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Help for the helpers: cooperation between Group 2 innate lymphoid cells and T helper 2 cells in allergic asthma

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Running title: ILC2 and T helper 2 cell cooperation

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Allergic asthma is a heterogeneous chronic lung disease characterized by reversible airway obstruction and hyperreactivity and is typically associated with eosinophilic inflammation (1-3). Classically, asthma is thought to arise from a T helper 2 (Th2)-driven immune response to airborne allergens such as house-dust mite, molds or animal dander (**Figure 1**). Th2 cells produce vast

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amounts of inflammatory cytokines that play a central role in orchestrating an allergic immune response: interleukin-4 (IL-4) stimulates B cells to produce antigen-specific IgE, IL-5 induces activation and differentiation of eosinophils and their recruitment from the bone marrow, and IL-13 acts on airway epithelial and smooth muscle cells to mediate mucus hypersecretion and bronchial hyperresponsiveness, respectively. Cellular differentiation of naïve T cells into Th2 effector cells requires dendritic cell (DC)-mediated antigen presentation in the T cell zones of lymph nodes. In asthma, DCs acquire the intrinsic capacity to drive Th2 immunity when activated either by pro-Th2 cytokines released by epithelial cells, including IL-1 α , GM-CSF, IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) (See for recent review: Ref. 4) or alternatively by allergens, such as fungal products from *Alternaria* (5), as they sample the airway lumen by forming dendritic extensions (Figure 1).

The central role of Th2 cells in allergic airway inflammation was however challenged by the recent discovery of group 2 innate lymphoid cells (ILC2), which have the capacity to produce large amounts of IL-5 and IL-13 when activated by the pro-Th2 cytokines IL-25, IL-33 or TSLP (Reviewed in: Ref. 6, 7). Although it was previously known that innate immune cells could function as an early source of Th2 cytokines, only in 2010 these cells were characterized as CD45⁺IL-7R⁺IL-25R⁺IL-33(T1ST2)⁺IL-2R⁺ cells with lymphocyte morphology that do not express antigen receptors or lineage markers (6, 7). Several mouse models were employed to demonstrate their critical role in allergic airway inflammation, particularly in RAG-deficient mice in the absence of functional B and T lymphocytes. These included inflammation induced by intranasal administration of IL-25 and IL-33, as well as ovalbumin (OVA) protein, *Alternaria*, glycolipids from *Sphingomonas* bacteria and papain protease (reviewed in: Ref. 8). Although the contribution of ILC2 in allergy in the context of an intact adaptive Th2 response is less well studied, experiments whereby wild-type mice were exposed to HDM or OVA showed that ILC2 in the lung and in bronchoalveolar lavage are a major source of IL-5 and IL-13 in allergic airway inflammation (9, 10). ILC2 can enhance Th2 responses when transferred

into recipient mice (11, 12), but the central question how ILC2 interact with cells of the adaptive immune system remained unanswered. In particular, cross-talk between ILC2 and Th2 lymphocytes was largely unexplored.

In the current issue of *Allergy*, Drake *et al.* provide evidence that ILC2 promote adaptive immunity by enhancing Th2 effector function, whereby interactions between ILC2 and CD4⁺ T cells involve the TNF-family member OX40L as well as ILC2-derived IL-4 (13). To explore possible interactions *in vitro*, lung-derived IL-33-activated ILC2 were co-cultured with naïve anti-CD3/anti-CD28-activated splenic CD4⁺ T cells. Addition of ILC2 enhanced T cell proliferation and resulted in robust increases in the levels of IL-4, IL-5 and IL-13 in the supernatants, but not of IFN γ . An analysis of cytokine expression in sorted ILC2 and CD4⁺ T cells by RT-PCR showed that co-culture with T cells induced a ~13-fold upregulation in ILC2 of IL-4, while the presence of ILC2 increased the levels of IL-5 mRNA in T cells by ~30-fold. IL-5 production was also increased when ILC2 were co-cultured with CD4⁺ T cells derived from *IL5*^{-/-} mice, albeit to a lesser extent. Therefore, these co-culture experiments indicate that ILC2 and CD4⁺ T cells enhance each other's IL-5 production.

