Background: Allergic inflammation involves the sensitization of naive CD4+ T cells to allergens, resulting in a Th2-skewed inflammatory response. Although antigen presentation by dendritic cells to T cells in the lymph node is crucial for Th2 cell development, the innate signals that initiate adaptive type 2 inflammation and the role of group 2 innate lymphoid cells (ILC2s) are poorly understood.

Objective: We sought to investigate the influence of ILC2s and the route of priming on the development of an adaptive type 2 immune response to lung allergens.

Methods: Wild-type and ILC2-deficient mice were exposed intranasally or systemically to the Th2-inducing antigens house dust mite or ovalbumin in a model of allergic airway inflammation or the Th17-inducing bacterial antigen Saccharopolyspora rectivirgula in a model of hypersensitivity pneumonitis. The formation of an adaptive immune response was evaluated based on serum antibody titers and production of T cell-derived cytokines (IL-4, IL-5, IL-13 and IL-17A).

Results: We find that lung ILC2s play a critical role in priming the adaptive type 2 immune response to inhaled allergens, including the recruitment of eosinophils, Th2 cytokine production and serum IgE levels. Surprisingly, systemic priming with ovalbumin, with or without adjuvants, circumvents the requirement for ILC2s in inducing Th2-driven lung inflammation. ILC2s were also found to be dispensable for the sensitization to Th1- or Th17-inducing antigens.

Conclusion: These data highlight a critical role for ILC2s in the development of adaptive type 2 responses to local, but not systemic, antigen exposure. (J Allergy Clin Immunol 2014;133:1142-8.)

Key words: Innate lymphocytes, allergy, asthma, house dust mite, Th2, IgE

Allergic airway inflammation (AAI)/asthma is a chronic inflammatory disease characterized by a Th2-biased immune response, antibody class-switching to IgE, and reversible airflow obstruction. Its prevalence has increased by 12% between 2001 and 2009, and it currently affects 8.2% of the population in the United States alone, resulting in an annual economic burden of $56 billion. The most prevalent therapeutic strategies were developed in the 1970s and include the use of inhaled corticosteroids to dampen inflammation and long-acting β-agonists to induce smooth muscle cell relaxation. Although effective, these drugs do not target the underlying immune dysregulation that leads to disease. Similarly, in developing new therapeutics, the current emphasis has been on biologics targeting downstream effectors of allergic responses, primarily antibodies directed at IgE and Th2 cytokines. These new treatment options have shown only marginal clinical success, and the data suggest that targeting the effector response of allergic inflammation might not represent an optimal approach. In summary, there is a current unmet need in the treatment of allergic airway disease. It is likely that a greater understanding of the mechanisms involved in the initial priming and sensitization phase could unveil novel therapeutic targets.

House dust mite (HDM) is one of the most common indoor allergens, and up to 85% of asthmatic patients respond to this allergen. Inhaled HDM acts on airway epithelial cells in a Toll-like receptor 4-dependent manner, inducing the release of cytokine danger signals, including IL-25, IL-33, thymic stromal lymphopoietin (TSLP), and GM-CSF. Understanding how allergens are able to promote naïve CD4+ T-cell polarization to a Th2 phenotype has been hampered by the lack of a clearly defined innate cell type that produces Th2-associated cytokines (eg, IL-4, IL-5, and IL-13) during the sensitization phase. Studies with Rag2−/− mice, which lack T and B cells, identified a novel innate cell type that produced IL-5 and IL-13 in response to IL-25. Several groups have since characterized populations of innate lymphoid cells that act as robust sources of IL-5 and IL-13, but not IL-4, in various tissues, including the spleen, adipose tissue, lymph nodes, lungs, and nasal mucosa. Although initially referred to as nuocytes, natural helper cells, or innate helper type 2 cells, they are now more simply referred to as group 2 innate lymphoid cells (ILC2s) based on their unique expression profile of cell-surface antigens (CD45 +Lin−CD127+CD25−CD90.2−ST2+Sca1−) and their production of the type 2 cytokines IL-5 and IL-13 in response to...
to epithelium–derived IL-25, IL-33, and TSLP. ILC2s also require the transcription factors retinoic acid receptor–related orphan receptor α (RORα),15,16 GATA-3,17,18 and T-cell factor 119 for their specification and maturation. Previously, we showed that RORα-deficient mice have an impaired innate response to protease allergens in the lung.15 However, the selective role of ILC2s in the priming of different CD4⁺ T H subsets and the development of adaptive immune responses in the lung has yet to be examined. Using distinct models of adaptive immunity induction, we demonstrate a specific and highly selective role for ILC2s in pro-\textit{\textbf{ductive}} immune responses in the lung.15

