Group 2 innate lymphoid cells license dendritic cells to potentiate memory $T_H2$ cell responses

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Rapid activation of memory CD4$^{+}$ T helper 2 (T$_H2$) cells during allergic inflammation requires their recruitment into the affected tissue. Here we demonstrate that group 2 innate lymphoid (ILC2) cells have a crucial role in memory T$_H2$ cell responses, with targeted depletion of ILC2 cells profoundly impairing T$_H2$ cell localization to the lungs and skin of sensitized mice after allergen re-challenge. ILC2-derived interleukin 13 (IL-13) is critical for eliciting production of the T$_H2$ cell-attracting chemokine CCL17 by IRF4$^{+}$CD11b$^{+}$CD103$^{−}$ dendritic cells (DCs). Consequently, the sentinel function of DCs is contingent on ILC2 cells for the generation of an efficient memory T$_H2$ cell response. These results elucidate a key innate mechanism in the regulation of the immune memory response to allergens.

Allergic airway disease affects millions of people worldwide. Although the disease is heterogeneous in etiology, a misguided acquired type 2 immune response to allergens underlies its pathology in most people. Memory T$_H2$ cells are crucial for recall responses to antigens and the subsequent inflammation driven by type 2 cytokines, although the innate immune system is also intricately involved in coordinating this process. At the mucosal barrier, innate immune cells are rapidly activated by damage or microbe-associated molecular patterns to produce cytokines, chemokines and cell-surface costimulatory molecules. Although this inflammatory milieu enables rapid homing, efficient activation and survival of memory T$_H2$ cells, the exact mechanism is not completely understood.

Innate lymphoid cells (ILCs) serve as part of the innate immune system. In the context of infection, ILCs function as sentinels that precede the generation of antigen-specific adaptive immune responses. ILC2 cells are an important early source of type 2 cytokines and are essential for the memory T$_H2$ cell response 2,3. Despite their role in promoting inflammatory responses, the exact mechanism is not completely understood 2,3.

We hypothesized that ILC2 cells, as an innate source of type 2 cytokines rapidly produced locally after allergen exposure, might have a role in initiating the memory T$_H2$ cell response by creating a chemokine milieu that promotes T$_H2$ cell recruitment.

Here we demonstrate that the innate response mediated by both ILC2 cells and DCs is required for the memory T$_H2$ cell response in allergen-sensitized mice. We used iCOS-T mice, which carry a laxP-flanked Diphtheria toxin receptor (DTR) gene inserted into the Icos locus that enables Cd4-Cre–mediated excision of the DTR gene from T cells and its retention in ILC2 cells, enabling IL2 depletion through Diphtheria toxin while sparing T cells 15, to ablate ILC2 cells before the antigen-recall response while leaving intact their critical functions during T$_H2$ cell priming. Allergen sensitized mice in which ILC2 cells were depleted did not recruit memory T$_H2$ cells to the lung and skin after allergen re-challenge. We find that ILC2 cells act upstream of DCs and are essential for their production of CCL17. Taken together, these results indicate that ILC2 cells are critical in the orchestration of an efficient localized memory T$_H2$ cell response together with tissue-resident DCs.

RESULTS

Protease allergen induces a memory T$_H2$ cell recall response

To induce a robust memory T$_H2$ cell–mediated immune response, we primed and re-challenged mice intranasally with papain, a protease allergen 16 that shares similarities with parasitic protozoan clan CA peptidases and requires intrinsic enzymatic activity to elicit innate and adaptive allergic responses 13,21,22 (Fig. 1a). Priming induced acute eosinophilia and increased numbers of ILC2 cells, which were largely

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H2 cell response to allergens. (a) WT or iCOS-T mice were sensitized to and re-challenged with papain and 2W1S peptide at intervals (a) and analyzed every 2 d for BAL eosinophils, neutrophils and alveolar macrophages (MΦ). (b) (Supplementary Fig. 1a). Flow cytometry detection (c) and quantification (d) (Supplementary Fig. 1e) of lung ILC2 cells (live CD45⁺CD3⁺CD4⁺ lineage GATA-3⁺) and 2W1S tetramer⁺ (Tet⁺) or Tet⁻ T₁₂ cells (live CD45⁺CD3⁺CD4⁺Foxp3⁻ GATA-3⁻) cells. (e) Numbers of total lung eosinophils, measured on day 135, in sensitized mice after re-challenge with 2W1S + papain (PAP) or heat-inactivated papain (HP) on day 132. (f,g) Numbers of lung eosinophils (f) or CCL17 levels in BAL fluid (g) on day 16 in papain + 2W1S–sensitized mice re-challenged with PAP or HP on day 15. Data are representative of at least two independent experiments (mean ± s.e.m. of at least three animals per group (b,d,f,g) or each point represents one animal (e)). *P < 0.001; **P < 0.0001 (statistical details are presented in Online Methods).

resolved by day 15, whereas re-challenge elicited greatly amplified eosinophilic inflammation (Fig. 1b and Supplementary Fig. 1a–e).