In the interactions of ILC2 and T cells cellular contacts play a key role, because Th2 cytokine production was significantly reduced in co-cultures in a transwell system in the absence of cell-cell contacts. Inferred from the critical role in Th2 polarization of the co-stimulatory molecule OX40L that is induced on DCs by TSLP (14, 15), the authors hypothesized that the interaction between ILC2 and T cells may involve OX40L expression on ILC2. Indeed, blocking OX40/OX40L interactions significantly inhibited Th2 cytokine production in the co-cultures. Nevertheless, other molecular mechanisms are likely involved, because only a partial inhibition was observed (13). Although surface expression of OX40L is readily detected upon *in vitro* activation of B cells or DCs (15, 16), Drake *et al.* could only detect it intracellularly and speculate that OX40L may only be transiently expressed on the ILC2 cell membrane upon encounter of CD4⁺ T cells. Addition of anti-IL-4R α to the co-cultures significantly

inhibited IL-5 and IL-13 production. Co-culture of IL-4-deficient ILC2 with wild-type CD4⁺ T cells resulted in significantly decreased IL-5 and IL-13 production compared with co-culture of wild-type ILC2 and CD4⁺ T cells, suggesting that ILC2-derived IL-4 plays a key role in ILC2/CD4⁺ T cell interaction.

Finally, synergistic interactions between T cells and ILC2 were studied *in vivo* in an airway inflammation model whereby mice were intranasally exposed to OVA protein plus the cysteine protease bromelain. Lung ILC2 and CD4⁺ T cells from non-sensitized WT mice were transferred into naïve *Il7r^{-/-}* mice, which are deficient in both T cells and ILC2. Whereas mice reconstituted with either T cells or ILC2 showed limited inflammation, transfer of both cell populations resulted in robust airway eosinophilia and increased IL-13 in the bronchoalveolar lavage. ILC2 were shown to promote antigen-specific CD4⁺ T cell responses, as only lung cells from mice reconstituted with both ILC2 and CD4⁺ T cells (and not with each population alone) produced high levels of IL-5 and IL-13 when re-stimulated with OVA *in vitro*.

Taken together, these findings provide evidence for bidirectional interactions between ILC2 and CD4⁺ T cells that likely involve IL-4 and OX40L. The identification of a functional role for ILC2-derived IL-4 in promoting Th2 cytokine production is interesting (**Figure 1A**). Although IL-25 or IL-33 stimulation of ILC2 does not induce IL-4 (6, 7), they do have the capacity to produce IL-4 in response to TSLP or leukotriene D (17-19). As ILC2 appear to upregulate their IL-4 expression particularly upon interaction with CD4⁺ T cells, it is conceivable that ILC2/CD4⁺ T cell interactions may establish a positive feedback loop that increases IL-4 production by ILC2. In this context, it may be important that IL-2 produced by activated CD4⁺ T cells has the ability to stimulate ILC2 proliferation and cytokine production *in vitro* and *in vivo* (18, 20). ILC2 could be an important early source of IL-4, which is the canonical cytokine for the induction of the expression of the Th2 key transcription factor Gata3, and thus the initiation of Th2 cell polarization in naïve T cells. It would be worthwhile to

investigate the relevance of ILC2-derived IL-4 in *in vivo*, e.g. in allergic airway inflammation models involving adoptive transfer of IL-4-deficient and wild-type ILC2.

A role for the OX40/OX40L axis in the interactions between ILC2 and naïve CD4⁺ T cells is unlikely, as OX40 is only expressed on activated or memory T cells. Direct support of Th2 cells by ILC2 (**Figure 1B**) would parallel the role of the closely related ILC3 population, which was reported to be essential for maintenance of memory CD4⁺ T cells via direct OX40/OX40L interactions in the absence of persistent antigen following *Listeria monocytogenes* infection (21). Because *in vivo* OX40L blockade will also interfere with the ability of DC to interact with CD4⁺ T cells, it may prove challenging to directly examine the importance of OX40L expression on ILC2 for memory T cell maintenance. Again, complex *in vivo* reconstitution experiments employing OX40L-deficient ILC2 would be needed.

