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Collaborative Interactions between Type 2 Innate Lymphoid Cells and Antigen-Specific CD4+ Th2 Cells Exacerbate Murine Allergic Airway Diseases with Prominent Eosinophilia

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Type-2 innate lymphoid cells (ILC2s) and the acquired CD4+ Th2 and Th17 cells contribute to the pathogenesis of experimental asthma; however, their roles in Ag-driven exacerbation of chronic murine allergic airway diseases remain elusive. In this study, we report that repeated intranasal rechallenges with only OVA Ag were sufficient to trigger airway hyperresponsiveness, prominent eosinophilic inflammation, and significantly increased serum OVA-specific IgG1 and IgE in rested mice that previously developed murine allergic airway diseases. The recall response to repeated OVA inoculation preferentially triggered a further increase of lung OVA-specific CD4+ Th2 cells, whereas CD4+ Th17 and ILC2 cell numbers remained constant. Furthermore, the acquired CD4+ Th17 cells in Stat6−/−/IL-17–GFP mice, or innate ILC2s in CD4+ T cell–ablated mice, failed to mount an allergic recall response to OVA Ag. After repeated OVA rechallenge or CD4+ T cell ablation, the increase or loss of CD4+ Th2 cells resulted in an enhanced or reduced IL-13 production by lung ILC2s in response to IL-25 and IL-33 stimulation, respectively. In return, ILC2s enhanced Ag-mediated proliferation of cocultured CD4+ Th2 cells and their cytokine production, and promoted eosinophilic airway inflammation and goblet cell hyperplasia driven by adoptively transferred Ag-specific CD4+ Th2 cells. Thus, these results suggest that an allergic recall response to recurring Ag exposures preferentially triggers an increase of Ag-specific CD4+ Th2 cells, which facilitates the collaborative interactions between acquired CD4+ Th2 cells and innate ILC2s to drive the exacerbation of a murine allergic airway diseases with an eosinophilic phenotype. The Journal of Immunology, 2015, 194: 3583–3593.

In developed countries, exacerbation of allergic asthma often results in significant morbidity (1). The recurrent episodes of allergic asthma are often triggered by repeated allergen exposures that can occur upon a background of chronic allergic inflammation and structural changes (2). Repeated exposures to allergens may mediate the maintenance of a persistent inflammatory process, which often results in an increased severity of chronic allergic asthma (3). It has long been postulated that lung resident CD4+ Th2 cells induced after primary allergen exposure are responsible for the exacerbation of allergic asthma after allergen reexposures (4–6). These Ag-experienced CD4+ Th2 effector memory cells produce IL-4, IL-5, and IL-13 that mediate the eosinophilic inflammation that defines a major subtype of asthma (7–9). The discovery of the CD4+ Th17 cell lineage and its function in driving airway neutrophilic inflammation has provided insight into the understanding of the heterogeneity of chronic asthma (10–12). Increased expression of IL17 transcripts was found to be associated with patients with severe asthma (13, 14). In murine models of allergic lung diseases, IL-17 produced by CD4+ Th17 or IL-17–producing Th2 cells was also shown to contribute to the exacerbation of experimental allergic asthma (15–17). Although many studies have demonstrated the essential roles of Th2 and Th17 immune responses in the pathogenesis of murine allergic airway diseases, little is known about their relative contributions to the Ag-driven exacerbation of murine allergic airway diseases.

In addition to acquired Th2 cell immunity, recent studies identified a novel innate cell lineage, type 2 innate lymphoid cells (ILC2s), as potent Th2 cytokine producers involved in the allergic immune response (18–22). Subsequent studies revealed that ILC2s could develop from common lymphoid progenitors and that their differentiation and maintenance require the transcription factors ROR-α and GATA-3 (23–25). Notably, ILC2s lack Ag-specific receptors and express high levels of an array of cytokine receptors, including IL-25R (IL-17RB), IL-33R (ST2), IL-7Rα, and IL-2Rα (19, 20). ILC2s can rapidly elicit large amounts of IL-5 and IL-13 in response to IL-25 and IL-33 stimulation in the presence of IL-7 and/or IL-2 (19, 26). Indeed, ILC2s were functionally impaired in the Il17rb−/−, St2−/−, or common γ-chain (γc)−/−/Rag2−/− mice, which failed to expel helminthic infection effectively (19, 20). These studies highlight the importance of ILC2s in mediating the protective type 2 immune response against parasitic infection by acting as the early cellular source of Th2 cytokines before the development of acquired T cell immunity.

Accumulating evidence also suggests a critical role of ILC2s in initiating airway hyperresponsiveness (AHR) and eosinophilic inflammation. Intranasal papain or IL-33 administration induced robust

Abbreviations used in this article: AHR, airway hyperresponsiveness; BALF, bronchoalveolar lavage fluid; ILC2, type 2 innate lymphoid cell; Lin, lineage; WT, wild-type.

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IL-5 and IL-13 production by adoptively transferred ILC2s that were sufficient to drive airway eosinophilia in common γ-chain (γc)+/Rag2−/− recipient mice or AHR in Il13−/− mice (26, 27). Four days after the initial exposure to papain and preceding the peak of T cell response, ILC2-deficient mice reconstituted with ROR-α−/− bone marrow displayed significantly less pulmonary inflammation than ILC2-deficient mice transplanted with wild-type (WT) bone marrow did (24). Although these studies highlight the importance of ILC2s in initiating Th2 inflammation in response to intranasal stimuli, the involvement of ILC2s in driving Ag-induced exacerbation of chronic murine allergic airway diseases have been unclear.

Notably, Rag2−/− mice that developed functional ILC2s, but lacked the T cell compartment, also exhibited less severe eosinophilic airway inflammation than WT mice did after repeated papain inhalation (28). These findings suggest that both the innate and adaptive branches of type 2 immunity are required for the maximum exacerbation of chronic allergic inflammation induced by response to repeated allergen.

In this report, we examine the immune response of innate ILC2s and OVA-specific CD4+ Th2 and Th17 cells during the course of the acute, rest, and recall phases of murine allergic airway diseases. We found that in rested mice that were previously sensitized with allergens, repeated OVA Ag rechallenge preferentially induced the accumulation of lung OVA-specific CD4+ Th2 cells, not Th17 cells or ILC2s, and that this accumulation was associated with the profound eosinophilic inflammation and significant increase of OVA-specific IgE and IgG1. Although ILC2s are not sufficient to mount an Ag recall response, these cells enhanced the eosinophilic inflammation induced by Ag-specific CD4+ Th2 cells. Thus, our results suggest that lung-resident, Ag-specific Th2 cells cooperate with ILC2s to drive OVA Ag recall-induced exacerbation of murine allergic airway diseases.