Accordingly, allergen-induced CD4⁺ T₁₂ cells, identified by their expression of the transcription factor GATA-3 (ref. 23), promoted an amplified antigen-recall response (Fig. 1c,d and Supplementary Fig. 1f). 2W1S-1Aβ tetramer-traceable memory T₁₂ cells were generated by the administration of the 2W1S peptide together with papain to mice. Priming efficiently induced tetramer⁺ T₁₂ cells, and re-challenge provoked a rapid increase in lung tetramer⁺ T₁₂ cells (Fig. 1d). We observed similar inflammation kinetics

![Figure 1](image1.png)

**Figure 1.** Protease allergen induces a robust memory T₁₂ cell-mediated recall response in sensitized mice. (a–d) Mice were sensitized and re-challenged with papain and 2W1S peptide at intervals (a) and analyzed every 2 d for BAL eosinophils, neutrophils and alveolar macrophages (MΦ) (b) (Supplementary Fig. 1a). Flow cytometry detection (c) and quantification (d) (Supplementary Fig. 1e) of lung ILC2 cells (live CD45⁺CD3⁺CD4⁺ lineage GATA-3⁺) and 2W1S tetramer⁺ (Tet⁺) or Tet⁻ T₁₂ cells (live CD45⁺CD3⁺CD4⁺Foxp3⁻ GATA-3⁻) cells. (e) Numbers of total lung eosinophils, measured on day 135, in sensitized mice after re-challenge with 2W1S + papain (PAP) or heat-inactivated papain (HP) on day 132. (f,g) Numbers of lung eosinophils (f) or CCL17 levels in BAL fluid (g) on day 16 in papain + 2W1S–sensitized mice re-challenged with PAP or HP on day 15. Data are representative of at least two independent experiments (mean ± s.e.m. of at least three animals per group (b,d,f,g) or each point represents one animal (e)). *P < 0.001; **P < 0.0001 (statistical details are presented in Online Methods).

![Figure 2](image2.png)

**Figure 2.** ILC2 cells are required for memory T₁₂ cell response to allergens. (a–e) WT or iCOS-T mice were sensitized, re-challenged and treated with DTx (Supplementary Fig. 4a). (a,b) Lung ILC2 cells detected by flow cytometry (a) and quantified (b) as in Figure 1c. Numbers above outlined areas in a indicate percentage of gated population. (c) Numbers of lung T₁₂ cells in ILC2-replete (black), ILC2-depleted (gray) or WT control mice on day 15, detected as in Figure 1c. (d) Numbers of ILC2 cells and T₁₂ cells in the lungs of iCOS-T mice treated with PBS (black) or DTx (blue) on day 16. Two iCOS-T mice failed ILC2 depletion (red), as indicated by increased ILC2 numbers over the threshold of average naive lung ILC2 cells (dotted line). Points represent individual animals (Pearson’s test). (e) Total numbers of lung CD4⁺ T cells. (f,g) Total numbers of tetramer⁺ T₁₂ cells in the lungs of WT or iCOS-T mice administered DTx and/or papain (Supplementary Fig. 4b) (f). Intracellular Ki67 expression in lung CD4⁺ T cell populations (g). (h) Numbers of lung ILC2 and T₁₂ cells on day 135 in sensitized iCOS-T mice re-challenged on day 132 and administered PBS (black) or DTx (blue) (Supplementary Fig. 4e). (i) IL-4 concentrations in BAL fluid on day 16 in WT or iCOS-T mice administered with DTx and/or papain (Supplementary Fig. 4a). n.s., not significant; *P < 0.001; **P < 0.0001 (statistical details are presented in Online Methods).
when we used an alternative allergen, Alternaria alternata extract (Supplementary Fig. 2). We observed similar results when we assayed the persistence of the memory T\(_{\text{H}2}\) cell response by delaying re-challenge with allergen for 130 d (Fig. 1e and Supplementary Fig. 3a–d). Enzymatically active papain induced antigen-recall responses and increased T\(_{\text{H}2}\) cell numbers in the lung that were greatly amplified compared to those induced by heat-inactivated papain or 2W1S peptide alone (Fig. 1e and Supplementary Fig. 3e–i). Active papain also induced higher numbers of eosinophils and greater amounts of CCL17 (Fig. 1f,g). As papain protease activity is essential for ILC2 cell activation, these results raised the possibility that ILC2 cells are important for an efficient memory T\(_{\text{H}2}\) cell response to inhaled protease allergens.

ILC2 cells promote memory T\(_{\text{H}2}\) cell responses to allergens

To assess the role of ILC2 cells during the memory T\(_{\text{H}2}\) cell response without affecting their critical functions during T\(_{\text{H}2}\) cell priming, we treated iCOS-T mice with diphtheria toxin (DTx) to ablate ILC2 cells temporarily while sparing other blood lineages, including CD4\(^+\) T cells\(^1\) (Supplementary Fig. 4a,b). We used flow cytometry to confirm that administration of DTx before antigen re-challenge efficiently depleted ILC2 cells in iCOS-T mice (here referred to as ILC2-depleted mice) (Fig. 2a,b and Supplementary Fig. 4c). T\(_{\text{H}2}\) cell numbers were substantially lower in ILC2-depleted mice than in iCOS-T mice untreated with DTx (ILC2-replete mice) after allergen re-challenge on day 16 (Fig. 2c). This decrease in T\(_{\text{H}2}\) cell numbers was equivalent to the baseline levels observed in papain-sensitized wild-type (WT) mice. We also observed a strong correlation between numbers of ILC2 cells and tissue-infiltrating T\(_{\text{H}2}\) cells (Fig. 2d). The total number of lung CD4\(^+\) T cells after re-challenge was unaffected in ILC2-depleted mice (Fig. 2e). By day 20, the lungs of re-challenged ILC2-depleted mice showed lower numbers of antigen-specific 2W1S tetramer\(^{+}\) memory T\(_{\text{H}2}\) cells than did those of ILC2-replete controls (Fig. 2f). We obtained similar results for 2W1S tetramer\(^{+}\) T\(_{\text{H}2}\) cells (Supplementary Fig. 4d). The decrease in T\(_{\text{H}2}\) cell numbers was not due to impaired T cell proliferation in the ILC2-depleted mice, as indicated by staining for proliferation marker Ki67 (Fig. 2g). Additionally, we re-challenged and analyzed iCOS-T mice 130 d after priming (Supplementary Fig. 4e), and found that ILC2 depletion resulted in a significant decrease in total T\(_{\text{H}2}\) cell numbers in the lungs after re-challenge (Fig. 2h) and lower numbers of 2W1S tetramer\(^{+}\) T\(_{\text{H}2}\) cells than in ILC2-replete mice (Supplementary Fig. 4f). After re-challenge we also observed an increase in lineage-CD127\(^{+}\) non-ILC2 and non-ILC3 cells, which did not express IL-13; this induction did not occur after ILC2 cell depletion (Supplementary Fig. 4g.h). T\(_{\text{H}2}\) cell numbers were also reduced after re-challenge in ILC2-depleted mice treated with A. alternata (Supplementary Fig. 4i). We also noted lower concentrations of IL-4 in bronchoalveolar lavage (BAL) fluid after papain re-challenge in ILC2-depleted mice (Fig. 2i), T\(_{\text{H}2}\) cells are the major source of IL-4 after papain re-challenge (Supplementary Fig. 4j). These results indicate that ILC2 cells have an important role in the generation of memory T\(_{\text{H}2}\) cell responses.