### Abbreviations used

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>AAI</td>
<td>Allergic airway inflammation</td>
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<tr>
<td>BAL</td>
<td>Bronchoalveolar lavage</td>
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<td>BMT</td>
<td>Bone marrow transplant</td>
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<td>DC</td>
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<td>House dust mite</td>
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<td>HP</td>
<td>Hypersensitivity pneumonitis</td>
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<td>ILC2</td>
<td>Group 2 innate lymphoid cell</td>
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<td>OVA</td>
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<td>RORα</td>
<td>Retinoic acid receptor–related orphan receptor α</td>
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<td>Thymic stromal lymphopoietin</td>
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### METHODS

#### Mice and bone marrow transplantation

C57BL/6J, B6.SJL-Ppic<sup>+</sup> Pep<sup>−/−</sup>BoyJ (Ly5.1), and B6.C3(Cg)-Rora<sup>−/−</sup> mice were purchased from the Jackson Laboratories (Bar Harbor, Me) and were bred and maintained in a specific pathogen-free environment at the Biomedical Research Centre. Pups from Rora<sup>−/−</sup> littermates, and 1 naïve bone marrow cells were exposed intranasally to 40 mg of total protein 3 times a week for 3 weeks. Mice were sacrificed 4 days after the last intranasal exposure.

#### Antibodies and flow cytometry

Staining and antibody dilutions were prepared in PBS containing 2% FCS, 2 mmol/L EDTA, and 0.05% sodium azide. Samples were first blocked in buffer containing 5 μg/mL anti-CD16/32 (2.4G2) to block nonspecific antibody binding. Alexa Fluor 450–conjugated CD3e (145-2C11), CD11c (N418) CD11b (M1/70), CD19 (1D3), CD45R/B220 (RA3-6B2), NK1.1 (PK136), Gr1 (RB6-8C5), and Ter119 (TER-119); phycoerythrin (PE)–conjugated CD45, CD45R/B220, Siglec-F (E50-2440), PerCP-Cy5.5–conjugated CD45.2 (104), and V500–conjugated CD45 (30-F11) were purchased from BD Biosciences (San Jose, Calif). Fluorescein isothiocyanate–conjugated B220; and efluor650-conjugated CD90.2 (53-2.1) were purchased from eBioscience (San Diego, Calif). PE–conjugated CD45, CD45R/B220, Siglec-F, and 7/4. staining was performed with the Cytofix/Cytoperm kit (BD Biosciences), and viable cells were identified by using the efluor-450, 506, or 780 fixable viability dye (eBioscience). Samples were acquired on a BD LSRII, and data analysis was performed with FlowJo software (TreeStar, San Carlos, Calif).

### Lung explant culture

Mice were sacrificed by means of CO₂ asphyxiation, and lungs were inflated with 1.5 mL of Dulbecco modified Eagle medium containing 10% FCS, 2-mercaptoethanol, penicillin/streptomycin, and 1% low-melting-point agarose kept at 37°C and cooled on ice. Lungs were sliced with a razor into approximately 0.5-mm-thick sections and placed in 2 mL of culture media stimulated with either PBS or 50 μg/mL HDM antigen for 18 hours. Golgi-Plug (BD Biosciences) was added to the explant cultures 6 hours before collection, and a single-cell suspension was made by passing the lung slices through a 70-μm cell strainer. Lung ILC2s were identified as Lin⁻CD45⁺CD90.2⁺CD25⁻Sca1<sup>−</sup>ST₂⁺ viable cells and gated for IL-5 expression based on fluorescence minus one controls. Septated IL-5–positive cells in the cell-free supernatant were quantified by means of ELISA with purified and biotin antibody pairs to IL-5 (TRFK5 and TRFK4) from eBioscience.

