REVIEW

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T_H2, allergy and group 2 innate lymphoid cells

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The initiation of type 2 immune responses by the epithelial cell–derived cytokines IL-25, IL-33 and TSLP has been an area of extensive research in the past decade. Such studies have led to the identification of a new innate lymphoid subset that produces the canonical type 2 cytokines IL-5, IL-9 and IL-13 in response to IL-25 and IL-33. These group 2 or type 2 innate lymphoid cells (ILC2 cells) represent a critical source of type 2 cytokines *in vivo* and serve an important role in orchestrating the type 2 response to helminths and allergens. Further characterization of ILC2 cell biology will enhance the understanding of type 2 responses and may identify new treatments for asthma, allergies and parasitic infections. Interactions between ILC2 cells and the adaptive immune system, as well as examination of potential roles for ILC2 cells in the maintenance of homeostasis, promise to be particularly fruitful areas of future research.

The immune system evolved to protect the host from a huge diversity of pathogenic organisms, but it can also cause various pathologies, including autoimmune disease and allergy. Mammalian immune responses are often grouped into two main categories: type 1 and type 2. These responses differ broadly in their mechanisms of induction, as well as the innate cell types, cytokines, effector molecules, helper T cell types and immunoglobulin isotypes involved in pathogen elimination. Type 1 immune responses are induced in response to bacteria, viruses, fungi and protozoa and are mediated mainly by $CD4^+$ cells of the T_H1 and T_H17 subsets of helper T cells, cytotoxic $CD8^+$ T cells and antibodies of the immunoglobulin G (IgG) isotypes. When directed against self antigens, type 1 responses can cause autoimmune disorders[1–5].

In contrast to type 1 responses, type 2 responses are induced in response to infection with large extracellular parasites, such as helminths. Type 2 responses also mediate allergic inflammatory diseases such as asthma, allergic rhinitis and atopic dermatitis. Type 2 effector responses enhance barrier defenses at mucosal surfaces and induce the expulsion and/or killing of parasites. T_H2 cells regulate type 2 responses through the secretion of various type 2 cytokines, including IL-4, IL-5, IL-9 and IL-13. IL-4 from T_H2 cells promotes IgE production, which can bind to the high-affinity Fcε receptor on granulocytes, including basophils and mast cells. Activated basophils and mast cells release a variety of inflammatory mediators, including cytokines, chemokines, histamine, heparin, serotonin and proteases, which results in smooth-muscle constriction, greater vascular permeability and the recruitment of inflammatory cells. IL-5 and IL-9 promote tissue eosinophilia and mast cell hyperplasia, respectively. IL-13 promotes mucus production by goblet cells and airway hyper-responsiveness (AHR), which is a hallmark of allergic asthma[2–5].

In this Review we will discuss how type 2 responses are initiated, potentiated and maintained, focusing on group 2 innate lymphoid cells (ILC2 cells) and their interactions with other innate and adaptive cells that orchestrate and execute the type 2 response.

Initiation and effector subsets in type 2 responses

Type 2 responses at mucosal sites are initiated by epithelial cell–derived cytokines such as TSLP, IL-25 and IL-33, which are released during tissue damage, pathogen recognition or allergen exposure (**Fig. 1**). TSLP is expressed at steady state in the lungs, intestines and tonsils, and this expression seems to be an important inhibitory signal that prevents type 1 responses at these sites. Overexpression of TSLP in the lungs or skin leads to the development of AHR or atopic dermatitis, respectively[6,7]. Accordingly, TSLPR-deficient mice have impaired type 2 responses[8]. The cell types that respond to TSLP include dendritic cells (DCs), monocytes, B cells, mast cells and T cells[9]. T_H2 cells have higher expression of TSLPR than do T_H1 or T_H17 cells, and TSLP promotes the differentiation and cytokine secretion of T_H2 cells[10,11]. IL-25 is expressed in lung epithelial cells after exposure to allergens and during infection with some helminths[12,13]. Transgenic overexpression of IL-25 in airway epithelial cells, as well as intraperitoneal or intranasal administration of this cytokine, promotes type 2 responses[14,15]. Moreover, IL-25-deficient mice have diminished AHR in a model of asthma[16], and polymorphisms in the IL-25 receptor IL-17RB have been associated with asthma in humans[17]. Similarly, administration of IL-33 results in prototypical type 2 responses, such as eosinophilia, secretion of IgE and type 2 cytokines, mucus production and expulsion of parasites[18,19]. In agreement with that, IL-33-deficient mice have impaired AHR, and mice deficient in the IL-33 receptor IL-1RL1 have diminished worm expulsion and granuloma formation[20–22].