ILC2 cell activation precedes recruitment of memory T\(_{\text{H}2}\) cells

We hypothesized that IL-13 produced by ILC2 cells might be essential for the rapid secretion of DC-derived CCL17 (refs. 17,25) that promotes recruitment of CCR4\(^{+}\) memory T\(_{\text{H}2}\) cells to the site of allergen exposure. Indeed, we found that ILC2 cells rather than T\(_{\text{H}2}\) cells were the major producers of IL-13 before and immediately after allergen re-challenge (Fig. 3a). A time-course analysis showed that IL-13 and CCL17 were detected rapidly in the BAL fluid after allergen re-challenge and followed similar kinetics (Fig. 3b,c). The expression of these factors correlated with a rapid influx of CCR4\(^{+}\)CD4\(^{+}\) T cells into the lung tissue after allergen re-challenge (Fig. 3d,e). To investigate the involvement of IL-13 in T\(_{\text{H}2}\) cell recruitment to the lung...
we treated mice with neutralizing antibodies to block IL-13 during re-challenge with papain, which resulted in a significant reduction in the number of T1f2 cells (Fig. 3f and Supplementary Fig. 5a). Notably, blocking of CCL17 led to a similar reduction in lung T1f2 cell numbers (Fig. 3f). Both IL-13 and CCL17 neutralization resulted in a lower frequency of CD44+CD62LCCR4+CD4+ memory T cells in mice after allergen re-challenge (Fig. 3g,h). These data suggest that IL-13 and CCL17 contribute to the same pathway for memory T1f2 cell induction.

**Antigen challenge induces CCL17*CD103*CD11b* DCs**

We next investigated the cellular source of the CCL17 produced after allergen challenge. Conventional lung DCs were subdivided into CD11b*CD103* and CD11b*CD103* populations, with the latter being involved in allergic lung inflammation26 (Fig. 4a). CD11b*CD103* lung DCs also express the transcription factor IRF4, which is associated with type 2 allergic inflammation27,28. We found that acute challenge of the lungs with papain substantially increased the numbers of the CCL17-producing type 2–associated CD11b*CD103*IRF4* DCs (Fig. 4b). We conducted gene-expression analysis of CD11b*CD103* and CD11b*CD103* lung DC populations; CD11b*(Siglec-F) and CD11b*(Siglec-F) lung macrophages; monocytes; and activated and naive lung ILC2 cells (Fig. 4c). We observed that CD11b*CD103* lung DCs expressed *Ir4f* and showed higher expression of *Ccl17* than did CD11b*CD103* lung DCs and lung macrophages. Furthermore, although naive ILC2 cells expressed *Ili3* but not CD17, lung DCs expressed high levels of *Il4ra* (encoding IL-4 receptor-a (IL-4Rα)), *Ili3ra1* (encoding IL-13Rα1) and *Stat6* (encoding the transcription factor *Stat6*). Thus, B220*CD11c*MHC-II*CD11b*CD103*IRF4* lung DCs represent an important potential source of CCL17 in response to allergen challenge and express IL-13 receptors, which raises the possibility that these DCs respond to ILC2 cell–produced IL-13.

**Figure 5** ILC2 cells and IL-13 are critical for CCL17+ DC expansion and CCL17 production after allergen exposure. (a) IL-13Rx1 expression measured by flow cytometry on Siglec-F*CD11c*F4/80* lung macrophages (MΦ) and B220*MHC-II*CD11c*CD11b*CD11b+ or -CD11b+ DCs. Gray shaded areas indicate fluorescence-minus-one staining control, black line indicates *Il13ra1*–; blue line indicates WT. (b,c) BAL CCL17 concentrations (b) or CCL17*CD11b*CD103* DCs (c) measured on day 2 in WT, WT + anti–IL-13–treated or transgenic mice treated with papain (PAP), or heat-inactivated papain (HP; WT mice only) on days 0 and 1. Groups were compared to WT treated with papain (PAP). (d) Quantification of CCL17+ DC populations in the lungs of naive (Ctrl) or papain-treated (PAP) WT BMT and *Rora*fl/fl BMT mice on day 2. (e,f) Quantification of CCL17*CD11b*CD103* DCs in the lungs (e) and CCL17 concentration in the BAL (f) of WT and iCOS-T mice treated with DTx and/or papain (Supplementary Fig. 4a). (g) CCL17 concentration in co-culture supernatants of antigen-experienced OT-II–transgenic CD44+CD4+ T cells plus WT DCs and/or ILC2 cells. n.s., not significant; *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001. Data are representative of at least two independent experiments containing at least three animals or cultures per group (mean ± s.e.m. (b-g); individual points represent individual animals (c); statistical details are presented in Online Methods).
ILC2 cells are crucial for allergen-induced CCL17 production