Recently, other models by which ILC2 induce efficient Th2 cell-mediated allergic lung inflammation were proposed. It was shown that ILC2-derived IL-13 was critical for an adaptive Th2 immune response to inhaled papain allergen (22). ILC2-derived IL-13 promoted migration of activated CCR7⁺ lung DC into draining lymph nodes where they primed differentiation of naïve T cells into Th2 cells (**Figure 2C**). In addition, ILC2 may directly induce the activation of naïve T cells in a cell contact-dependent fashion through antigen presentation by MHC class II (11, 12, 20, 23) (**Figure 2D**). Although blockade of MHC class II interactions inhibited the ability of ILC2 to stimulate T cell proliferation (20), it is currently unknown whether ILC2 have the capacity to efficiently take up and process antigen. To assess the stimulatory function of ILC2 in Th2 differentiation or maintenance of memory Th2 cells, it is crucial to know the location of ILC2 during sensitization and challenge phases of allergic lung inflammation. Finally, the temporal activation of ILC2 and CD4⁺ T cells is likely to be very different between various asthma models, as T-cell dependency differs substantially between e.g. HDM and papain-driven allergic airway inflammation.

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Taken together, evidence is accumulating for a critical role of ILC2 in promoting Th2-mediated allergic lung inflammation. In a recent study, OX40L blockade in mild asthma patients did not significantly affect allergen-induced airway responses, but nevertheless a reduction of sputum eosinophils was found (24). It is therefore important to further investigate the role of the CD40/CD40L axis, although it is likely to be only one of many molecular mechanisms by which ILC2 drive Th2 cell responses.

Conflicts of interest

None.

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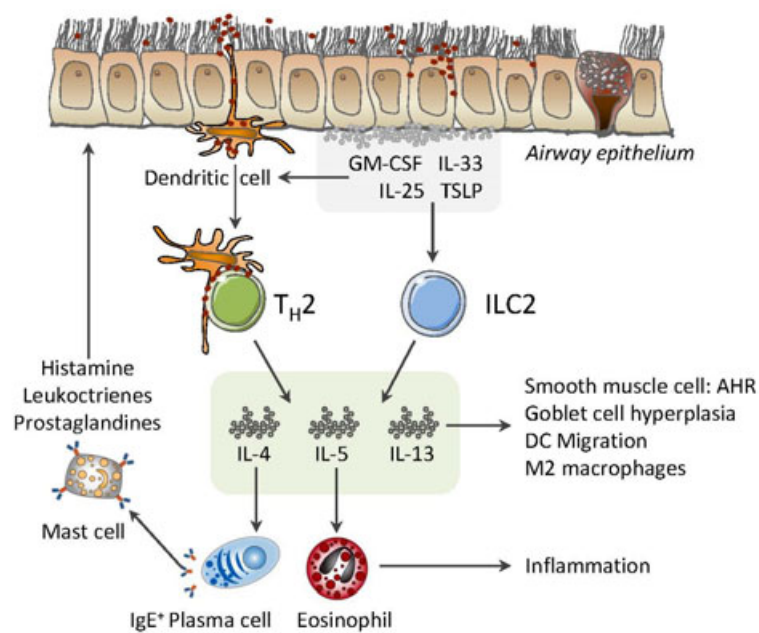
Legends to figures

Figure 1.

