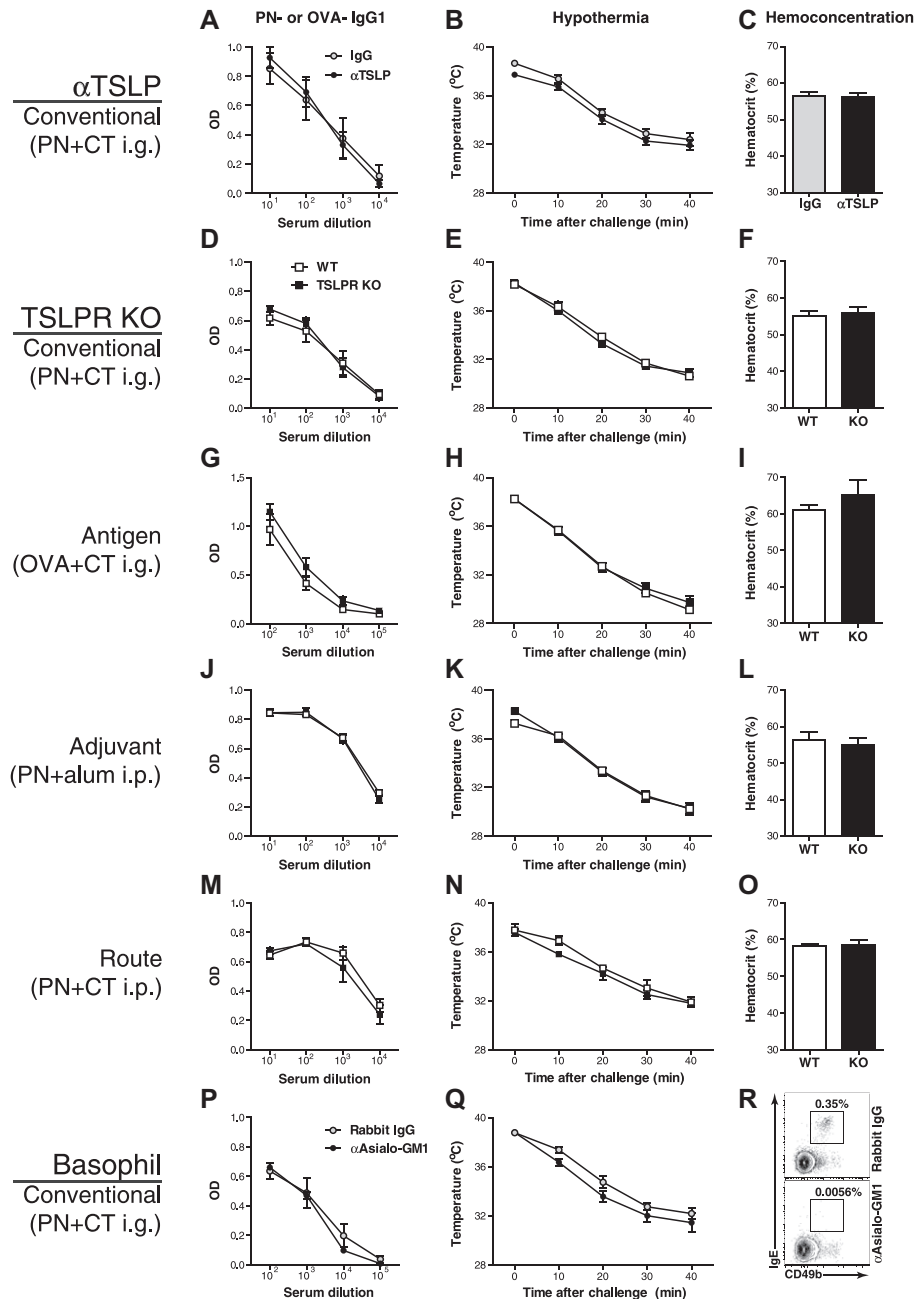


FIG E2. TSLPR independence does not alter with antigen, sensitizing adjuvant, or route of sensitization, and basophils are not required for peanut (PN) allergy. WT or TSLPR KO mice were sensitized with peanut plus CT intragastrically (*i.g.*; **A-F** and **P-R**), OVA plus CT intragastrically (*i.g.*; **G-I**), or peanut plus CT intraperitoneally (*i.p.*; **M-O**) and then challenged. Fig E2, **A-C**, Mice received anti-TSLP antibody or rat IgG 1 day before each gavage. Fig E2, **A-C**, Mice received anti-TSLP antibody or rat IgG 1 day before each gavage. **J-L**, Mice were sensitized with peanut plus alum intraperitoneally. Fig E2, **P-R**, Anti-asialo GM1 or rabbit IgG was injected 1 day before each gavage. Fig E2, **A, D, G, J, M, P**, Serum peanut- or OVA-specific IgG₁ 1 day before challenge. Anaphylactic assessment was based on core body temperature (Fig E2, **B, E, H, K, N**, and **Q**) and hematocrit levels (Fig E2, **C, F, I, L**, and **O**). Fig E2, **R**, Proportion of CD3⁺CD19⁺NK1.1⁺c-Kit⁺CD49b⁺IgE⁺ basophils in spleens. Shown are 2 experiments with 3 to 8 mice per group per experiment.

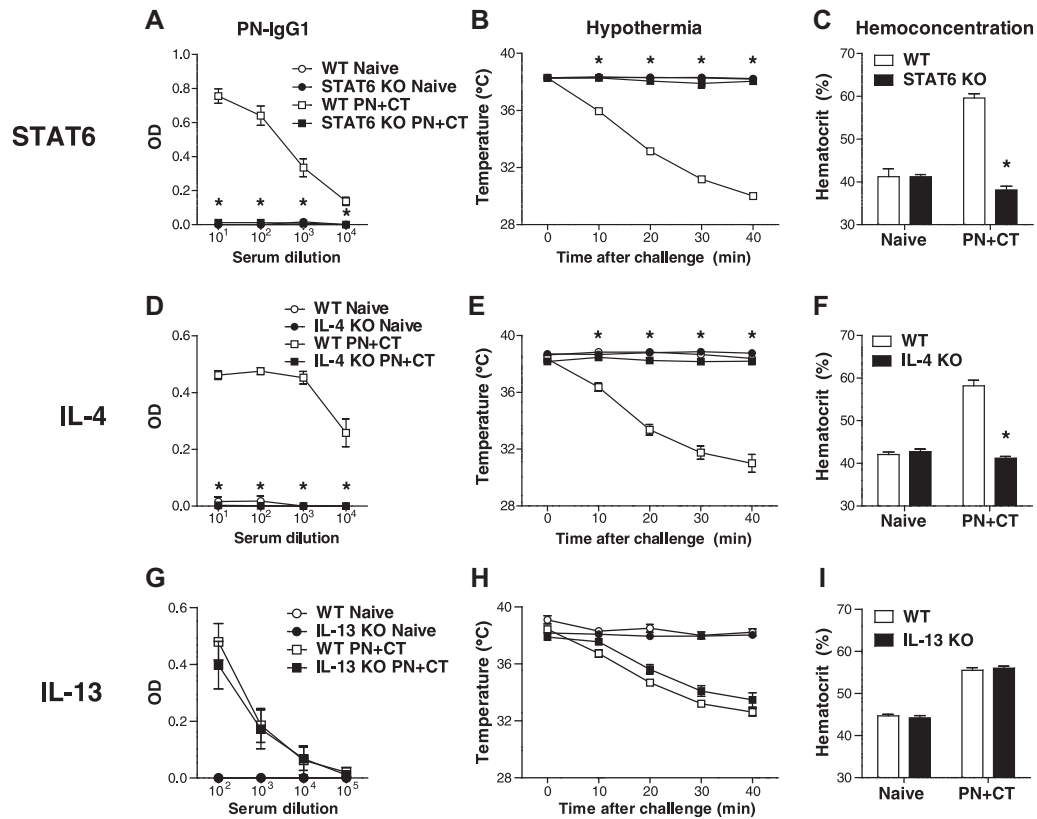


FIG E3. Peanut (PN) sensitization follows canonical T_H2 dependency on STAT6 and IL-4. WT (A-I), STAT6 KO (Fig E3, A-C), IL-4 KO (Fig E3, D-F), or IL-13 KO (Fig E3, G-I) mice received peanut plus CT intragastrically weekly for 4 weeks and were challenged with peanut intraperitoneally 1 week later. Fig E3, A, D, and G, Serum peanut-specific IgG₁ levels 1 day before challenge. Anaphylactic assessment was based on core body temperature (Fig E3, B, E, and H) and hematocrit levels (Fig E3, C, F, and I). Shown are 2 experiments with 3 to 8 mice per group per experiment. * $P < .05$ versus WT peanut plus CT.