Mirroring their crucial role in IIL3ra1, lung DCs also expressed surface IL-13Rα1, as analyzed by flow cytometry and compared to Siglec-F<sup>−/−</sup>CD11c<sup>F4/80</sup><sup>−/−</sup> macrophages (Fig. 5a). Moreover, mice in which IL-13 was neutralized by monoclonal antibody (IL-13–neutralized mice), IL13<sup>−/−</sup> and IIL3ra1<sup>−/−</sup> mice showed lower CCL17 concentrations in BAL fluid (Fig. 5b) and lower numbers of CCL17<sup>+</sup>CD11b<sup>+</sup>CD103<sup>−</sup> lung DCs (Fig. 5c) after papain stimulation than did WT controls. Similarly, IL33<sup>−/−</sup> mice, in which lung ILC2 cells are not efficiently activated, showed impaired CCL17 production (Supplementary Fig. 5b). Two types of ILC2-deficient mice (Rora<sup>−/−</sup> bone marrow–transferred (BMT) recipient mice<sup>29,30</sup> and Rora<sup>−/−</sup>Il7ra<sup>−/−</sup> Cre mice<sup>15</sup>, which carry a Rora deletion in Il7ra-expressing lymphocytes (Fig. 5c,d) and ILC2-depleted iCOS-T mice (Fig. 5e) had considerably fewer CCL17<sup>+</sup>CD11b<sup>+</sup>CD103<sup>−</sup> lung DCs after papain administration than did WT control mice. Furthermore, CCL17 concentrations were significantly lower in the BAL fluid of papain-challenged ILC2-deficient (Rora<sup>−/−</sup>Il7ra-Cre) and in re-challenged ILC2-depleted iCOS-T mice than in their ILC2-replete controls. (Fig. 5b,f). We obtained similar results in mice treated with the allergen A. alternata (Supplementary Fig. 5c). Furthermore, co-cultures of lung tissue–derived ILC2 cells and DCs together with CD4<sup>+</sup> T cells from ovalbumin-specific T cell antigen receptor–transgenic OT-II mice led to more efficient production of CCL17 than that of ILC2 cells and DCs individually (Fig. 5g). Taken together, these results demonstrate a crucial interplay between ILC2 cells, IL-13 and DC-produced CCL17.

IL-13 is crucial for DC-driven memory Th2 cell recruitment

Lung DCs serve an important sentinel function and are critical for Th2 cell induction and memory responses<sup>2</sup>. The depletion of lung DCs in CD11c-DTR mice, which express DTR from the Il7ra promoter, during papain re-challenge profoundly impaired Th2 cell recruitment while sparing ILC2 cells (Fig. 6a,b and Supplementary Fig. 6a–e). Thus we propose that ILC2 cells regulate DC activation and expression of CCL17 through their production of IL-13. To investigate the importance of IL-13 acting on DCs during the memory Th2 cell response, we used the bone marrow of WT, IIL3ra1<sup>−/−</sup> and CD11c-LuciDTR mice to generate CD11c-LuciDTR<sup>+</sup> WT and CD11c-LuciDTR<sup>+</sup> IIL3ra1<sup>−/−</sup> mixed bone-marrow chimeras (Supplementary Fig. 6f). In these bone-marrow chimeras, DTRx administration before re-challenge eliminates functionally normal DCs carrying the DTR transgene and, in the case of mixed chimeras, yields mice that contain primarily WT or IIL3ra1<sup>−/−</sup> DCs (Fig. 6c,d and Supplementary Fig. 6g). As predicted by the proposed upstream role of ILC2 cells and IL-13 in the response to allergens, the numbers of lung Th2 cells and the concentration of IL-13 in BAL fluid were unaffected by the depletion of DCs or by having predominantly IL-13–unresponsive DCs (Fig. 6e,f), as were the numbers of lung macrophages (Supplementary Fig. 6h). In contrast, the concentration of CCL17 in BAL fluid was markedly reduced in the mixed bone-marrow chimeras in which IL-13Rα1 was specifically absent on DCs, as compared to WT controls (Fig. 6g). Indeed, the absence of IL-13Rα1 signaling in the DC population resulted in concentrations of CCL17 similar to those observed after the ablation of DCs (Fig. 6g). Moreover, the absence of IL-13Rα1 expression by DCs also led to a reduction in the number of Th2 cells in the lungs after papain re-challenge (Fig. 6h). This impairment mirrored the effect of DC ablation (Fig. 6h), despite the equivalent numbers of lung DCs in WT and IIL3ra1<sup>−/−</sup> mixed chimeras (Fig. 6d). Thus, IL-13 signals are essential for the rapid activation of DCs and their production of the memory Th2 cell–recruiting chemokine CCL17.

Dermal and gut ILC2 cells mediate expansion of CCL17<sup>+</sup> DCs

CCL17 is important for Th2 cell infiltration into the skin<sup>3</sup>. By intracellular staining, we identified cutaneous CCL17<sup>+</sup> DCs, the majority of which expressed CD11b and IRF4 (Fig. 7a). Cutaneous IL-13–producing ILC2 cells have also been described<sup>31–33</sup>, which suggests that, in the lung, CCL17<sup>+</sup> DCs in the skin might be controlled by ILC2 cell–derived IL-13. Lineage–CD127<sup>+</sup>GATA-3<sup>−</sup> cutaneous ILC2 cells were readily detectable in naive and papain-stimulated WT or IIL3ra1<sup>−/−</sup> mice but were nearly absent in Rora<sup>−/−</sup>Il7ra-Cre mice (Supplementary Fig. 7a). Acute eosinophilic inflammation induced by papain challenge was significantly impaired in IIL33<sup>−/−</sup> or Rora<sup>−/−</sup>Il7ra-Cre mice (Supplementary Fig. 7b). Moreover, the increase in the number of CCL17<sup>+</sup> DCs after allergen challenge was not observed in the absence of ILC2 cells or IL-13 (Fig. 7b), mirroring our results in the lung. In addition to its role in the skin and lung, CCL17 is also important for Th2 cell homing to the gut, where ILC2-derived IL-13 is critical for responding to helminth infection via IL-25 or IL-33 (refs. 5–7). Infection of WT mice with the helminth Nippostrongylus brasiliensis (N.b.) induces a potent type 2 response<sup>34</sup>, and we observed increased numbers of peritoneal CCL17<sup>+</sup>CD11b<sup>+</sup>CD103<sup>−</sup>IRF4<sup>+</sup> DCs on day 6 after infection (Fig. 7c,d). The administration of IL-13 also resulted in a similar increase in CCL17<sup>+</sup>CD11b<sup>+</sup> DCs in the peritoneum (Fig. 7e). Stimulation with IL-33 yielded increased numbers...
ILC2 cells are important for TH2 cell responses in the skin