Materials and Methods

Mice

BALB/c mice (Thy1.2) were purchased from the National Cancer Institute (Bethesda, MD). BALB/c mice (Thy1.1; stock number 000443), Stat6−/− mice (stock number 002828), IL-4–GFP reporter (4get) mice (stock number 004910) and DO11.10 TCR transgenic mice (stock number 003303) were purchased from The Jackson Laboratory. IL-17–GFP reporter mice were purchased from Biocytogen. BALB/c mice (Thy1.2+) were purchased from the National Cancer Institute (Bethesda, MD). BALB/c mice (Thy1.1; stock number 005443), Stat6−/− mice (stock number 002828), IL-4–GFP reporter (4get) mice (stock number 004910) and DO11.10 TCR transgenic mice (stock number 003303) were purchased from The Jackson Laboratory. IL-17–GFP reporter mice were purchased from Biocytogen.

Induction of allergic airway diseases

Mice were intranasally sensitized with 70 μg papain (Calbiochem) or Aspergillus oryzae (Sigma-Aldrich) and in the presence of 43 μg OVA (Sigma-Aldrich) protein in 50 μl saline (mixed immediately before administration) or 50 μl saline only every other day for total of six times and then rested for 7 d before intranasal administration of OVA protein (100 μg in 50 μl saline) alone, 70 μg papain in 50 μl saline, or 50 μl saline every other day for a total of additional six times. Potential endotoxin contamination was removed from OVA by endotoxin-removing gel (Thermo Fisher Scientific). Mice were sacrificed 1 d after the last Ag challenge.

Assessment of airway inflammation by bronchoalveolar lavage fluid cellular analysis and histology

Lungs were washed with 1 ml PBS, bronchoalveolar lavage fluid (BALF) was collected, and total cells were counted with a hemocytometer. Slides were prepared by cytospin centrifugation and stained with Fisher HealthCare protocol Hema 3 solutions. BALF cell differential counts were determined using morphologic criteria under a light microscope with evaluation of more than 150 cells per slide. In some experiments, lung tissue was fixed with 10% formalin solution and then submitted to the Pathology Research Center at Cincinnati Children’s Hospital Medical Center for H&E and periodic acid–Schiff staining.

Assessment of airway hyperresponsiveness

AHR was studied in anesthetized mice 1 d after the last Ag challenge. Anesthesia was delivered by i.p. injection of ketamine/xylazine/acepromazine (4:1:1) solution (0.2 ml per animal). Changes in airway resistance to methacholine (acetyl-β-methylcholine chloride; Sigma-Aldrich) were assessed as described previously (29). A tracheostomy was performed, and the mouse was connected to a flexVent system (VCIREQ, Montreal, QC, Canada). Airway resistance was measured after nebulization of PBS (baseline) and then increasing doses of methacholine (25, 50, and 100 mg/ml).

Isolation of lung cells and flow cytometry

Lungs were dissected and forced through a 40-μm cell strainer to make single-cell suspensions and then analyzed by flow cytometry. In some experiments, lungs were first enriched for CD11b- and CD19-negative cells by magnetic anti-CD11b and anti-CD19 microbeads and then separated into two tubes for staining: T cells were stained with PE-Cy7–conjugated anti-CD3ε (145-2C11), Pacific Blue–conjugated anti-CD4 (RM4-5), anti-CD8 (53-6.7), Gr-1 (RB6-8C5), and CD335 (NKP46, 29A1.4), allophycocyanin–Cy7–conjugated anti-CD62L, and/or allophycocyanin–conjugated anti-CD11b (32). ILC2s were stained with allophycocyanin–conjugated rat anti-human/mouse IL-17R mAb (clone S13B, a gift from Dr. Kenji Izuhara, Saga Medical School, Japan), PerCP-Cy5.5–conjugated mAbs against Lin markers (NK1.1 [PKP136], CD11b [M1/70], CD11c [HL3], CD8 [53-6.7], B220 [RA3-6B2], Gr-1 [RB6-8C5], and CD335 [NKP46, 29A1.4]), PE-conjugated anti-ICOS (7E.1C9), allophycocyanin–Cy7–conjugated anti-CD25 (PC61), PE–Cy7–conjugated anti-CD127 (ART34), V500-conjugated anti-Sca1 (Ly6A/E, D7), and biotinylated anti-T1/ST2(DJ8) Abs and then further stained with Brilliant Violet 421–labeled Streptavidin (BioLegend) before analyses with a FACSCan II (BD Biosciences) or cell sorting with a FACSAria II (BD Biosciences) maintained by the Research Flow Cytometry Core in the Division of Rheumatology at Cincinnati Children’s Hospital Medical Center. Data were analyzed using FlowJo™ software.

ELISA

Single-cell suspensions were made from lungs of mice 1 d after the last Ag challenge: 3.0 × 106 cells were stimulated with medium only, OVA (10 μg/ml), or IL-25 (10 ng/ml) plus IL-33 (10 ng/ml), and in some experiments, anti–mIL-2 mAbs (clone: JES6-1A12, 10 μg/ml) were added for 3 d. Collected supernatants were assessed by ELISA for IL-5 (R&D Systems), IL-13 (Antigenix America), IL-4, IL-17, and IFN-γ (BD Biosciences).

Serum OVA-specific IgE and OVA-specific IgG1 were measured using an OVA-IgE ELISA kit (MD Bioproducts) or OVA-IgG1 ELISA kit (Alpha Diagnostic), respectively.

RNA isolation and quantitative real-time PCR analysis

RNA from sorted cell populations was isolated with an RNeasy Plus Mini kit (Qiagen), and cDNA templates were synthesized with SuperScript III reverse transcriptase (BioRad). Quantitative real-time PCR analyses were performed with SYBR Green Chemistry (Applied Biosystems) in an ABI Prism 7900 detection system using previously described primer sets (30, 31). Expression levels of target genes were normalized to endogenous Gapdh transcript levels, and relative quantification of samples was compared with the expression level of indicated genes in naive CD4+ T cells isolated from naive BALB/c mice as the baseline.