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The activation of T cells during type 2 responses is classically associated with the generation of effector T_H2 cells. T_H2 cells are characterized by expression of the transcription factor GATA-3 and the production of large amounts of IL-4, as well as of IL-5, IL-9, IL-10 and IL-13. DCs are critical for the initiation of adaptive T_H2 responses[23], and DCs with the intrinsic ability to promote such responses have been identified in the spleen, lungs and skin[4]. Furthermore, type 2–inducing cytokines can condition DCs to 'preferentially' prime T_H2 responses; for example, DCs exposed to TSLP *in vitro* efficiently polarize naive T cells toward a T_H2 effector phenotype[24].

The identification of additional specialized effector T cells that express type 2 cytokines, such as follicular helper T cells and T_H9 cells, has shown that particular subsets of T cells may be responsible for driving specific features of type 2 responses *in vivo*. Follicular helper T cells express the transcriptional regulator Bcl-6, secrete IL-21 and are specialized to help B cells in the germinal center; IL-4 secreted from these cells is probably critical for the production of IgE during allergic and anti-helminth responses[25]. T_H9 cells can be generated by the stimulation of naive cells *in vitro* with IL-4 and transforming growth factor-β, express the transcription factors PU.1 and IRF4 and secrete large amounts of IL-9 and small amounts of IL-4 (refs. 26,27). T_H2 cells can also be reprogrammed into T_H9 cells by transforming growth factor-β; notably, IL-9 expression has been observed in $CD4^+$ T cells from the bronchoalveolar lavage fluid of asthmatic patients[28,29]. Heterogeneity among T_H2 cells has also been addressed through the use of genetic tools to analyze the spatial and temporal expression of type 2 cytokines by $CD4^+$ T cells *in vivo*[30,31]. With this approach, it has been determined that IL-4, but not IL-13, is expressed in follicular helper T cells in lymphoid organs. In contrast, effector T_H2 cells in the tissues express both IL-4 and IL-13. Notably, compartmentalized expression of type 2 cytokines correlates with the functional importance of these cytokines and their specific roles in the allergic response. In the future, similar studies addressing the expression of IL-5, IL-9 and IL-10 by T cells *in vivo* will provide better understanding of the plasticity and identity of the specific subsets of $CD4^+$ T cells that regulate type 2 responses.

IgE secretion by B cells is an important effector mechanism of type 2 immunity, and the recognition of allergens by IgE on mast cells is responsible for induction of the cardinal features of classic allergic responses, including anaphylactic shock. A variety of cells of the innate immune system, including mast cells and basophils, express the high-affinity IgE receptor FcεRI. FcεR expression can also be induced in DCs after exposure to allergens[32], and expression of the high- and low-affinity IgE receptors FcεRI and FcεRII has also been reported in eosinophils[33].

Mast cells and basophils mediate the allergic response by releasing critical inflammatory mediators through degranulation in response to the recognition of allergens by IgE. Mast cells differentiate in the bone marrow from $CD34^+$ precursor cells in the presence of stem-cell factor, recirculate through the blood and home to peripheral tissues[34]. Mast cells are found mainly in epithelial barriers such as skin and mucosal tissues, including the lungs and gastrointestinal tract, and increase in number after exposure to allergens[35]. Asthmatic patients also have enhanced degranulation of mast cells[36]. Basophils are short-lived cells that differentiate in the bone marrow and recirculate in the blood. Basophil development was originally considered to be exclusively IL-3 dependent[37]; however, TSLP has now been shown to be able to promote the differentiation and proliferation of basophils in an IL-3 independent manner[38]. The degranulation of TSLP-generated basophils is IgE independent, and instead these cells respond to IL-33 and IL-18; interestingly, the effector molecules released in response to these various stimuli are also different and could potentially determine the heterogeneity and diversity of basophil function[39].