To examine the involvement of ILC2 cells on cutaneous TH2 cell responses in sensitized animals, we employed several models of allergen-induced TH2 cell recruitment. First, we sensitized animals by intranasal administration of papain on successive days (day 0 and day 1) and intradermal re-challenge on day 15 and analyzed skin-infiltrating TH2 cells on day 16 (Fig. 7g). Depletion of ILC2 cells before re-challenge resulted in a significant reduction in cutaneous TH2 cell numbers, with a strong correlation between numbers of ILC2 cells and TH2 cell numbers, and we observed an impairment in IL-13+ TH2 cell recruitment in the absence of ILC2 cells (Supplementary Fig. 7c–f), whereas a single intradermal challenge with papain into nonsensitized mice did not induce significant changes in TH2 cell infiltration as compared to naive controls, which indicates that sensitization is required. Thus, ILC2 cells are important for TH2 cell recruitment to the skin after allergen re-challenge in sensitized animals.

**DISCUSSION**

We have identified an ILC2 cell–dependent pathway for the rapid activation of memory TH2 cell responses in the lung after allergen re-exposure. We demonstrate that once activated, ILC2-derived IL-13 stimulates CD11b+CD103+ lung DCs to produce the chemokine CCL17, promoting the recruitment of CCR4+ memory TH2 cells. Targeted depletion of ILC2 cells in sensitized iCOS-T mice during re-challenge with papain (even after 130 d) resulted in significantly reduced numbers of IL-4–producing memory TH2 cells in the lung that persisted for at least 5 d after challenge.

We have found that ILC2 cells are the major cellular source of IL-13 in allergen-sensitized mice immediately before and after re-challenge and that ILC2 cell–derived IL-13 release precedes effector memory TH2 cell recruitment. Although tissue-resident memory T cells are found in distinct anatomical sites, where they function to alert and recruit other immune components upon re-challenge, there is little evidence yet for the existence of tissue-resident memory TH2 cells in the airways. Moreover, our results show that the enzymatic activity of papain, which is critical for activation of ILC2 cells, is required for memory TH2 cell recruitment. Attenuated or inert allergens such as heat-inactivated papain or ovalbumin might require substantially higher and prolonged dosing to induce inflammation, possibly owing to inefficient ILC2 cell activation. Although other mechanisms...
for early IL-13 production have been proposed, including mast cell–mediated recruitment of T$_H$2 cells or tissue-resident memory T$_H$2 cells, these models pre-date the discovery of ILC2 cells. Notably, IL-13 release after allergen re-challenge coincides with similarly rapid production of the memory T$_H$2 cell attracting chemokine CCL17. This IL-13 signal is essential for CCL17 production specifically from IRF4$^+$CD11b$^+$CD103$^-$ DCs. Using ILC2-deficient Rora$^{-/-}$ or Il7ra$^{-/-}$ BMT and Rora$^{-/-}$/Il7ra-Cre mice, we show that IRF4$^+$CD11b$^+$CD103$^-$ DCs do not produce CCL17 after papain challenge, demonstrating that ILC2 cells have a critical role in creating a type 2 inflammatory milieu. This is supported by co-culture experiments showing the instructive role of ILC2 cells for type 2 chemokine production from lung DCs. Indeed, the failure of DCs to release CCL17 after selective ILC2 depletion in sensitized animals indicates that ILC2 cells license DCs to promote memory T$_H$2 cell responses after allergen re-challenge.

The role of DCs for memory T$_H$2 cell responses is well established; here we confirm the central role of IL-13 in this process by generating mixed bone-marrow chimeras. Indeed, sensitized mice harboring only Il13ra1$^{-/-}$ DCs phenocopied DC-depleted mice in their inability to produce CCL17 and recruit memory T$_H$2 cells after re-challenge. Furthermore, the neutralization of IL-13 before allergen re-challenge in sensitized mice effectively prevented CCR4$^+$CD44$^+$CD62L$^-$ memory T$_H$2 cell recruitment to the lung to the same degree as in CCL17-neutralized animals. Together, these data provide strong evidence for the importance of ILC2-derived IL-13 in the efficient recruitment of memory T$_H$2 cells to the allergen-challenged lung through ‘collaboration’ with lung DCs.

These results also expand the understanding of DC function in type 2 immunity. As tissue-resident sentinels, DCs are among the first cells to respond to pathogens or allergens in the airs2. In naive animals, DCs are essential for antigen presentation to naive CD4 T cells, and subsequent T$_H$2 cell priming6,37,38 after direct interaction with cytokines such as TSLP39,40. Although alarmins can act directly on DCs, some scenarios of type 2 inflammation require ILC2 cells as a critical intermediate. For example, DC migration to the draining lymph nodes after initial allergen sensitization relies on the activation of ILC2 cells13. Likewise, in the present study, CCL17 production from DCs is contingent on IL-33–induced activation of ILC2 cells. Although consistent with the idea of DCs as essential modulators of adaptive T$_H$2 cell–mediated immunity, we reveal the central role of ILC2 cells as a critical upstream component of efficient memory T$_H$2 cell responses.