CD4+ T cell depletion

Mice were injected i.p. with anti-CD4 mAb (clone GK1.5; 300 μg/mouse in 100 μl PBS, Bio-X Cell) or Rat IgG (Jackson ImmunoResearch) 24 h before the first and the fourth OVA rechallenges.

Coculture and adoptive transfer of OVA-specific CD4+ Th2 cells and ILC2s

OVA-specific CD4+ Th2 cells were generated as described (15). DO11.10 TCR transgenic mice were first immunized i.p. with 100 μg of OVA323–339 peptide in 0.1 ml saline and OVA-protein/Alum (Thermo Fisher Scientific). CD4+ T cells were positively selected from splenocytes and cultured with APCs (irradiated CD4+ cells) pulsed with 10 μM OVA323–339 peptide in the presence of anti–IFN-γ mAb (20 μg/ml; XMG 1.2) and IL-4 (10 ng/ml; Peprotech) for 7 d.

CD4+ Th2 CELLS AND ILC2S EXACERBATE ALLERGIC LUNG DISEASES

Aspergillus oryzae

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ILC2s were induced as described previously (20). BALB/c mice were injected with 400 ng recombinant mouse IL-25 (R&D Systems) in PBS daily for total of 4 d. Mesenteric lymph nodes were collected and pooled together for single-cell suspensions. After staining the cells with purified rat anti-mouse CD19 (53.6.72), rat anti-mouse CD8, CD8, CD8, and CD4 cells were stained with ILC2s markers as described above, and IL-17RB/ST2/Lin- cells were sorted out as ILC2s. Purified ILC2s were assessed for surface MHC class II expression with FITC-conjugated anti-mouse MHC class II (NIMR-4) by flow cytometry; 5.0 \( \times 10^5 \) irradiated mouse MHC class II (NIMR-4) by flow cytometry; 5.0 \( \times 10^4 \) pulsed ILC2s (5.0 \( \times 10^5 \)) cocultured with ILC2s (5.0 \( \times 10^5 \)) pulsed with 1 \( \mu \)g/ml OVA23-339 peptide for 3 d before the analysis of intracellular cytokine production by flow cytometry or the collections of supernatants for the measurements of cytokine production using ELISA. In some experiments, BALB/c mice were labeled with CellTrace Violet (Molecular Probes) before coculture with or without OVA23-339 peptide-pulsed ILC2s for the analysis of proliferation. For in vivo experiments, Thy1.2BALB/c mice were injected i.v. with OVA-specific CD4 Th2 cells (1.0 \( \times 10^5 \)), ILC2s (5.0 \( \times 10^5 \)), or both cell types 1 d before intranasal challenge with OVA protein (100 \( \mu \)g) for 3 consecutive days. In some experiments, congenic Thy1.1BALB/c mice were used and treated with 250 \( \mu \)g anti-Thy1.1 mAb (clone 19E12; Bio-X Cell) i.p. on the day of and 2 d after cell transfer. Total and differential cell counts in BALF were evaluated 24 h after the last OVA challenge. The left lung was fixed with 10% formalin solution and then submitted to the Pathology Research Core at Cincinnati Children’s Hospital Medical Center for H&E staining and periodic acid-Schiff staining. The rest of the lung tissue was forced through 40-μm strainers to make single-cell suspensions and then stimulated with OVA protein (10 \( \mu \)g/ml) or medium as a control for 3 d. Secreted cytokines in the collected supernatants were assessed with ELISA.

**Results**

Repeated Ag rechallenges preferentially induce eosinophilic airway disease

To study CD4+ Th cell response to recurrent Ag exposures in the context of allergic inflammation, we first challenged mice six times intranasally with OVA Ag plus protease allergens papain to induce an acute phase of pulmonary allergic inflammation. After 7 d of rest, sensitized mice were rechallenged intranasally with OVA or saline only for an additional six times before subsequent analysis of the pulmonary CD4+ Th cell response (protocol diagramed in Fig. 1A). Compared with naive mice, the first episode of repeated intranasal challenge triggered substantial infiltration of the airway by inflammatory cells that included predominantly eosinophils (40%), neutrophils, macrophages, and lymphocytes, as shown in the total and differential cell counts of the BALF (Fig. 1B and data not shown). These sensitized mice produced detectable titers of serum OVA-specific IgE (>1.0 \( \mu \)g/ml) and IgG1 (>1.0 \( \times 10^7 \) U/ml; Fig. 1C), displayed pathological changes in the lung as evidenced by their prominent mucus production and goblet cell hyperplasia,

**FIGURE 1.** Repeated OVA Ag rechallenges preferentially induce eosinophilic murine allergic airway diseases. (A) Mice were exposed to OVA and papain (tick marks) every other day for a total of six times (acute phase, days 1–11), rested for 7 d (rest phase, days 12–18), and rechallenged with OVA or saline alone every other day (tick marks) for a total of six times (recall phase, days 19–29). Mice were sacrificed, and indicated samples were collected for analysis at selected time points (arrows, day 0, 12, 18, 19, 23, and 29). (B) BALF and (C) serum samples from naive mice or mice sacrificed 1 d after the indicated phase, or times of rechallenges were collected for the measurements of BAL total, eosinophil, or (B) neutrophil cell numbers and (C) titers of serum OVA Ag-specific IgE and IgG1. (D) Mice that experienced the acute and rest phase were then rechallenged with saline, BSA, or OVA every other day for total six times at the recall phase. One day after the last rechallenge, their AHR were measured with flexiVent (D) and lung sections were analyzed by H&E and periodic acid–Schiff staining (E). Data represent mean ± SEM. Scale bars, 100 μm. **p < 0.01, ***p < 0.001. OVA-, OVA Ag-specific.
and developed AHR as previously reported (data not shown) (15, 32). Thus, similar to previous studies of a murine allergic airway diseases (15, 32), the first episode of repeated intranasal challenge is sufficient to induce the development of murine allergic airway diseases promptly, termed the “acute phase.” After 7 d of rest, significantly fewer eosinophils and neutrophils were retained in the airway, and mucus production and goblet cell hyperplasia were reduced (Fig. 1B and data not shown). Notably, these rested mice produced similar amounts of OVA-specific IgE and IgG1 as mice in the acute phase of murine allergic airway diseases (Fig. 1C). These results suggest that the Ag OVA-specific Th2 immune response persists in the rested mice, despite the resolution of pulmonary inflammation. Indeed, reexposure only to OVA Ag rapidly induced a significant increase in the eosinophil, but not neutrophil, infiltration of the airway, which is positively associated with the number of OVA Ag rechallenges (Fig. 1B). Furthermore, these rechallenged mice also developed a robust humoral response against OVA Ag as evidenced by their elevated serum titers of OVA-specific IgE (>30 μg/ml) and IgG1 (>5.0 × 10^8 U/ml; Fig. 1C). Consequently, repeated rechallenge with OVA Ag only, but not saline or BSA, induced these rested mice to redevelop severe AHR and prominent mucus production and goblet hyperplasia, termed the “recall phase” of murine allergic airway diseases (Fig. 1D, 1E). Together, these data suggest that intranasal OVA rechallenge preferentially triggers airway eosinophilic inflammation and further increases the levels of Ag-specific serum IgE and IgG1 in rested mice that previously developed pulmonary allergic inflammation.