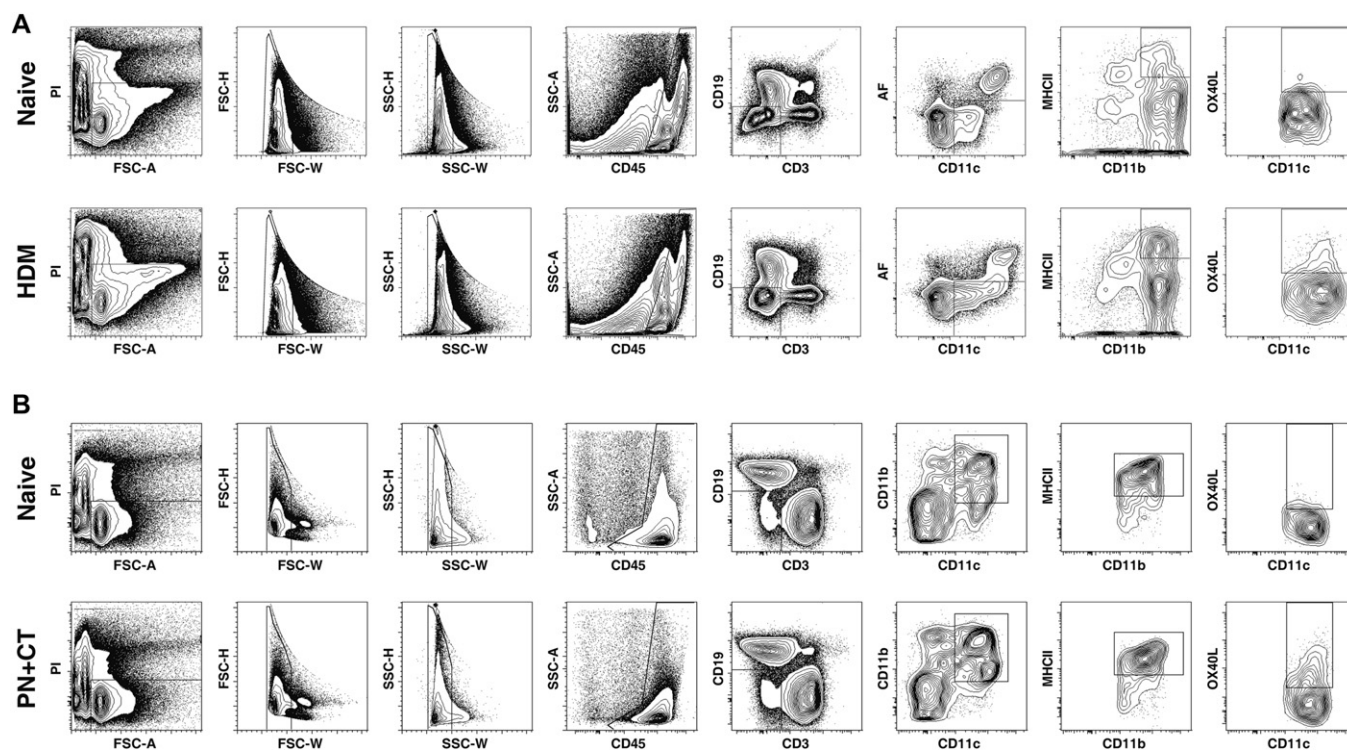


FIG E4. Myeloid DCs expand after 3 days of HDM or peanut (PN) plus CT exposure. Flow cytometric gating strategy for myeloid DCs in the lung (A) or mesenteric LNs (B). Representative plots are from 2 to 3 experiments. AF, Autofluorescence; FSC, forward scatter; PI, propidium iodide; SSC, side scatter.