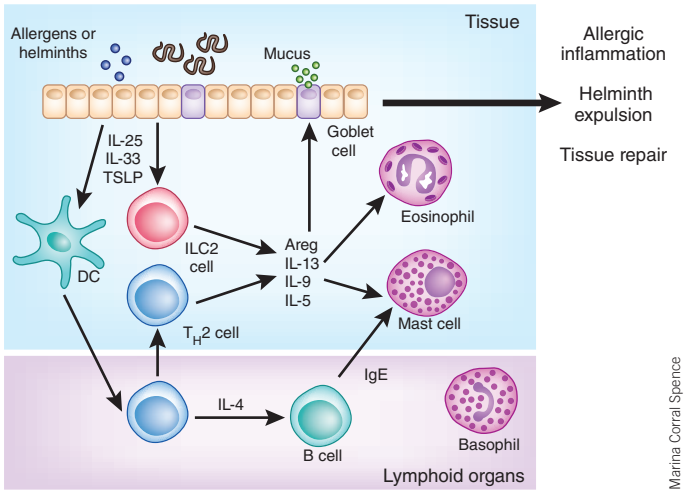

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Figure 1 Initiation and propagation of type 2 responses. Type 2 responses are initiated by allergens or helminths that disrupt the epithelial barriers and induce secretion of IL-25, IL-33 and TSLP. Those epithelium-derived cytokines activate ILC2 cells, which directly secrete type 2 cytokines, and DCs, which induce T_H2 responses. The secretion of type 2 cytokines by ILC2 cells feeds back on the epithelium to induce mucus secretion by goblet cells (IL-13) and tissue repair (amphiregulin (Areg)). Secretion of IL-9 and IL-5 by ILC2 cells leads to the recruitment and activation of mast cells and eosinophils. The activation of T cells in lymphoid organs further amplifies the secretion of type 2 cytokines, and the production of IL-4 by T cells in lymphoid organs leads to the production of IgE by B cells. Together, the responses triggered by secretion of type 2 cytokines from both ILC2 and T_H2 cells orchestrate allergic inflammation, helminth expulsion and tissue repair.

The infiltration of eosinophils into the airways is a characteristic feature of asthma and is critically dependent on IL-5 (ref. 40). The differentiation of eosinophils from bone marrow precursors depends on IL-3, IL-5 and the cytokine GM-CSF; under steady-state conditions, eosinophils migrate to the gastrointestinal tract[41]. Eosinophils secrete a variety of cytotoxic cationic proteins that promote oxidative stress–inducing tissue damage[42]. A previously unappreciated feature of eosinophils is the regulation and maintenance of another type 2 effector cell, the alternatively activated macrophage, which further confirms the function of the eosinophil as a critical regulator of type 2 responses. Interestingly, the maintenance of alternatively activated macrophages by eosinophils is also critical for the regulation of glucose homeostasis[43]. In the future it will important to determine if similar interactions are involved in pathogenic responses, including asthma and allergic responses.

The activation of macrophages by IL-4 and IL-13 promotes differentiation toward the specialized alternatively activated macrophage subset noted above (also called 'M2 macrophages'), characterized by expression of arginase-1 and Relm-α/β in mice[44]. These macrophages are generally considered anti-inflammatory; however, they also contribute to the expulsion of parasites and promote pathogenic type 2 responses as well as angiogenesis and tissue repair[45].

A variety of previously unappreciated populations of innate cells that can also serve as important sources of T_H1-, T_H2- and T_H17-type cytokines have been identified. These so-called 'innate lymphoid cells' (ILCs) have morphological characteristics similar to those of lymphocytes, but they do not express T cell or B cell antigen receptors and lack cell-surface markers associated with other cell lineages of the immune system.