Repeated Ag reexposure preferentially enhance CD4+ Th2 immune response

We next examined the frequency of CD4+ T cell compartments before and after repeated OVA Ag reexposures in IL-4–GFP and IL-17–GFP reporter mice. Although few lung GFP+CD4+ T cells could be detected in naive reporter mice (<1% of total CD4+ T cells), the initial set of OVA Ag plus papain challenges, not saline only, induced similar percentages or numbers of CD4+ Th2 (15.1 ± 1.2% or 127.8 ± 5.7 × 10^3, mean ± SEM) or CD4+ Th17 (14.8 ± 1.1% or 140.6 ± 7.8 × 10^3, mean ± SEM) cells in the lung of challenged IL-4–GFP or IL-17–GFP reporter mice, respectively (Fig. 2A, 2B, Supplemental Fig. 1B). We confirmed that purified CD4+CD62L−GFP+ cells from challenged IL-4–GFP or IL-17–GFP reporter mice produced indicated Th2 or Th17 cytokines, respectively, demonstrating that the GFP expression by these reporter mice faithfully marked indicated T cell immune response (Supplemental Fig. 1A). After 7 d of rest, the numbers of both CD4+ Th cell subsets (67.2 ± 3.9 × 10^3 versus 74.8 ± 5.6 × 10^3, Th2 versus Th17, mean ± SEM) decreased significantly (Fig. 2A, 2B). Notably, repeated OVA Ag reexposures triggered a substantial increase in lung resident CD4+ Th2 cells in both percentage (37.1 ± 1.3%, mean ± SEM) and number (341.2 ± 15.5 × 10^3, mean ± SEM), which were much fewer than those of CD4+ Th17 cells (80.9 ± 4.2% or 403.1 ± 2.5 × 10^3, mean ± SEM). Indeed, reexposure only to OVA Ag rapidly induced a significant increase in the eosinophil, but not neutrophil, infiltration of the airway, which is positively associated with the number of OVA Ag rechallenges (Fig. 1B). Furthermore, these rechallenged mice also developed a robust humoral response against OVA Ag as evidenced by their elevated serum titers of OVA-specific IgE (>30 μg/ml) and IgG1 (>5.0 × 10^8 U/ml; Fig. 1C). Consequently, repeated rechallenge with OVA Ag only, but not saline or BSA, induced these rested mice to redevelop severe AHR and prominent mucus production and goblet hyperplasia, termed the “recall phase” of murine allergic airway diseases (Fig. 1D, 1E). Together, these data suggest that intranasal OVA rechallenge preferentially triggers airway eosinophilic inflammation and further increases the levels of Ag-specific serum IgE and IgG1 in rested mice that previously developed pulmonary allergic inflammation.

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Trophils; OV A-, OV A Ag-specific. Lym, lymphocytes; Mac, macrophages; Neut, neutrophils; OD, ovalbumin.

induce an increase in percentage and number of KJ1-26+CD4+CD62L−IL-4+Th2 cells were significantly increased from the acute phase (0.011 ± 0.005% and 192.7 ± 64.2, mean ± SEM) after repeated OVA Ag rechallenge (0.063 ± 0.006% and 783.3 ± 121.0, mean ± SEM; Fig. 2C, 2D). In contrast, repeated OVA Ag rechallenge failed to induce an increase in percentage and number of KJ1-26+CD4+CD62L−IL-17+Th17 cells (0.021 ± 0.008% and 168.3 ± 49.9 at the acute phase; 0.031 ± 0.003% and 264.0 ± 54.8 at the recall phase; Fig. 2C, 2D). In concert with these observations, ex vivo OVA or papain Ag restimulation induced lung CD4+ T cells to produce significantly higher amounts of the Th2 cytokines IL-5 and IL-13 at the recall phase than at the rest or acute phase (Fig. 2E and Supplemental Fig. 1C). These results suggest that lung resident CD4+ Th2 cells are the primary cells responsible for mounting an Ag recall response and that the percentage of Ag-specific CD4+ Th2 cells, not Th17 cells, increased significantly after Ag reexposure in rested mice that previously developed allergic airway diseases.

**Th17 cells are not sufficient to mount an Ag-induced recall allergic response in the absence of the Th2 cell compartment**