#### Induction and assessment of AAI

HDM (Derma\textit{\textbf{tophagoides} pteronyssinus}) extract was obtained from Greer Laboratories (Lenoir, NC; containing 0.034 μg of Der p 1 and 0.095 EU of total protein), and disease was induced, as previously described. Mice were treated intranasally with endotoxin-free PBS or HDM on days 0, 1, and 2 with 25 μg of total protein and on days 14, 15, 16, and 17 with 5 μg, and mice were sacrificed 24 hours after the final challenge.

For ovalbumin (OVA)/alum–induced AAI, mice were injected intraperitonially with 50 μg of chicken OVA (grade III; Sigma, St Louis, Mo) adsorbed to 650 μg of aluminum hydroxide (Sigma) on days 0 and 7. OVA contained less than 0.125 EU/100 ng of protein, as measured using the PYROGENT Gel Clot assay (Lonza, Walkersville, Md). Mice were treated intranasally on days 21, 22, 23, 25, and 27 with 50 μg of OVA, and mice were sacrificed on day 28.

### Detection of antigen-specific IgG<sub>1</sub>, IgG<sub>2a</sub>, and IgE

ELISA for total serum IgE was performed according to the manufacturer’s instructions (BD Biosciences). For detection of antigen-specific IgE, IgG<sub>1</sub>, and IgG<sub>2a</sub> plates were coated with 25 μg/mL S. rect\textit{\textbf{rivula}} or HDM antigen in carbonate buffer (0.1 mol/L sodium carbonate, pH 9.5). Serum was diluted in assay diluent (PBS with 3% BSA) and added to the plates for 2 hours at 37°C. Antigen-specific IgGs were detected with horseradish peroxidase–conjugated antibodies to mouse IgG<sub>1</sub> or IgG<sub>2a</sub> biotin-conjugated anti-mouse IgE (R35-72), streptavidin–horseradish peroxidase, and TMB substrate (BD Phar\textit{\textbf{Mingen}}, San Jose, Calif).

#### Immune analysis

Lungs were minced and digested with 200 U/mL collagenase IV (Sigma) for 1 hour at 37°C, a single-cell suspension was obtained by passing the tissue through a 70-μm cell strainer, red blood cells were lysed, and leukocytes were
enriched by means of Percoll separation. Leukocytes were counted and stimulated at 4 to 8 \(3\times10^6\) cells/mL in culture media (Dulbecco modified Eagle medium with 10% FCS, penicillin/streptomycin, 50 \(\mu\)mol/L 2-mercaptoethanol, and 25 mmol/L HEPES) with the indicated concentration of HDM or \textit{S rectivirgula} antigens for 72 hours. Cell-free supernatants were collected, and cytokine levels were measured by means of ELISA with purified and biotin antibody pairs to IL-13 (eBio13A, eBio1316H) or IL-17A (eBio17CK15A5, eBio17B7) from eBioscience.

RNA isolation and quantitative RT-PCR

Lungs were homogenized in Trizol (Life Technologies, Carlsbad, Calif) by using a Qiagen TissueLyser II (Valencia, Calif). Total RNA was extracted and reversed transcribed with a high-capacity cDNA kit (Life Technologies), and quantitative RT-PCR was performed with Sybr green (KAPA Biosystems, Woburn, Mass). The primers used for quantitative PCR are listed in the Methods section in this article’s Online Repository at www.jacionline.org. Reactions were carried out in an ABI 7900 real-time PCR machine (Life Technologies), and values are expressed relative to \textit{Actb}.