ILCs can be categorized into three groups on the basis of their ability to produce cytokines associated with T_H1, T_H2 or T_H17 cells[46]. The ILC1 group is defined by production of interferon-γ and includes natural killer cells and interferon-γ-producing non–natural killer cell ILCs. The ILC2 group produces IL-5, IL-9 and IL-13 in response to IL-25 and IL-33. The ILC3 group produces IL-17A and IL-22 and includes both ILCs and lymphoid tissue–inducer cells. In the following sections, we will focus on ILC2 cells and discuss their discovery, characteristics and functions. In addition, we will discuss their role in allergic responses and speculate on their potential interactions with adaptive immunity.

Table 1 Molecular surface markers and location of innate type 2 cytokine–producing cells

	ILC2 cells			
	Natural helper cells	Nuocytes	Innate helper 2 cells	MPP^{type2} cells
	Fat-associated lymphoid structures; lungs	Mesenteric lymph nodes; intestines; lung	Spleen; liver; mesentery	Gut-associated lymphoid tissue
MHC class II	–	+	+/–	+
FcεRI	–	–	–	–
ST2	+	+	+	+/–
IL-17RB	+	+	ND	+
CD4	–	–	–	–
CD45	+	+	+	+
CD25 (IL-2Rα)	+	+	+	–
Thy-1	+	+	+	+/–
Sca-1	+	+	+	+
c-Kit	+	+	+	+/–
IL-7Rα	+	+	+	Low
ICOS	ND	+	+	ND

Tissue location (above columns) and the presence (+) or absence (–) of markers (far left column) on the surface of ILC2 cells (natural helper cells, nuocytes and innate helper 2 cells) and MPP^{type2} cells. ND, not detected; placeholder +/–, heterogeneous expression; Low, low expression.

Discovery of ILC2 cells

In 2001, IL-25 was identified as a cytokine structurally related to IL-17 (ref. 14). The administration of IL-25 to mice induces the expression of IL-4, IL-5 and IL-13 and secretion of IgE, IgG1 and IgA, as well as blood eosinophilia, and enhanced mucus production and epithelial cell hyperplasia and/or hypertrophy. Notably, IL-25 still induces the production of IL-5 and IL-13 in RAG-deficient mice, which lack B cells and T cells. Shortly thereafter, IL-5 was found to be produced by non-B–non-T cells after intranasal administration of IL-25 and infection of mice with *Nippostrongylus brasiliensis*[13]. In 2006, it was established that non-B–non-T cell c-kit$^+$FcεR1$^-$ cells respond to IL-25 and produce IL-4, IL-5 and IL-13 during helminth infection[47]. Finally, in 2010, three independent groups identified and further characterized type 2 cytokine–producing ILCs as cells that produce IL-5 and IL-13 in response to IL-25 and IL-33 (refs. 22,48,49).

ILC2 cells were discovered in lymphoid structures dependent on the common γ-chain in the peritoneal cavity, called 'fat-associated lymphoid clusters'[48]. These 'natural helper' cells have characteristics of lymphoid cells; however, they do not express lineage (Lin) markers (CD3ε, CD4, CD8α, TCRβ, TCRδ, CD5, CD19, B220, NK1.1, Ter119, Gr-1, Mac-1, CD11c and FcεRIα) but do express c-Kit, Sca-1, IL-7R and IL-33R[48]. They produce IL-5 and IL-13 in response to IL-33 or a combination of IL-2 and IL-25 (ref. 48). At the same time those cells were identified, a different subset of ILC2 cells, called 'nuocytes', was found through the use of reporter mice that express enhanced green fluorescent protein as a surrogate for IL-13 expression[22]. These cells expand their populations *in vivo* in response to IL-25 and IL-33 and produce IL-13 mainly at the early stage of helminth infection with *N. brasiliensis*[22]. Subsequently, innate Lin$^-$cells, called 'innate helper 2 cells', that arise in response to IL-25 and IL-33 or infection with *N. brasiliensis* were identified through the use of dual IL-4- and IL-13-reporter mice[49]. Although there are some differences among natural helper cells, nuocytes and innate helper 2 cells in terms of their phenotypes and tissue distribution, it has been agreed that these cells should all be referred to as 'ILC2 cells'[46,50,51].