ILC2 cells in other anatomical sites may similarly influence the recruitment of memory T$_H$2 cells in sensitized animals. T$_H$2 cells have an important role in atopic dermatitis3 and anti-helminth immunity41, and ILC2 cells have been reported in the skin11–13 and gut5–7. Although memory T$_H$2 cell homing to the lung, skin and gut involve different mechanisms, it is believed that CCR4 and CCL17 have a shared role in promoting their recruitment. Although we found that the skin and peritoneum contain a CCL17$^+$ DC population, which, like that in the lung, is CD11b$^+$IRF4$^+$, we also found that IL13$^{-/-}$ or ILC2-deficient Rora$^{-/-}$/Il7ra-Cre mice injected with papain intra-dermally contained fewer CCL17$^+$ DCs. Similarly, the number of CCL17$^+$ DCs was increased in the peritoneum upon infection with N. br. or after administration of recombinant IL-13, providing evidence that ILC2 cells, the predominant source of IL-13 during parasitic helminth infection5–7, may be driving this expansion. Indeed, administration of the ILC2-activating cytokine IL-33 results in increased numbers of peritoneal CCL17$^+$ DCs in WT, but not ILC2-deficient Rora$^{-/-}$/Il7ra-Cre mice. Thus, it is clear that ILC2 cells in other tissues share the ability to influence the induction of IRF4$^+$CCL17$^+$ DCs. Furthermore, ILC2 cell–depletion in allergen-sensitized iCOS-T mice markedly reduced memory T$_H$2 cell recruitment to the skin after re-challenge with papain allergen, which indicates a role for ILC2 cells in memory T$_H$2 cell responses in additional tissues.

Our data also expand on the role of IL-13, a type-2 cytokine widely regarded for its ‘effector’ functions42,43. We now reveal that its early production from ILC2 cells is essential for CCL17-driven recruitment of memory T$_H$2 cells. These results further support IL-13 as a target for therapeutic development1,44. Other experimental drugs that neutralize epithelial-derived IL-25, IL-33 and TSLP42 are likely to influence ILC2 cell activation and may have important additional indirect effects on memory T$_H$2 cells.

Thus, our results illustrate an ILC2 cell–dependent mechanism by which memory T$_H$2 cells are recruited to the airways and skin after secondary allergen challenge. Whereas DCs are known to be essential for T$_H$2 responses, we now show that ILC2 cells situated at the epithelial barrier regulate IL-13–dependent DC expression of the memory T$_H$2 cell–attracting chemokine CCL17. Hence, ILC2 licensing of DCs is a critical component of the memory T$_H$2 cell response to allergens at barrier sites.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

T.Y.H. designed and performed experiments, and wrote the paper. Y.Y.H. and N.G. designed experiments and wrote the paper. N.N.M. and N.A.J.M. performed experiments and wrote the paper. A.N.J.M. helped with experiments, and wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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1. Kim, H.Y., DeKruyff, R.H. & Uematsu, D.T. The many paths to asthma: phenotype shaped by innate and adaptive immunity. Nat. Immunol. 11, 577–584 (2010).
2. Lambreicht, B.N. & Hammad, H. The immunology of asthma. Nat. Immunol. 16, 45–56 (2015).
3. Islam, S.A. & Luster, A.D. T cell homing to epithelial barriers in allergic disease. Nat. Med. 18, 705–715 (2012).
4. McKenzie, A.N., Spits, H. & Eberl, G. Innate lymphoid cells in inflammation and immunity. Nature 484, 366–374 (2014).
5. Moro, K. et al. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit+$^+$Sca-1+$^+$ lymphoid cells. Nature 463, 540–544 (2010).
6. Neill, D.R. et al. Innate effector lymphocytes that mediate type-2 immunity. Nature 454, 1367–1370 (2010).
7. Price, A.E. et al. Systemically disseminated innate IL-13-expressing cells in type 2 immunity. Proc. Natl. Acad. Sci. USA 107, 11489–11494 (2010).
8. Chang, Y.J. et al. Innate lymphoid cells mediate influenza-induced airway hyper-reactivity independently of adaptive immunity. Nat. Immunol. 12, 631–638 (2011).
9. Halim, T.Y., Krauss, R.H., Sun, A.C. & Takei, F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. *Immunity* 36, 451–463 (2012).

10. Monticelli, L.A. et al. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat. Immunol.* 12, 1045–1054 (2011).

11. Drake, L.Y., Iijima, K. & Kita, H. Group 2 innate lymphoid cells and CD4+ T cells cooperate to mediate type 2 immune response in mice. *Allergy* 69, 1300–1307 (2014).

12. Gold, M.J. et al. Group 2 innate lymphoid cells facilitate sensitization to local, but not systemic, TSLP2-inducing allergen exposures. *J. Allergy Clin. Immunol.* 133, 1142–1148 (2014).

13. Halim, T.Y. et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity* 40, 425–435 (2014).

14. Mirchandani, A.S. et al. Type 2 innate lymphoid cells drive CD4+ Th2 cell responses. *J. Immunol.* 192, 2442–2448 (2014).

15. Oliphant, C.J. et al. MHCIImediated dialog between group 2 innate lymphoid cells and CD4(+) T cells potentiate type 2 immunity and promotes parasitic helminth expulsion. *Immunity* 41, 283–295 (2014).

16. van Rijt, L.S. et al. In vivo depletion of lung CD11c+ dendritic cells during allergen challenge abrogates the characteristic features of asthma. *J. Exp. Med.* 201, 981–991 (2005).

17. Crapster-Pregont, M., Yeo, J., Sanchez, R.L. & Kuperman, D.A. Dendritic cells and alveolar macrophages mediate IL-13-induced airway inflammation and chemokine production. *J. Allergy Clin. Immunol.* 129, 1621–1627.e3 (2012).