Histology

Lung tissue was fixed in 10% buffered formalin overnight and embedded in paraffin. Sections (5 \(\mu\)m thick) were stained with hematoxilin and eosin. A histologic disease score from 0 to 4 was attributed based on severity for peribronchial, perivascular, and parenchymal immune cell infiltration, resulting in a maximum score of 12.

Statistics

Results are presented as means \(\pm\) SEMs. The Student \(t\) test was used to determine statistical significance.

RESULTS

HDM induces lung ILC2 activation and IL-5 secretion

To test whether HDM could elicit ILC2-dependent production of \textit{T\(_2\)} cytokines, we used lung explant cultures, which maintain the lung microenvironment and epithelium-leukocyte interactions. Lung explants were stimulated with HDM or PBS. D, IL-5 released in the supernatant of PBS- or HDM-stimulated lung explants was measured by ELISA at 6, 12, and 24 hours. APC, Allophycocyanin.

ILC2s facilitate HDM-induced AAI

To examine the role of ILC2s in the development of allergic asthma, we used a HDM mouse model of AAI (Fig 2, A). In this model repeated intranasal exposure of HDM antigen induces several hallmark features of clinical asthma, including airway and lung parenchymal eosinophilia; a \textit{T\(_2\)}-biased immune response with high levels of IL-4, IL-5, and IL-13 secretion; and antibody class-switching to IgE. \(^6\)

Although ILC2s are known to produce IL-5 in response to protease antigens in the lung, \(^10\) their role in regulating the
FIG 2. HDM-induced AAI in WT and ILC2-deficient mice. A, Schematic of HDM disease model. B and C, Total cells (Fig 2, B) and eosinophils (Fig 2, C) collected in BAL samples of WT or ILC2-deficient mice. D, Total CD45.2+ lung ILC2s from PBS- and HDM-exposed WT or ILC2-deficient mice. E, Serum total IgE and HDM-specific IgE and IgG1 levels were quantified by ELISA. F, Transcript levels of Il4 and Il5 from lung RNA relative to Actb. G, Lung leukocytes were isolated and restimulated ex vivo with 50 μg/mL HDM for 72 hours. IL-13 levels were quantified by ELISA. Data are representative of 3 independent experiments (Fig 2, B and C and E-G; n = 4-8) or combined from 2 independent experiments (Fig 2, D; n = 4-5). Error bars are means ± SEMs. *P < .05. i.n., Intranasal.

Development of an adaptive immune response to physiologically relevant antigens is still unknown. To address this issue, we generated mice selectively lacking ILC2s by transplanting WT mice (CD45.1) with bone marrow from Rorasg/sg mice (CD45.2) to produce hematopoietic chimeras. These chimeric mice have a very selective defect in ILC2 production (although rare, residual, radioresistant CD45.1+ ILC2s can be detected in transplanted chimeras; see Fig E1 in this article’s Online Repository at www.jacionline.org). Indeed, RORα expression is tightly regulated in the hematopoietic compartment, with high expression found only on ILC2s and Tγδ17 cells but not Th2 cells (see Fig E2, A, in this article’s Online Repository at www.jacionline.org). Additionally, we found that isolated CD4+ T cells from WT or Rorasg/sg bone marrow transplanted (BMT) mice displayed no difference in their ability to polarize to Th2 cells, as marked by equal production of the Th2 cytokines IL-5 and IL-13, as well as equal expression of the transcription factor Gata3 (see Fig E2, B and C). When exposed to a HDM model of AAI, Rorasg/sg chimeras exhibited a significant impairment in Tγδ2 response induction, with a marked reduction in leukocyte infiltration, particularly eosinophils, into the airways 18 days after initial HDM exposure, whereas challenge with saline (PBS) resulted in very low levels of airway infiltrate comprised almost exclusively of macrophages (Fig 2, B and C). Additionally, HDM exposure resulted in a significant expansion of ILC2s in the lungs of WT BMT mice and a low level expansion in Rorasg/sg BMT mice (Fig 2, D). ILC2-deficient mice also exhibited significantly reduced levels of both total serum IgE and HDM antigen–specific IgE and IgG1 (Fig 2, E and F) and significantly reduced expression of lung transcripts for the Tγδ2-associated cytokines Il4 and Il5 (Fig 2, F). Isolated lung leukocytes were restimulated ex vivo with HDM antigen to evaluate the activity of Tγδ2 cells in the inflamed lung tissue. ILC2-deficient mice displayed a strikingly reduced recall response, as indicated by decreased antigen-induced production of IL-13 (Fig 2, G).