In parallel with those three reports describing natural helper cells, nuocytes and innate helper 2 cells, it was observed that administration of IL-25 promotes the accumulation of a Lin$^-$ multipotent progenitor (MPP) cell population in gut-associated lymphoid tissue that promotes T_H2 responses[52]. These cells were defined by their expression of Sca-1 and intermediate expression of c-Kit and were called 'MPP^{type2} cells'. Notably, these cells differ substantially from natural helper cells, nuocytes and innate helper 2 cells in many ways, including the precursors they are derived from, the expression of unique cell surface markers and their ability to differentiate into cells of the monocyte and granulocyte lineages[52] (**Table 1**). Thus, MPP^{type2} cells seem to be functionally distinct from ILC2 cells.

Development of ILC2 cells

Because of the absence of many lineage-defining surface markers on ILC2 cells, there has been a great deal of interest in the developmental requirements of these cells (**Fig. 2**). Similar to the development of other ILCs, the development of ILC2 cells is dependent on the transcription factor Id2 ('inhibitor of DNA binding 2')[48,51]. In addition, ILC2 cells specifically require the T_H2-defining transcription factor GATA-3 (refs. 53,54) and the transcription factor RORα[55,56]. ILC2 cells and their precursors in bone marrow have continuous high GATA-3 expression[53]. ILC2 cells are also completely lost in the absence of GATA-3, and their precursors in bone marrow fail to repopulate the peripheral pool of ILC2 cells, which suggests that GATA-3 is required for the maintenance of mature ILC2 cells and their precursors in bone marrow[53]. Furthermore, GATA-3 has been shown to be essential for the function of human ILC2 cells[54]. RORα is also critical for ILC2 development, as RORα-deficient mice lack ILC2 cells[55]. So far, a unique transcriptional regulator that distinguishes ILC2 cells from classic T_H2 cells has not been discovered, and transcriptional profiles of these cells are needed for identification of the molecular differences between them.

ILC2 cells are present in bone marrow and are differentiated from common lymphoid progenitors, which are Lin$^-$IL-7Rα$^+$Flt3$^+$. Thymocytes at CD4$^-$CD8$^-$ double-negative stages 1 and 2 maintain the potential to differentiate into ILC2 cells *in vitro*; however, athymic mice still develop ILC2 cells[48] and therefore the role of the thymus in ILC2 development *in vivo* remains unclear[56]. Finally, ILC2 cells require IL-33, IL-7 and Notch signaling for their generation *in vitro*[56]. Unlike prototypical ILC2 cells, MPP^{type2} cells can differentiate into cells of the monocyte-macrophage and granulocyte lineages; therefore, these cells are probably developmentally distinct from classic ILC2 cells and may be derived from common myeloid progenitors rather than common lymphoid progenitors.

ILC2 cells in humans

Human ILC2 cells were discovered in a study focusing on Lin$^-$ cells that express the IL-7 receptor α-chain (CD127)[57]. As ILC2 cells in mice

secrete IL-13 in response to IL-33 or IL-25 (with or without IL-2), IL-13 expression in an ILC population isolated from fetal gut was examined. Lin^-CD127^+ cells expressing CRTH2 (a chemoattractant receptor expressed on T_H2 cells) that express transcripts encoding IL-13 were found, which suggested that these cells may be ILC2 cells. These cells also express the IL-33 receptor IL-1RL1 (ST2), IL-17RB (a subunit of the IL-25 receptor), IL-17RA (the common subunit of the receptor for IL-25 and IL-17) and the common ILC and natural killer cell marker CD161. Human $Lin^-CD127^+CRTH2^+CD161^+CD25^+$ ILC2 cells also have high expression of GATA-3. Human ILC2 cells can also respond directly to TSLP, in addition to responding to IL-25 and IL-33. Ectopic expression of GATA-3 in $Lin^-CD127^+CRTH2^-$ cells also induces CRTH2 expression and expression of T_H2 cytokines[57]. Such studies have established the existence of ILC2 cells in humans and suggest that ILC2 cells serve an important role in allergic responses in humans. Thus, ILC2 cells are potential targets for therapeutic intervention; notably, lipoxin A4 has been identified as a negative regulator of ILC2 cells from asthmatic patients[58].