Allergen-specific CD4+ Th2 cells that reside in the lung after primary allergen exposure have been suggested to be responsible for the exacerbation of allergic asthma after allergen reexposures (4, 5). To evaluate the essential role of lung resident Th2 cells in mediating OVA recall–induced murine allergic airway diseases, we examined mice deficient in STAT-6, a key transcription factor for Th2 but not Th17 cell differentiation, that were further crossed with IL-17−GFP reporter mice (Stat6−/−/IL-17−GFP) (33, 34). Indeed, compared with IL-17−GFP WT mice, Stat6−/−/IL-17−GFP mice lacking the CD4+ Th2 cell compartment exhibited substantially reduced infiltration by inflammatory cells, including eosinophils, macrophages, and lymphocytes but not neutrophils, in the airway after repeated intranasal OVA Ag rechallenge (Fig. 3A). Notably, repeated OVA Ag rechallenge failed to induce robust increases of serum OVA Ag-specific IgE and OVA Ag-specific IgG1, as well as AHR, in the Stat6−/−/IL-17−GFP mice (Fig. 3B, 3C). In concert with the observation of normal neutrophil recruitment, the number of infiltrated CD4+ Th17 cells (159.3 ± 26.9 × 103, mean ± SEM) in the Stat6−/−/IL-17−GFP mice was comparable to that (173.1 ± 9.5 × 103, mean ± SEM) in IL-17−GFP WT mice (Fig. 3D, 3E). In addition, we did not observe an increase of the OVA Ag-specific CD4+ Th17 cell recall response to repeated OVA Ag rechallenge (Fig. 3E). Consequently, low levels of IL-5 and IL-13 cytokines were produced by OVA Ag–stimulated lung cells of Stat6−/−/IL-17−GFP mice ex vivo, whereas their IL-17 and IFN-γ production were higher than those in the lungs of mice at the acute phase (Fig. 2A, 2B). We also observed increased infiltration of CD4+ Th17 cells; however, their percentage (15.4 ± 1.1%, mean ± SEM) and number (165.7 ± 6.7 × 103, mean ± SEM) appeared similar to those in the lungs of mice at the acute phase (Fig. 2A, 2B). Similarly, repeated papain Ag reexposures also preferentially triggered a significant increase of CD4+ Th2 number (227.6 ± 21.6 × 103, mean ± SEM), not CD4+ Th17 cells (144.0 ± 3.2 × 103, mean ± SEM), compared with those in the lungs of mice at the acute phase (Supplemental Fig. 1B). Taking advantage of the KJ1-26 mAb that reacts with TCRs that recognize the MHC class II–restricted OVA323–339 peptide specifically, subsequent analysis further revealed that the percentage and number of lung KJ1-26+CD4+CD62L−IL-4+Th2 cells were significantly increased from the acute phase (0.011 ± 0.005% and 192.7 ± 64.2, mean ± SEM) after repeated OVA Ag rechallenge (0.063 ± 0.006% and 783.3 ± 121.0, mean ± SEM; Fig. 2C, 2D). In contrast, repeated OVA Ag rechallenge failed to induce an increase in percentage and number of KJ1-26+CD4+CD62L−IL-17+Th17 cells (0.021 ± 0.008% and 168.3 ± 49.9 at the acute phase; 0.031 ± 0.003% and 264.0 ± 54.8 at the recall phase; Fig. 2C, 2D). In concert with these observations, ex vivo OVA or papain Ag restimulation induced lung CD4+ T cells to produce significantly higher amounts of the Th2 cytokines IL-5 and IL-13 at the recall phase than at the rest or acute phase (Fig. 2E, Supplemental Fig. 1C), whereas the amounts of IL-17 and IFN-γ produced by lung CD4+ T cells were comparable between the acute and recall phase (Fig. 2E and Supplemental Fig. 1C). These results suggest that lung resident CD4+ Th2 cells are the primary cells responsible for mounting an Ag recall response and that the percentage of Ag-specific CD4+ Th2 cells, not Th17 cells, increased significantly after Ag reexposure in rested mice that previously developed allergic airway diseases.

**FIGURE 3.** Th17 cells are not sufficient to mount OVA Ag–induced eosinophilic murine allergic airway diseases. One day after the last OVA rechallenge, (A) total and differential cell counts in BALF; (B) serum OVA-specific IgE and IgG1 levels (OVA-IgE, OVA-IgG1); and (C) airway resistance to increasing doses of methacholine were measured in WT IL-17−GFP or Stat6−/−/IL-17−GFP reporter mice. (D) Expression of IL-17−GFP and CD62L by CD4+ T cells (gated on Lin−CD3+CD4+ cells) from lungs of WT IL-17−GFP or Stat6−/−/IL-17−GFP reporter mice were examined with flow cytometry. (E) Numbers of GFP+CD62L−CD4+ Th17 cells and GFP−KJ1-26−CD4+ Th17 cells in lungs from WT IL-17−GFP or Stat6−/−/IL-17−GFP reporter mice. (F) Lung cells were restimulated with medium (Med) or OVA for 3 d, and the indicated cytokines in supernatant were examined using ELISA. Data represent one of three independent experiments (n = 4 mice per group). Error bars denote mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001. Eos, eosinophils; Lym, lymphocytes; Mac, macrophages; Neut, neutrophils; OVA-, OVA Ag-specific.
similar to those in the IL-17–GFP WT mice (Fig. 3F). Collectively, these results suggest that the CD4+ Th2, not Th17, cell compartment is required for mounting the Ag recall allergic immune response.

Repeated OVA Ag rechallenge promotes IL-13 production by ILC2s

Recent studies demonstrated a pivotal role of ILC2s in the initiation of allergic inflammation (26, 27, 35); however, their frequency and function at the acute and recall phase of murine allergic airway diseases have not been addressed. Multicolor flow cytometric analysis identified a dominant cell population that expressed ST2 and IL-17RB but not known cell lineage markers (Lin-2) in the lungs of mice sensitized with papain plus OVA (Fig. 4A). These Lin-2ST2+IL-17RB+ cells also expressed IL-2Rα (CD25), IL-7Rα (CD127), ICOS, and Sca-1, the signature markers of ILC2s (19, 20, 36) (Fig. 4A). Furthermore, purified Lin-2ST2+IL-17RB+ cells from the lungs of mice with allergic airway diseases expressed high levels of the transcription factor Rora, Gata3, Il2, Il5, Il13, and Amphiregulin transcripts (Fig. 4B). Thus, these Lin-2ST2+IL-17RB+ cells detected in our murine model of allergic lung diseases are the recently described ILC2s (36). Next, we examined the percentage and number of these lung resident ILC2s at the acute, resting, and recall phase of allergic lung diseases and compared their capability to produce the Th2 cytokines IL-5 and IL-13 in response to IL-25 plus IL-33 stimulation ex vivo. Although few ILC2s (frequency, 0.5% ± 0.2%; number, 3.3 × 10^3 ± 1.3 × 10^3; mean ± SEM) could be detected in the lung of naive or saline-challenged mice, the initial intranasal OVA plus papain challenge triggered a significant increase in their percentage (2.5% ± 0.3%, mean ± SEM) and number (64.6 × 10^3 ± 4.2 × 10^3, mean ± SEM; Fig. 4C and data not shown). Intriguingly, the percentage (2.2% ± 0.3%, mean ± SEM) and number (63.7 × 10^3 ± 1.8 × 10^3, mean ± SEM) of ILC2s persisted in the lungs of challenged mice after 7 d of rest (Fig. 4C), although the pulmonary inflammation had largely resolved (Fig. 1B). Notably, compared with their frequency at the acute phase, repeated saline or OVA Ag rechallenge did not further increase lung ILC2 percentage (2.5% ± 0.4%, mean ± SEM) or number (66.8 × 10^3 ± 3.4 × 10^3, mean ± SEM) at the recall phase (Fig. 4C and data not shown). Treatment with IL-25 plus IL-33 induced ILC2s within lung homogenates from mice at each phase of allergic airway diseases to produce comparable amounts of IL-5 (Fig. 4D). Intriguingly, although their numbers remained constant, ILC2s from mice at the recall phase, which developed a significantly increased number of