Histologically, we found that loss of ILC2s also resulted in a significant reduction in lung histopathology, with reduced leukocyte infiltration into the lung tissue, particularly in the peribronchiolar and perivascular space (Fig 3, A and B). Consistent with the significant reduction in BAL eosinophil numbers in ILC2-deficient mice, the frequency of tissue-infiltrating parenchymal eosinophils was also reduced, as quantified by the evaluation of the eosinophil-specific mRNA Prg2 (encoding major basic protein 1) in the lung (Fig 3, C). Thus our results suggest that RORα-dependent ILC2s are critical for the development of allergic lung inflammation.

ILC2s are dispensable for the development of Tγδ2 responses to systemically delivered antigens

Although these data suggest that ILC2s are essential for the induction of Tγδ2 cell responses to physiologically relevant antigens when delivered locally through the natural intranasal route, they do not address whether there is a similar requirement for these cells in priming the adaptive immune system to systemically administered antigens in the presence of adjuvants. To test this, we used the well-described OVA/alum model of AAI (Fig 4, A). In this model mice are primed intraperitoneally (systemically) with OVA and alum, followed by intranasal challenge (local) with OVA. In contrast to our results with intranasal HDM antigen, we found that ILC2s were completely dispensable for the induction of AAI in this model. ILC2-deficient mice had similar numbers of airway-infiltrating leukocytes and displayed normal recruitment of eosinophils into the airways and lung tissue (Fig 4, B, C, and E). Likewise, we detected no significant differences in serum IgE levels or Il5 expression (Fig 4, D and E), suggesting normal development of an adaptive Tγδ2 response.

Systemic delivery of antigens without adjuvant elicits ILC2-independent Tγδ2 responses

Alum acts as a strong Tγδ2-inducing adjuvant that causes release of various danger signals, such as uric acid, that could bypass ILC2s and act on resident macrophages and dendritic cells (DCs). To examine whether ILC2s are required for systemic antigen priming of allergic responses in the absence of external adjuvant, we primed Rorasg/sg chimeras intraperitoneally with OVA in the absence of alum, followed by intranasal challenges with OVA (Fig 5, A). Surprisingly, ILC2-deficient mice had equivalent levels of leukocyte and eosinophilic infiltrates in the airways and tissues and exhibited no difference in IgE levels or Il5 expression.
(Fig 5, B-E). This suggests that systemic antigen delivery, irrespective of the addition of an exogenous adjuvant, is able to induce an ILC2-independent allergic airway inflammatory response.

**ILC2s are dispensable for local priming of T\textsubscript{H}1/T\textsubscript{H}17 responses**

Although we find ILC2s are essential for T\textsubscript{H}2 sensitization to locally delivered antigens in the lung, it remains unclear whether
they play any positive or negative role in the development of T_{H1}/T_{H17} responses. HP is a chronic lung inflammatory disease caused by repeated airborne exposure to predominantly organic antigens and is characterized by a T_{H1}/T_{H17}-biased immune response. In human subjects exposure to *S. rectivirgula*, a bacteria present in moldy hay, leads to farmer’s lung (a subtype of HP), and this is recapitulated in mice after repeated intranasal exposure to this antigen (Fig 6, A).