ILC2 in allergy

Intranasal administration of IL-25 or IL-33 can induce an allergic response in the lungs even in the absence of T cells and B cells, which suggests the potential involvement of ILC2 cells in this response[13,59]. Indeed, one of the first direct observations of ILC2 cells was made in a study examining the cellular response to the intranasal administration of IL-25, which showed production of IL-13 by non-B–non-T cells in the lungs[14]. In addition, subsequent studies have begun to establish a critical role for ILC2 cells in responses to aeroallergens.

Many allergens contain protease activity, including the protease allergen papain from papaya and house dust mite (HDM) allergen[60]. The allergenic activity of HDM allergen is largely protease dependent, and up to 85% of asthmatic patients are allergic to HDM allergen[61]. Notably, papain induces allergic lung inflammation even in RAG-deficient mice, which suggests that ILC2 cells might mediate allergic lung inflammation in response to protease-containing allergens[21]. Indeed, ILC2 cells seem to have a critical role in lung inflammation in response to papain and HDM allergen. In the absence of T cells, ILC2 cells are the main mediators of lung inflammation in response to intranasal administration of papain or HDM allergen; ILC2 cells are the main source of IL-5 and IL-13 in RAG-deficient mice, and transfer of ILC2 cells into mice deficient in T cells and ILC2 cells is sufficient to restore lung inflammation[62]. Together these data show that ILC2 cells are sufficient to induce many features of the allergic response in the absence of adaptive immunity.

ILC2 cells also have a critical role in AHR stimulated by glycolipids and infection with influenza virus. ILC2 cell–derived IL-13 is sufficient to mediate glycolipid-induced AHR when wild-type ILC2 cells are transferred into IL-13-deficient mice[63]. ILC2 cells are also sufficient to mediate AHR in response to infection with influenza virus[64]. Thus, ILC2 cells are able to induce a cardinal feature of asthma even in the absence of adaptive immunity.

So far, studies of the role of ILC2 cells in allergy have focused almost exclusively on allergic responses in the lungs, which are largely dependent on type 2 cytokines and are mostly independent of IgE. Thus, the role of ILC2 cells in allergic responses in other tissues and in IgE-dependent allergic responses remains unclear. Notably, whereas T cell–derived IL-4 and IL-13 are dispensable for the expulsion of worms, AHR and tissue inflammation, IgE production is entirely dependent on T cell–derived type 2 cytokines[65]. This suggests that if ILC2 cells have any role in IgE-dependent allergic sensitization, it is probably simply a supporting role. However, this does not exclude the possibility of a role

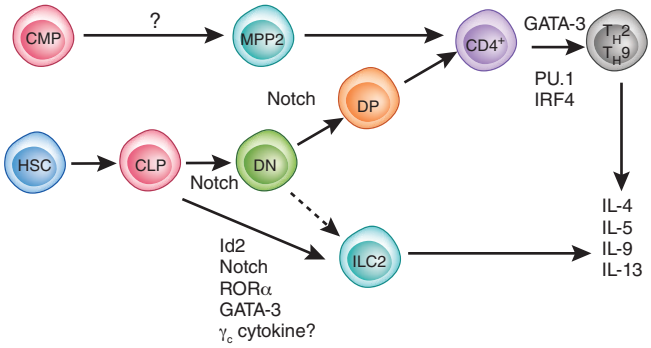

Figure 2 Innate and adaptive type 2 cell ontogeny. Cells of the type 2 response include T_H2, T_H9, ILC2 and MPP^{type2} cells. T_H2, T_H9 and ILC2 cells derive from common lymphoid progenitors in the bone marrow in a Notch-dependent manner. The differentiation of naive $CD4^+$ T cells into T_H2 cells is controlled by GATA-3, whereas that of T_H9 cells is controlled by PU.1 and IRF4. ILC2 differentiation depends on Id2, RORα, GATA-3 and common γ-chain (γ_c) cytokines. ILC2 cells can also differentiate from $CD4^-$ $CD8^-$ double-negative (DN) thymocytes *in vitro*. Given their ability to further differentiate into monocytes-macrophages and granulocytes, MPP^{type2} cells are probably generated from common myeloid progenitors (CMP); however, the factors that control MPP^{type2} differentiation still need to be determined. HSC, hematopoietic stem cell; CLP, common lymphoid progenitor; DP, double-positive thymocyte.