![Image](http://www.jimmunol.org/DownloadedFrom/3588CD4-Th2-CELLS-AND-ILC2S-EXACERBATE-ALLERGIC-LUNG-DISEASES.png)
lung CD4+ Th2 cells, produced a substantially larger amount of IL-13 than cells within the lungs of mice after the acute or rest phase after IL-25 and IL-33 or PMA/ionomycin stimulation (Fig. 4D, 4E). The increased IL-13 production by lung cells at the recall phase was not primarily contributed by the increased CD4+ Th2 cells that also expressed surface ST2 (data not shown), because these CD4+ Th2 cells produced 100-fold less IL-13 than that produced by ILC2s upon IL-25 and IL-33 stimulation (Supplemental Fig. 2A). These results suggest that, although ILC2 frequency remains constant in the lungs of mice during the development of chronic murine allergic airway diseases, their capability of producing IL-13 increases significantly and is positively associated with the number of lung CD4+ Th2 cells at the recall phase.

ILC2s failed to mount OVA Ag recall response in mice ablated of CD4+ T cells

ILC2s were shown to initiate the early phase of the type 2 immune response against parasitic infection and trigger the allergic immune response (20, 26). To study whether these cells alone can trigger an allergic response in the inflamed lung at the recall phase of allergic lung diseases, we injected anti-CD4 mAb i.p. to deplete CD4+ T cells 1 d before the first and fourth OVA Ag rechallenges (Fig. 5A). Indeed, anti-CD4 mAb treatments resulted in a significant reduction in the percentage and numbers of CD4+ Th2 cells but not ILC2s in the lungs of OVA Ag-rechallenged mice (Fig. 5B). Consequently, OVA-induced IL-5 and IL-13 production by Ag-specific CD4+ Th2 cells in the lung of anti-CD4 mAb–treated mice were significantly less than those of isotype mAb-treated mice. Intriguingly, although ILC2s remaining intact after the loss of lung CD4+ T cells (Fig. 5B), these cells responded poorly to IL-25/IL-33 stimulation and produced substantially less IL-13, but not IL-5, IFN-γ, or IL-17, than that produced by ILC2s of isotype mAb-treated mice (Fig. 5C). Furthermore, despite comparable frequency or numbers between Stat6−/−/IL-17–GFP mice that lacked CD4+ Th2 cell compartment and IL-17–GFP WT reporter mice at the acute and recall phases (Supplemental Fig. 2B), ILC2s in the lung of rechallenged Stat6−/−/IL-17–GFP mice were less capable of producing IL-13 than those in rechallenged IL-17–GFP WT reporter mice in response to IL-25 and IL-33 stimulation ex vivo (Supplemental Fig. 2C). In addition, anti–IL-2 mAb treatments abrogated the enhanced IL-13 production by ILC2 in the lung of rechallenged IL-17–GFP WT reporter mice in response to IL-25 and IL-33 stimulation ex vivo (Supplemental Fig. 2D). Therefore, repeated OVA Ag rechallenge failed to induce a significant increase in inflammatory cell infiltrations, particularly eosinophils in the BALF, or their serum OVA Ag-specific IgE and OVA Ag-specific IgG1 levels in the mice ablated of CD4+ T cells or deficient of Stat6 (Figs. 3A, 3B, 5D, 5E). Furthermore, histologic examination showed that these anti-CD4 mAb–treated or Stat6-deficient mice exhibited attenuated peribronchial inflammation and goblet cell hyperplasia (data not shown). Together, these results suggest that ILC2s in the inflamed lung are not sufficient to mediate allergic

**FIGURE 5.** ILC2s failed to mount an OVA Ag recall response in mice ablated of CD4+ T cells. (A) IL-4–GFP reporter mice were exposed to OVA and papain every other day for a total of six times during the acute phase, rested for 7 d, and then rechallenged with OVA alone every other day for a total of six times during the recall phase. Anti-CD4 or isotype control mAbs were injected i.p. into mice 1 d before the first (day 17) and 1 d before the fourth OVA rechallenges (day 23). Mice were sacrificed 1 d after the last OVA rechallenge when samples were collected for analysis. (B) Frequency and numbers of CD4+Th2 (Lin−CD3+CD62L+GFP+) and ILC2s (Lin−CD3−CD4+ST2−IL-17RB−) cells in the lung of mice treated with indicated mAbs were analyzed 1 d after the last OVA rechallenge. (C) Lung cells were restimulated with medium (Med), OVA, or IL-25 plus IL-33 for 3 d, and the indicated cytokines in supernatant were examined using ELISA. (D) Total and differential cell counts in BALF. (E) Serum OVA-IgE and IgG1 levels were measured using ELISA. Data represent one of three independent experiments (n = 4 mice per group). Error bars denote mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001. Eos, eosinophils; Lym, lymphocytes; Mac, macrophages; Neut, neutrophils; OVA-, OVA Ag-specific.
immune response against repetitive OVA rechallenges in mice lacking the CD4+ Th2 cell compartment, which cannot only mount Ag recall response but also enhance ILC2 function in their IL-13 production, possibly via their IL-2 production.