WT and ILC2-deficient mice were treated as outlined in Fig 6, A, and clinical features of HP were assessed on day 20 after induction. We found that loss of ILC2s had no effect on the ability to mount an adaptive T_{H1}/T_{H17} response to bacterial *S. rectivirgula* antigens. ILC2-deficient mice had similar levels of alveolar infiltrate with no difference in alveolar lymphocyte accumulation (Fig 6, B and C). Unlike allergic asthma, which is characterized by high serum IgE and IgG1 levels typical of a T_{H2} immune response, HP induces production of antigen-specific IgG2a. ILC2-deficient mice had normal *S. rectivirgula*-specific IgG2a and IgG1 levels (Fig 6, D). There was also no difference in T_{H17} responses in the lung, as measured based on Il17a transcript levels and IL-17A production from restimulated lung cultures (Fig 6, E and F). We conclude that ILC2s are largely dispensable for T_{H1}/T_{H17} inflammatory responses.

**DISCUSSION**

The initiation of T_{H2} responses and the innate cell types responsible for the production of cytokines before the polarization of naive CD4+ T cells to a T_{H2} phenotype is not well understood and is an important area of investigation. Lung ILC2s represent a recently characterized leukocyte population that share several key features of ILC2s (eg, TSLP-mediated cytokine IL-5 and IL-13 transcription), and are more abundant in sputum samples from allergic patients, highlighting their clinical importance.

Finally, genome-wide association studies have also found polymorphisms in the *ROA* locus to be associated with greater risk of asthma (along with *IL33* and *IL13*), suggesting a potential link between ILC2s and disease.

To test whether these cells are also required in models of systemic exposure to allergens, we primed mice intraperitoneally with OVA as a model antigen. Surprisingly, we found that systemic administration of antigen, along with an alum adjuvant, obviated the requirement for ILC2s in the development of a robust T_{H2} response. It is possible that the strong immune-stimulatory effects of alum, such as release of uric acid and stimulation of inflammatory monocytes, overcame any requirement for ILC2s in the sensitization phase. To test this, we repeated the same model of systemic antigen delivery in the absence of adjuvant and again found that this too led to an ILC2-independent T_{H2} response. Thus our data with *Rora^{+/−}* bone marrow chimeras now provide a formal link between these previously detected innate cytokine-producing cells and the generation of adaptive allergic immune responses to local antigen stimulation.
highlights the important interplay between lung-resident ILC2s and the epithelium in shaping the innate and adaptive immune response to inhaled allergens. The lung epithelium is a potent producer of ILC2-activating cytokines, such as IL-25, IL-33, and TSLP, and HDM-induced experimental asthma is dependent on epithelium-derived signals. 4

Although we found ILC2s to facilitate sensitization to the T(H)2-inducing HDM antigen, they were completely dispensable for sensitization to T(H)1/T(H)17-inducing antigens in a murine model of HP. This was surprising because RORα and its related transcription factor, RORγt, are important for proper T(H)17 differentiation, 30 and mice deficient in T(H)17 or its receptor are protected from the development of HP. 22,23 Although we found IL-17A production to be reduced in Rora-deficient BMT mice in both the HDM and OVA models of AAI (data not shown), we found no difference in IL-17A production in the T(H)17 model of HP. Thus it is likely that S. rectivirgula exposure is able to induce a strong T(H)17-polarizing stimulus and that the RORγt expression in RORα-deficient T cells is sufficient for T(H)17 development, whereas HDM and OVA produce a weak T(H)17-inducing signal that requires synergistic activity of both RORα and RORγt for proper T(H)17 differentiation. However, the ability of ILC2-deficient mice to mount a normal response in a T(H)17/T(H)17 model of lung inflammation does not preclude the potential role for other lung-resident innate lymphoid cell populations in modifying responses to bacterial antigens. However, our data rule out a significant role for ILC2s in this process.

In summary, our data highlight a pivotal role for ILC2s in the initiation of T(H)2 responses to mucosal routes of antigen exposure. They also highlight these cells as potential targets for therapeutics aimed at blocking the initiation of allergic responses before the development of adaptive immunity.