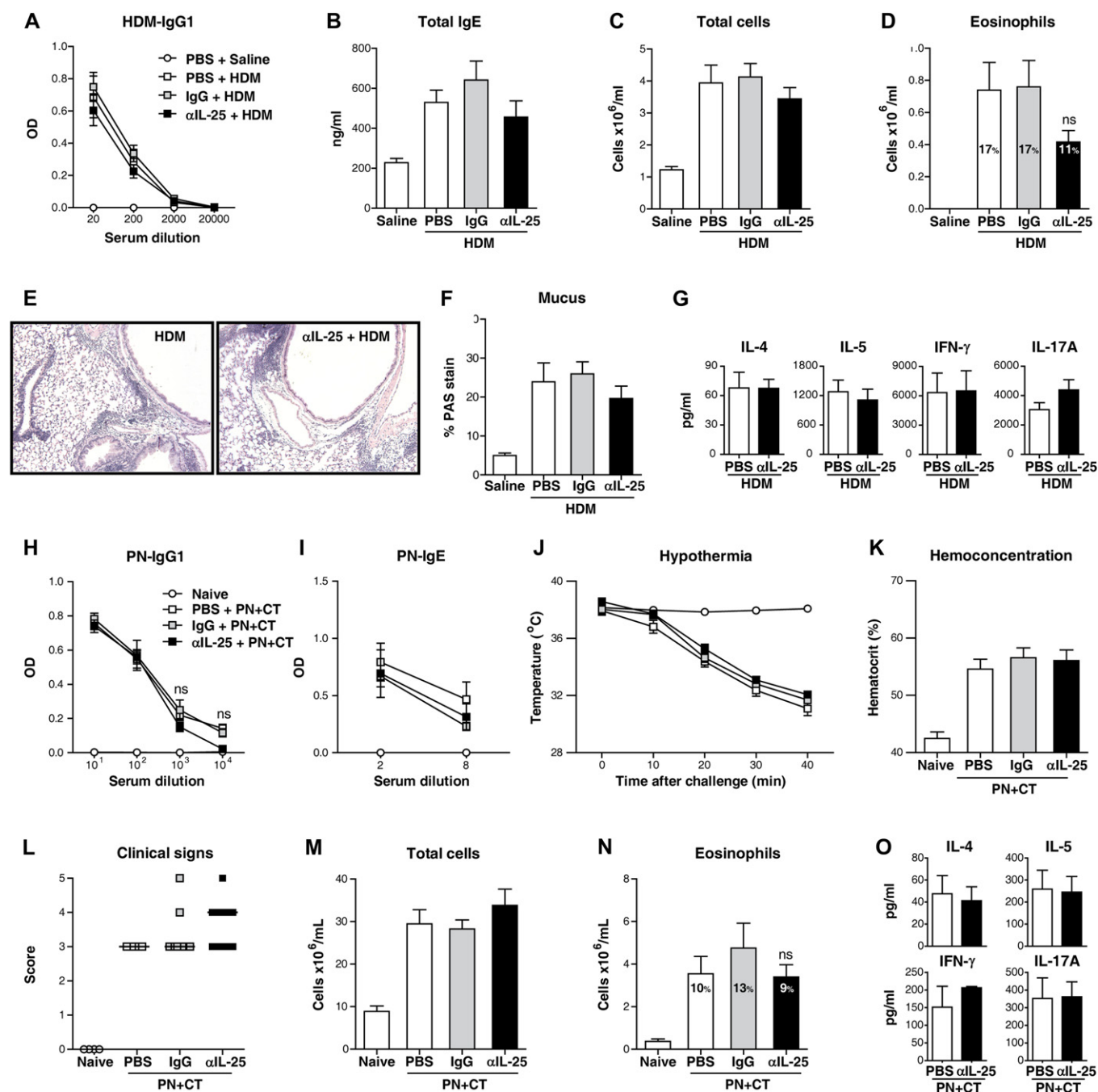
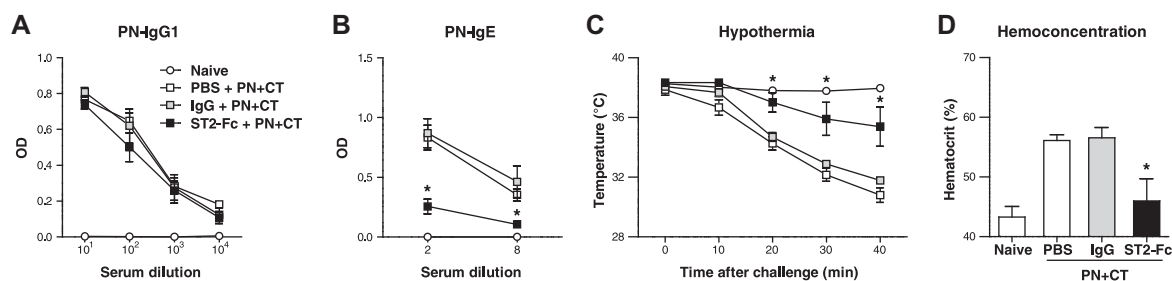