for ILC2 cells during the IgE-dependent allergic response itself. In this context, it is notable that mast cells are reported to produce IL-33 (ref. 66), which raises the possibility that the recognition of allergens by IgE on mast cells may also lead to the activation of ILC2 cells.

Nasal polyps from patients with chronic rhinosinusitis show enrichment for ILC2 cells, which suggests that ILC2 cells could have a critical role in allergic responses in humans[57]. Furthermore, lesions from patients with atopic dermatitis also show enrichment for ILC2 cells, which suggests their involvement in allergic responses in the skin[67]. Interestingly, ILC2 cells isolated from human skin are activated mainly by TSLP and are largely impervious to activation by IL-25 and IL-33; this suggests that the innate cytokines responsible for the activation of ILC2 cells may differ in their activation requirements depending on the tissue in which they reside.

ILC2 cells and T cells in allergy

Because many of the initial reports of ILC2 cells in allergy were studies of RAG-deficient mice, the role ILC2 cells in allergy in the context of an intact adaptive immune system has remained largely unclear. However, studies have begun to shed light on the relative contributions of ILC2 cells and T cells to the allergic response.

ILC2 cells have been found to represent a large proportion of the total IL-5- and IL-13-producing cells in the lungs in various models of asthma[68]. Over half of all IL-5- and IL-13-producing cells in the lungs after intranasal administration of IL-25 or IL-33 are ILC2 cells. However, directly induction of asthma via the administration of IL-25 and IL-33 elicits a largely innate asthmatic response, with little contribution from T cells, which might bias the allergic response toward ILC2 cells. Thus, the role of ILC2 cells after the induction of asthma through the use of ovalbumin and alum, which is thought to be largely T cell dependent, has also been examined[68]. Notably, even in asthma induced with ovalbumin and alum, ILC2 cells and T cells seem to provide at least equal contributions to the production of IL-5 and IL-13 in the lungs of wild-type mice. Furthermore, ILC2 cells contribute to the production of IL-5 and IL-13 after asthma is induced in wild-type mice by HDM

allergen, albeit less so than do T cells. ILC2 cells have also been detected in lung-draining lymph nodes after the induction of asthma with IL-25 or IL-33 (ref. 68). However, the role of ILC2 cells in the lymph nodes in asthma remains unclear.

ILC2 cells also seem to have an important role in papain-induced lung inflammation in mice with an intact adaptive immune system. Mice that lack ILC2 cells (because of a deficiency in RORα in the hematopoietic compartment) but that have an intact adaptive immune system have much less lung inflammation than do mice with both ILC2 cells and cells of the adaptive immune system, after intranasal administration of papain[55]. However, it is worth noting that lung inflammation in these studies was measured 4 days after initial exposure to papain, which probably precedes the peak of the T cell response.

Together, the studies noted above show that ILC2 cells produce large amounts of IL-5 and IL-13 in the tissues during an allergic response; under certain conditions and at specific time points, those concentrations are equal to or greater than the amounts of IL-5 and IL-13 made by T cells. Therefore, ILC2 cells seem to be critical for the induction of robust allergic responses in the lung, even in cases in which T cells were thought to be the main mediators of the allergic response.

In contrast to IL-5 and IL-13, which are produced by both T cells and ILC2 cells, IL-4 is produced mainly by conventional T cells, and ILC2 cells represent only a very small proportion of the cells that produce IL-4 during asthma[68,69]. Thus, ILC2 cells seem to be similar to effector T_H2 cells in the tissues in their cytokine expression pattern. T cells in the tissues and those in the lymph nodes also show divergent cytokine expression patterns during helminth infection: T cells in the tissues express both IL-4 and IL-13, whereas those in the lymph nodes express mainly IL-4 (ref. 31).