18. Mikhak, Z. et al. Contribution of CCR4 and CCR8 to antigen-specific Th2 cell trafficking and allergic pulmonary inflammation. *J. Allergy Clin. Immunol.* 123, 67–73.e3 (2009).

19. Imai, T. et al. Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. *Int. Immunol.* 11, 81–88 (1999).

20. Novy, H.S., Marchioll, L.E., Sokol, W.N. & Wells, I.D. Papain-induced asthma–physiological and immunological features. *J. Allergy Clin. Immunol.* 63, 98–103 (1979).

21. Mottram, J.C., Helms, M.J., Coombs, G.H. & Sajid, M. Clan CD cysteine peptidases of parasitic protozoa. *Trends Parasitol.* 19, 182–187 (2003).

22. Kamiyo, S. et al. IL-33-mediated innate response and adaptive immune cells contribute to maximum responses of protease allergen-induced allergic airway inflammation. *J. Immunol.* 190, 4489–4499 (2013).

23. Zheng, W. & Flavell, R.A. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* 89, 587–596 (1997).

24. Moon, J.J. et al. Naive CD4+ T cell frequency varies for different epitopes and predicts repertoire diversity and response magnitude. *Immunity* 27, 203–213 (2007).

25. Medoff, B.D. et al. CD11b+ myeloid cells are the key mediators of Th2 cell homing into the airway in allergic inflammation. *J. Immunol.* 182, 623–635 (2009).

26. Plantinga, M. et al. Conventional and monocye-derived CD11b+ dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. *Immunity* 38, 322–335 (2013).

27. Gao, Y. et al. Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. *Immunity* 39, 722–732 (2013).

28. Williams, J.W. et al. Transcription factor IRF4 drives dendritic cell to promote Th2 differentiation. *Nat. Commun.* 4, 2990 (2013).

29. Halim, T.Y. et al. Retinoic-acid-receptor-related orphan nuclear receptor-a is required for natural helper cell development and allergic inflammation. *Immunity* 37, 463–474 (2012).

30. Wong, S.H. et al. Transcription factor RORα is critical for nuocyte development. *Nat. Immunol.* 13, 229–236 (2012).

31. Kim, B.S. et al. A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *J. Exp. Med.* 210, 2939–2950 (2013).

32. Maizels, R.M., Hewitson, J.P. & Smith, K.A. Susceptibility and immunity to helminth parasites. *Curr. Opin. Immunol.* 24, 459–466 (2012).

33. Kumar, R.K., Herbert, C. & Foster, P.S. The “classical” ovalbumin challenge model of asthma in mice. *Curr. Drug Targets* 9, 485–494 (2008).

34. Stephens, R. & Chaplin, D.D. IgE cross-linking or lipopolysaccharide treatment induces recruitment of Th2 cells to the lung in the absence of specific antigen. *J. Immunol.* 169, 5468–5476 (2002).

35. Hamm, H. 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.* 207, 2097–2111 (2010).

36. MacDonald, A.S., Straw, A.D., Dalton, N.M. & Pearce, E.J. Cutting edge: Th2 response induction by dendritic cells: a role for CD40. *J. Immunol.* 168, 537–540 (2002).

37. Wills-Karp, M. Interleukin-13 in asthma pathogenesis. *J. Allergy Clin. Immunol.* 124, 2990 (2013).

38. MacDonald, A.S., Straw, A.D., Dalton, N.M. & Pearce, E.J. Cutting edge: Th2 response induction by dendritic cells: a role for CD40. *J. Immunol.* 168, 537–540 (2002).

39. Ito, T. et al. StLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J. Exp. Med.* 202, 1213–1223 (2005).

40. Eiwegger, T. & Akdis, C.A. IL-33 links tissue cells, dendritic cells and Th2 cell response through OX40 ligand. *Nat. Rev. Immunol.* 12, 459–466 (2012).

41. Ito, T. et al. StLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J. Exp. Med.* 202, 1213–1223 (2005).

42. Fahy, J.V. Type 2 inflammation in asthma—present in most, absent in many. *Nat. Rev. Immunol.* 15, 57–65 (2015).
ONLINE METHODS

Mice. C57Bl/6 (B6), B6.Tg[Il2rgtm1Ghe/X] (Il2rg−/−), B6.129S2-Roratm1Jfs2Crt (Rora−/−), B6.129S7-Il7ratm1Mjj/Luc (Il7r−/−), B6.129S5-Il7ratm1Gjk/Jcr (Il7r−/−), B6.129S7-Il13ratm1Mmu/Luc (Il13r−/−), B6.129S6-Il15ratm1Jfs3Gjh/Crl (Il15r−/−), B6.129S3-Il17ratm1Mmu/Luc (Il17r−/−), B6.129S5-Cd11btm1Nmk/Wtsu (Cd11b−/−), B6.129S1-Itgaxtm1Jfr/J (Itgax−/−), B6.129S1-Cd11ctm1Jfr/J (Cd11c−/−) and B6.129S1-Cd11ctm1Jfr/J (Cd11c−/−) were prepared from lung by mechanical dissociation and digestion in 3 ml RPMI-1640 containing collagenase I (500 U/ml) and DNase I (0.2 mg/ml) for 45 min at 37 °C on a shaker (220 r.p.m.), followed by filtration through a 70-µm strainer and 30% Percoll gradient enrichment of leukocytes. Peritoneal cells were collected by flushing the peritoneal cavity with 2 ml of PBS. Single cells were re-stimulated and stained for surface and intracellular markers as described.15

Flow cytometry. Single cells were incubated with anti-mouse CD16/32 (eBioscience) to block Fc receptors and then stained with eFluor 450–conjugated lineage marker monoclonal antibodies (mAbs) (CD3, CD5, CD19, NK1.1, CD11b, FcRn, F4/80, Ly-6C/G and Ter119; 1:250), APC-conjugated CD4, APC-conjugated CD45; FITC-conjugated CD7, and PE-conjugated CD44 mAbs (Becton Dickinson, San Jose, CA). We used the following reagents to treat mice: Baytril (0.1 mg/L) in drinking water for 4 weeks and used for analysis at 24–32 weeks after transplant. Diphtheria toxin was used for ILC2 depletion through the intranasal injection of rmIL-33 (0.5 µg) or vehicle (0.1% CMC-Na in water). Recipient mice were lethally irradiated (2 doses of 4.5 Gy) followed by intravenous transplantation of 107 whole bone marrow cells from 4- to 6-week-old mice. Mice were given Baytril (0.1 mg/L) in drinking water for 4 weeks and used for analysis at 24–32 weeks after transplant.