We thank Les Rollins and the BRC Animal Care Facility, Taka Murakami and the BRC genotyping service, Andy Johnson and the UBC Flow Cytometry Facility and Rupinder Dhesi and Michael Williams for core support.

Key messages

- ILC2s facilitate sensitization to local, but not systemic, T(H)2-inducing allergen exposures.
- ILC2s are dispensable for the development of an adaptive immune response to local bacterial antigens that induce T(H)1/T(H)17 responses.

REFERENCES

METHODS

In vitro T-cell polarization

$CD^4^+T$ cells were isolated from the spleens and lymph nodes of naive WT or $Roralpha^{-/-}$ BMT mice by means of negative selection using RoboSep (STEMCELL Technologies, Vancouver, British Columbia, Canada). $CD^4^+\text{cells}$ ($2.5 \times 10^5$) were cultured for 6 days in culture media (Iscove modified Dulbecco medium supplemented with 10% FBS, 100 U/mL penicillin, 100 $\mu$g/mL streptomycin, and 150 $\mu$mol/L monothioglycerol) with 1 $\mu$g/mL each of plate-bound anti-CD3 (145-2C11) and anti-CD28 (37.51) in the presence of neutral (10 ng/mL IL-2), Th2 (10 ng/mL IL-2, 10 ng/mL IL-4, and 10 $\mu$g/mL anti–IFN-\gamma [XMG1.2]) or Th17 (10 ng/mL IL-1\beta, 20 ng/mL IL-6, 10 ng/mL IL-23, 10 ng/mL TNF-\alpha, 1 ng/mL TGF-\beta, 10 $\mu$g/mL anti–IL-4 [11B11], and 10 $\mu$g/mL anti–IFN-\gamma [XMG1.2]) conditions. Cell-free supernatants were collected at day 6 for cytokine measurements, and total RNA was extracted with Trizol for quantitative RT-PCR. Immature ILC2s were sort purified from C57Bl/6 bone marrow ($CD45^-Lin^-CD127^-CD25^-ST2^+$) and cultured for 7 days in media supplemented with 10 ng/mL each of IL-7 and IL-33.
FIG E1. Reduced lung ILC2s in Rora<sup>−/−</sup> BMT mice. A, Flow cytometric analysis of lung ILC2s from WT and Rora<sup>−/−</sup> BMT mice. B, Quantification of lung CD45.2<sup>+</sup> ILC2s from donor mice (WT or Rora<sup>−/−</sup>) or residual radioresistant ILC2s (CD45.1<sup>+</sup>). Data are representative of 2 independent experiments. Error bars are means ± SEMs.
FIG E2. RORγt expression profile and function in Th2 polarization. CD4⁺ T cells isolated from WT or Rorasg/sg BMT mice were stimulated under neutral, Th2- or Th17-polarizing conditions for 6 days. A, Expression of Rora transcript from T-cell polarization cultures or purified ILC2s was measured by using quantitative PCR relative to Actb. B, Secreted IL-5 and IL-13 from Th2 polarization conditions was quantified by means of ELISA. C, Expression of Gata3 transcript from Th2 polarization conditions was measured by using quantitative PCR relative to Actb. Data are representative of 2 independent experiments. Error bars are means ± SEMs.
FIG E3. Model for ILC2 function in lung inflammation. Exposure of the lung epithelium to HDM antigens results in loss of barrier integrity and release of innate danger signals (TSLP, IL-25, and IL-33), which in turn activate lung-resident ILC2 release of IL-5 and IL-13. This results in eosinophil recruitment and activation of immature lung resident dendritic cells (iDC). Activated lung DCs pick up antigen for transport to the draining lymph nodes and activation of a Th2 adaptive response. Systemic immunization with allergen obviates the need to ILC2s to mount an adaptive Th2 response. Local exposure to bacterial Th1/Th17-inducing antigens (S rectivirgula) activate a pathway independent of group 2 ILCs but might rely on other lung-resident innate lymphocyte populations, such as the IL-17A- and IL-22-producing ILC3s. Eos, Eosinophil; mDC, myeloid dendritic cell.
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