FIG E5. IL-25 neutralization does not significantly affect pulmonary and gastrointestinal allergic sensitization to HDM and peanut (PN). **A-G**, WT mice received PBS vehicle, rat IgG, or 1 mg of anti-IL-25 neutralizing antibody 1 day before and throughout 10 days of HDM exposure. Serum HDM-specific IgG₁ (Fig E5, A) and total IgE (Fig E5, B) levels. Total cell (Fig E5, C) and eosinophil (Fig E5, D) counts in bronchoalveolar lavage fluid; mean percentages of eosinophils are shown in bars. Fig E5, E, Representative lung sections stained with hematoxylin and eosin ($\times 10$ magnification) or periodic acid-Schiff (PAS) insets ($\times 40$ magnification) to assess leukocyte infiltration and mucus production. Fig E5, F, Morphometric analysis of periodic acid-Schiff staining. Fig E5, G, Cytokine production from HDM-stimulated splenocytes. **H-O**, WT mice received rat IgG or anti-IL-25 antibody 1 day before peanut plus CT intragastrically weekly for 4 weeks and then were challenged with peanut intraperitoneally 1 week later. Serum peanut-specific IgG₁ (Fig E5, H) and IgE (Fig E5, I) levels 1 day before challenge. Anaphylactic assessment was based on core body temperature (Fig E5, J), hematocrit levels (Fig E5, K), and clinical signs (Fig E5, L). Total (Fig E5, M) and eosinophil (Fig E5, N) cell counts in peritoneal lavage; mean percentages of eosinophils are shown in bars. Fig E5, O, Cytokine production from peanut-stimulated splenocytes. Shown are 2 to 3 independent experiments with 3 to 6 mice per group per experiment. *ns*, Not significantly different.

ST2-Fc
during
sensitization



ST2-Fc
before
challenge

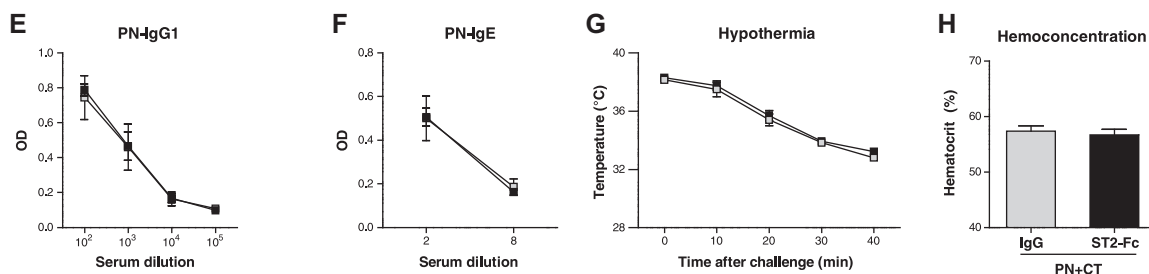


FIG E6. Differential effect of ST2-Fc treatment on peanut (PN) sensitization versus challenge. WT mice received PBS vehicle, human IgG, or ST2-Fc 1 day before each sensitization (**A-D**) or challenge (**E-H**). Serum peanut-specific IgG₁ (Fig E6, **A** and **E**) and IgE (Fig E6, **B** and **F**) levels 1 day before challenge. Anaphylactic assessment was based on core body temperature (Fig E6, **C** and **G**) and hematocrit levels (Fig E6, **D** and **H**). Shown are 2 independent experiments with 3 to 8 mice per group per experiment. * $P < .05$ versus control PBS- or IgG-treated mice exposed to peanut plus CT.