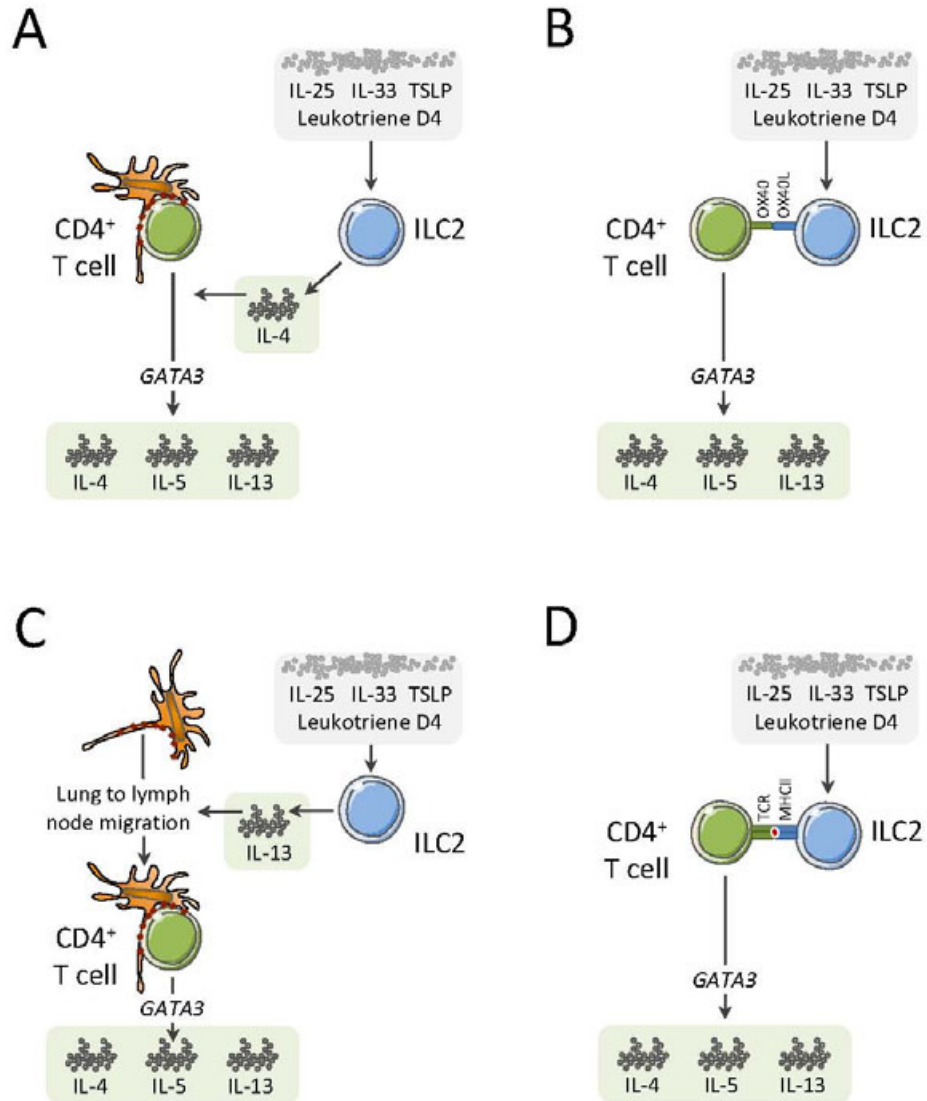
Initiation of Th2 differentiation and the central role of Th2 cytokines in allergic airway inflammation. DCs acquire the capacity to drive Th2 immunity when activated either by pro-Th2 cytokines released by epithelial cells or alternatively directly by allergens as they sample the airway lumen by forming dendritic extensions.

Figure 2.

Four different, not mutually exclusive models for cooperation between ILC2 and CD4⁺ T cells in allergic lung inflammation. (A) ILC2-derived IL-4 promotes Th2 differentiation; (B) OX40-OX40L-mediated direct interactions between ILC2 and CD4⁺ T cells; (C) ILC2-derived IL-13 enhances migration of DC to draining lymph nodes; (D) ILC2 present antigen through MHC class II and thereby activate CD4⁺ T cells. See text for details.



Hendriks - Figure 1



Hendriks - Figure 2