Bone marrow transplantation. Recipient mice were lethally irradiated (2 doses of 4.5 Gy) followed by intravenous transplantation of 107 whole bone marrow cells from 4- to 6-week-old mice. Mice were given Baytril (0.1 mg/L) in drinking water for 4 weeks and used for analysis at 24–32 weeks after transplant.

Gene-expression analysis. We obtained microarray data sets for the listed cell types in Figure 4c from data assembled by the ImmGen consortium and compared them to our own ILC2 microarray data. Data analysis was performed with FlexArray 1.5 (Genome Quebec). Microarray data sets for ILC2 cells are available in the GEO database under accession number GSE36057; all other data sets are available under GSE15907.

Statistics. Data were analyzed using GraphPad Prism 6. Pearson’s test was performed to determine goodness of fit, as denoted by r2. One-way analysis of variance (ANOVA), two-tailed Student’s t-test or Mann-Whitney test was used to determine statistical significance between groups, with P ≤ 0.05 being considered significant. Statistical details for figures (including number of samples or mice per group (n), details of experimental replicates and statistical tests done, where applicable) were as follows: Figure 1b, n = 3 per time point, representative of 2 independent experiments; Figure 1d, n = 3 per time point, representative of 2 independent experiments; Figure 1e, n = as indicated, representative of 3 independent experiments, one-way ANOVA; Figure 1f, g = n = 8, 5, representative of 3 independent experiments, two-tailed Student’s t-test; Figure 2b, n = 5, 5, 6, 5, representative of 3 independent experiments, one-way ANOVA; Figure 2c, n = 11, 13, 14, 5, pooled results from 3 independent experiments, one-way ANOVA; Figure 2d, n = as indicated, pooled results from 3 independent experiments, Pearson’s test. Figure 2e, n = 5, 5, 6, 6, representative of 3 independent experiments, ANOVA; Figure 2f, n = 7, 7, 6, 6, representative of 2 independent experiments, one-way ANOVA; Figure 2h, n = as indicated, single experiment, one-way ANOVA; Figure 2i, n = 5, 5, 6, representative of 2 independent experiments, ANOVA; Figure 2j, n = as indicated, single experiment, one-way ANOVA; Figure 2k, n = 5, 5, 6, 6, representative of 2 independent experiments, one-way ANOVA.
of 2 independent experiments, Mann-Whitney test. Figure 3a, n = 3, 3, 3, representative of 2 independent experiments, two-tailed Student's t-test. Figure 3b, c, e, n = 3 per time point, representative of 2 independent experiments; Figure 3f, n = 6, 6, 6, representative of 2 independent experiments, one-way ANOVA. Figure 4b, n = 3, 3, 3, 3, representative of 2 independent experiments. Figure 5b, n = 4, 4, 4, 4, 4, representative of 2 independent experiments, one-way ANOVA. Figure 5c, n = as indicated, representative of 2 independent experiments, one-way ANOVA; Figure 5d, n = 5, 6, 3, 3, representative of 3 independent experiments, two-tailed Student's t-test. Figure 5e, n = 7, 6, 8, 8, representative of 3 independent experiments, one-way ANOVA; Figure 5f, n = 5, 5, 6, 6, representative of 2 independent experiments, one-way ANOVA. Figure 5g, n = 3, 3, 3, 3, representative of 3 independent experiments.

Figure 6a, b, n=5, 3, representative of 3 independent experiments, two-tailed Student's t-test. Figure 6d, n = 5, 6, 6, representative of 2 independent experiments, one-way ANOVA. Figure 6f, n = 5, 6, 6, representative of 2 independent experiments; Figure 6g, n = 5, 6, 6, representative of 2 independent experiments, one-way ANOVA. Figure 6h, n = 9, 11, 11, pooled results from 2 independent experiments, one-way ANOVA. Figure 7b, n = 3, 4, 4, 4, representative of 2 independent experiments, one-way ANOVA. Figure 7d, n = 3, 4, representative of 2 independent experiments, two-tailed Student's t-test. Figure 7e, n = 3, 4, representative of 2 independent experiments, two-tailed Student's t-test. Figure 7f, n = 4, 4, 4, representative of 2 independent experiments, one-way ANOVA. Figure 7h, n = as indicated, pooled results from 2 independent experiments, Pearson's test. Figure 7i, n = 11, 11, 11, 12, pooled results from 2 independent experiments, one-way ANOVA.

43. Tittel, A.P. et al. Functionally relevant neutrophilia in CD11c diphtheria toxin receptor transgenic mice. Nat. Methods 9, 385–390 (2012).
44. Haymaker, C.L. et al. Bone marrow–derived IL-13Rα1-positive thymic progenitors are restricted to the myeloid lineage. J. Immunol. 188, 3208–3216 (2012).
45. Schlenner, S.M. et al. Fate mapping reveals separate origins of T cells and myeloid lineages in the thymus. Immunity 32, 426–436 (2010).
46. Heig, T.S. & Painter, M.W. Immunological Genome Project C. The Immunological Genome Project: networks of gene expression in immune cells. Nat. Immunol. 9, 1091–1094 (2008).