ILC2s facilitate Ag-specific Th2 cell–driven allergic airway inflammation by enhancing type 2 cytokine production

Recent studies suggest that ILC2s may have the capacity to modulate CD4+ T cell differentiation (37). To address whether ILC2s can augment an Ag-specific Th2 immune response, we examined Th2 cytokine production by in vitro–generated OVA Ag-specific CD4+ Th2 cells cocultured with or without purified ILC2s in vitro (Supplemental Fig. 3A). Consistent with recent reports (37), ILC2s were found to express detectable surface MHC class II and had the potential to process OVA Ag, thus inducing a moderate level of OVA-specific Th2 cell proliferation (Supplemental Fig. 3B–D). Furthermore, cocultured ILC2s pulsed with OVA323–339 peptide induced OVA Ag-specific CD4+ Th2 cells to produce significantly larger amounts of IL-4, IL-5, and IL-13, but not IFN-γ production, than those produced by OVA Ag-specific CD4+ Th2 cells or ILC2s alone stimulated with OVA323–339 peptide ex vivo (Fig. 6A, Supplemental Fig. 3E). Conversely, these activated OVA Ag-specific CD4+ Th2 cells also enhanced the function of cocultured ILC2s in producing IL-13 cytokine as revealed by intracellular cytokine staining (Supplemental Fig. 3E). To demonstrate whether ILC2s can enhance an Ag-specific Th2 cell immune response in vivo, allergic responses exhibited by mice that were adoptively transferred with ILC2s alone, OVA Ag-specific CD4+Th2 cells with or without ILC2s were examined and compared after repeated Ag challenge. One day after a regimen of three OVA Ag inhalation challenges, the number of infiltrated eosinophils, but not neutrophils, macrophages, or lymphocytes, in the airway of mice transferred with both OVA Ag-specific CD4+Th2 cells and ILC2s were significantly higher than in mice transferred with OVA Ag-specific CD4+ Th2 cells alone or ILC2s alone (Fig. 6B). Correspondingly, mice given both OVA Ag-specific CD4+ Th2 cells and ILC2s exhibited much more pronounced mucus production and goblet cell hyperplasia compared with mice given OVA Ag-specific CD4+ Th2 cells or ILC2s alone (Fig. 6C). To confirm that donor-derived ILC2s indeed infiltrated into lung and facilitated OVA Ag-specific CD4+ Th2 cell–mediated airway inflammation, congenic Thy1.1+ mice were treated with anti-Thy1.1 mAb to deplete en-

**FIGURE 6.** ILC2s facilitate Ag-specific Th2 cell–driven allergic airway inflammation by enhancing Ag-driven type 2 cytokine production. (A) In vitro–generated OVA-specific Th2 cells, ILC2s, and a combination of OVA-specific Th2 cells and ILC2s were stimulated with OVA323–339 Peptide for 3 d; the indicated cytokines in the supernatant were examined using ELISA. (B–D) BALB/c mice were injected i.v. with in vitro–generated OVA-specific Th2 cells, ILC2s, and a combination of OVA-specific Th2 and ILC2s and were challenged intranasally 24 h later with OVA alone for 3 consecutive days. One day after the last OVA challenge, (B) total and differential cell numbers in BALF were counted, (C) histology of lung sections were examined by H&E and periodic acid–Schiff staining, and (D) lung cells from mice in the indicated groups were restimulated with OVA ex vivo for 3 d, and cytokines in the supernatant were examined using ELISA. Data represent one of three independent experiments (n = 4 mice per group). Error bars denote mean ± SEM. Scale bars, 100 μm. *p < 0.05, **p < 0.01, ***p < 0.001. Eos, eosinophils; Lym, lymphocytes; Mac, macrophages; Neut, neutrophils.
dogenous ILC2s before adoptive transfer with Thy1.2+ cells. Consis-
tently, the numbers of infiltrated inflammatory cells in the airway of
anti-Thy1.1 mAb-treated congenic Thy1.1+ mice that were later
transferred with both OVA Ag-specific Thy1.2+CD4+Th2 cells and
Thy1.2+ILC2s were significantly higher than those in mice that
received Thy1.2+CD4+Th2 cells alone (Supplemental Fig. 4A–C).
In agreement with the observations from the in vitro coculture as-
say, the lung cells from mice transferred with both cell types pro-
duced much greater amounts of IL-5 and IL-13, but not IL-17 and
IFN-γ, after ex vivo OVA protein restimulation than lung cells from
mice transferred with only Th2 cells or only ILC2s (Fig. 6D).
Collectively, these results suggest that innate ILC2s can facilitate
Ag-driven reactivation of lung-resident Ag-specific CD4+ Th2 cells,
which in return promote ILC2 function to produce increased IL-13
cytokine during the Ag recall response.

Discussion

Allergic asthma is a heterogeneous inflammatory disease of the
airway. A subgroup of patients with severe asthma often has per-
sistent symptoms and frequent exacerbations, leading to poor dis-
ease control and considerable morbidity (1, 38–40). Accumulating
clinical evidence suggests that airway eosinophilic inflammation is
associated with a subphenotype of asthma that is prone to become
exacerbated (41–45); however, the underlying immunologic mech-
anisms that preferentially drive the eosinophilic phenotype of severe
asthma remain elusive. In this study, we established a murine model
of chronic allergic lung diseases with prominent airway eosinophilic
inflammation. We observed that the increase of airway eosinophils
was positively associated with the number of repeated intranasal
OVA Ag rechallenges, which preferentially drive the OVA Ag-
specific humoral and cellular Th2 immune response. Although
both OVA Ag-specific CD4+Th2 and Th17 cells were induced in the
lungs of mice that developed acute allergic airway diseases, re-
peated Ag rechallenge preferentially drove the increase of lung
OVA Ag-specific CD4+ Th2, not CD4+ Th17, cell numbers. Nota-
bly, the increased number of lung CD4+ Th2 cells resulted in en-
hanced IL-13 production by lung ILC2s in response to IL-25 and
IL-33 stimulation. Reciprocally, ILC2s could facilitate CD4+ Th2
cells to mediate the exacerbation of allergic lung diseases by pro-
moting Ag-specific type 2 cytokine production.

Several animal studies have demonstrated that both CD4+ Th2
and Th17 immune responses can occur concomitantly in the lung
after aerallergen exposures and have suggested their roles in
driving the heterogeneity and severity of allergic airway diseases
(15, 16, 46, 47). However, few studies explore the frequency of an
Ag-specific CD4+ Th2 and Th17 recall response and the relative
contributions of Th2 and Th17 cells in the Ag-driven exacerbation
of murine allergic airway diseases. Similar to previous observa-
tions (48), we showed that the presence of the protease allergens
papain or Aspergillus oryzae facilitated the development of lung
CD4+ Th2 and Th17 immune responses to repeated intranasal
OVA Ag challenge and resulted in allergic inflammation in the
airway. Intriguingly, after 7 d of rest and the resolution of airway
inflammation, repeated intranasal OVA Ag rechallenges alone
were sufficient to drive the exacerbation of murine allergic lung
diseases with prominent airway eosinophilia in mice that previ-
ously experienced airway inflammation. Notably, we found that
the number of OVA Ag rechallenges is positively associated with
the number of airway eosinophils and that reexposure to OVA Ag
induced a significant increase of lung OVA Ag-specific CD4+ Th2
immune response but not CD4+ Th17 immune response. Fur-
thermore, Stats3−/−/IL-17-GFP mice, which lack the Th2 immune
compartment, did not exhibit exacerbation after repeated OVA Ag
rechallenges, despite their OVA Ag-specific Th17 cell immune
response developing normally. Thus, our findings suggest that in-
haled allergens may preferentially induce the expansion of lung-
resident, Ag-specific CD4+ Th2 cells, but not Th17 cells, supporting
the notion that the Ag-specific CD4+ Th2 cells are the primary cell
type responsible for mounting the Ag recall response during allergic
inflammation. Similar to our observations, another study demon-
strated that OVA Ag-specific CD4+ Th2 cells induced by inhaled
OVA and lipid Ags can recirculate and seed in the lung of a para-
biotic partner to mount an OVA Ag-specific Th2 immune response
and drive allergic inflammation (49). The underlying mech-
anisms that preferentially mediate the increase of OVA Ag-specific
CD4+ Th2 immune response after repeated OVA Ag rechallenge are
currently unclear. Whether the OX40 ligand-expressing pulmonary
dendritic cells endowed by thymic stromal lymphopoietin protein or
pulmonary Th2-permissive microenvironment have a critical role in
determining the Ag-specific allergic immune response remains to be
addressed (50).

The ILC2, a new member of the innate lymphoid cell lineage, was
recently identified in a search for non-B/non–T cells that
respond to IL-25 stimulation by initiating type 2 immune re-
ponses against parasitic infection or by driving acute airway in-
flammation (18–20, 51, 52). Systemic administration of IL-25 or
IL-33 cytokine in mice is sufficient to trigger a substantial influx
of ILC2s into the peripheral lymphoid tissues and to activate these
cells to produce large amounts of IL-5 and IL-13 that initiate acute
allergic inflammation in the absence of Ags and prior to the de-
velopment of adaptive immunity (19–21, 51, 53). However,
the frequency and function of ILC2s during the manifestations
of chronic allergic disorders driven by the adaptive immunity after
prolonged allergen exposures have not been addressed. In our
murine model of chronic allergic lung diseases, we observed that
after the lung infiltration triggered by the initial allergen expo-
sures during the acute phase, the number of lung ILC2s remained
constant during the resting and recall phases, regardless of the
number of Ag rechallenges or the number of lung-resident CD4+
Th2 cells. Indeed, ablation of CD4+ T cells in mice that developed
acute allergic airway diseases failed to affect ILC2s frequency
after repetitive OVA Ag rechallenge. Notably, the function of lung
ILC2s to produce IL-13 in response to IL-25/IL-33 stimulation
ex vivo correlated positively with the number of lung CD4+ Th2
cells after repeated OVA Ag rechallenge. These intriguing findings sug-
gest that the lung ILC2s function can be regulated by environmental
cues induced by the acquired CD4+ Th2 cell compartment and are in
concert with a recent finding showing that although ILC2s consti-
tutively secrete IL-5, their capability to produce IL-13 depends on
allergic inflammation (54). The molecular mechanisms underlying
how Th2 cells might influence the function of ILC2s are currently
unclear. However, IL-2 from CD4+ T cells has been suggested as
a key factor in this process (37, 55). These results suggest that the
frequency and number of ILC2s are sustained after mice develop
allergic airway diseases regardless of the inflammatory status in the
lung and that the number of CD4+ Th2 cells correlates positively
with their IL-13 production.

Originally termed as “accessory cells” because of their surface
expression of MHC class II (51), ILC2s have been suggested to
have a function in modulating CD4+ T cell–mediated immune re-
response (56, 57). Indeed, recent studies demonstrate that ILC2s can
facilitate the initiation of CD4+ Th2 cell immune response at the
sensitization phase of allergic lung inflammation (58, 59), possibly
via a cell-cell contact– and MHC class II–dependent manner (37).
Similarly, MHC class II–expressing ILC2s are also shown to pro-
mote protective CD4+ Th2 immune response against parasitic hel-
minth infection (60). In support of these findings, our results also
show that cocultured ILC2s that expressed MHC class II could
enhance type 2 cytokine production by OVA-specific CD4+ Th2 cells in the presence of OVA peptide. Furthermore, we showed that cotransfer of ILC2s facilitated the Ag-driven CD4+ Th2 cell immune response, which exacerbated airway inflammation, mucus production, and goblet cell hyperplasia in mice. Our results suggest that in addition to the role in facilitating the initiation of CD4+ Th2 immune response, ILC2s may promote the reactivation of Ag-specific CD4+ Th2 effector memory cells, resulting in the exacerbation of chronic airway allergic inflammation. Thus, our findings point out a view that after allergic sensitization by dendritic cells (61), infiltrated ILC2s could have an important accessory role to promote local allergen recall response at the chronic phase of allergic asthma, as recently suggested by Gasteiger et al. (62). The detailed molecular mechanisms by which ILC2s interact with Ag-specific CD4+ Th2 cells to promote allergen recall response remain to be determined.

Clinical studies suggest that a subgroup of patients with early-onset asthma exhibits eosinophilic inflammation that is associated with the increased number of lung T cells and decreased lung function and that this subgroup can be categorized as a major subphenotype of asthma driven by Th2 inflammation (1, 8, 9, 63). The observations from our studies using a murine model of chronic allergic lung diseases suggest that the increased lung-resident, Ag-specific CD4+ Th2 cells augment the capability of ILC2s to produce IL-13. Reciprocally, ILC2s potentiate the response of Ag-specific Th2 cells to Ag reexposure to produce elevated type 2 cytokines and to exacerbate allergic airway inflammation. Our intriguing findings suggest that the persistent eosinophilic inflammation may be a result of the collaborative interactions between infiltrating ILC2s and the preferentially accumulated Ag-specific CD4+ Th2 cells driven by recurrent allergen exposures. Whether such reciprocal interplays between innate ILC2s and adaptive CD4+ Th2 cells underlie the mechanisms by which allergic individuals with early-onset asthma, and the persistent eosinophilia phenotype later develop severe asthma remain to be examined. Further characterization of the molecular mechanisms involved in the crosstalk between lung-resident CD4+ Th2 cells and ILC2s may provide additional insights into the design of therapeutic approaches to prevent the exacerbation of severe allergic diseases.

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