In addition to expressing IL-5 and IL-13, ILC2 cells can express IL-9. In a study of an IL-9-fate-mapping reporter mouse, ILC2 cells were found to be an important source of IL-9 after the administration of papain, whereas IL-9 production by T cells was largely undetectable with this particular reporter system[70]. In that study, IL-9 expression was transient in ILC2 cells, and ILC2 cells that had expressed IL-9 became producers of IL-5 and IL-13 after restimulation *ex vivo*. That suggests that IL-9-producing ILC2 cells may also be the precursors of the traditional ILC2 cells observed in other models. Thus, in contrast to IL-4, which is made mainly by T cells, and IL-5 and IL-13, which are made by both T cells and ILC2 cells, IL-9 may be produced mainly by ILC2 cells in response to protease allergens. Determination of the relative contributions of various sources of type 2 cytokines, including IL-9, in other models of allergy as well as during helminth infection is needed.

The studies discussed above have begun to address the relative contributions of ILC2 cells versus T cells to the allergic response. However, definitive answers to these questions will probably require the construction of new tools to manipulate ILC2 cells in the presence of an intact adaptive immune system. In particular, there is a critical need for a convenient model of ILC2 cell deficiency; furthermore, construction of mice with ILC2 cell–specific expression of Cre recombinase would allow the deletion of particular critical cytokines (such as IL-5, IL-9, IL-13 and amphiregulin) specifically in ILC2 cells in the presence of the adaptive immune response. Careful examination of gene-expression patterns in ILC2 cells and T_H2 cells may identify differences between these two cell types that may be exploited for the construction of such tools.

Interactions between ILC2 cells and the adaptive immune system

Although ILC2 cells are sufficient to induce type 2 inflammation under certain conditions, T_H2 cells and IgE remain the 'major players' in classic allergic responses. Thus, the extent to which ILC2 cells contribute to allergic responses probably depends on their interactions with the adaptive immune system, either as initiators of adaptive immunity or as responders to signals produced by T cells and B cells. Although the importance of ILC2 cells in the control of adaptive immunity remains unclear, many mechanisms by which ILC2 cells could potentially interact with T cells and B cells have already been discovered.

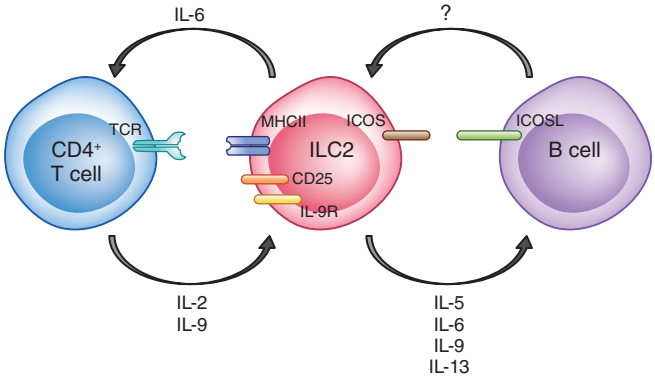

Figure 3 Potential interactions of ILC2 cells with T cells and B cells. ILC2 cells may interact with T cells and B cells through both cytokine secretion and the expression of molecules at the cell surface. ILC2 cells may regulate T cell differentiation through antigen presentation via MHC class II (MHCII) and through the secretion of IL-6. In turn, T cells support the maintenance and proliferation of ILC2 cells via the production of IL-2 and IL-9. ILC2 cells also support B cell survival and isotype switching through cytokine production and interactions of ICOS with its ligand ICOSL. The identification of B cell–derived mediators that feed back on ILC2 cells will require additional studies.

One mechanism through which ILC2 cells could activate T cells is antigen presentation. At least some ILC2 cells are reported to express major histocompatibility complex (MHC) class II, which suggests that these cells could potentially be involved in the activation of $CD4^+$ T cells, either in the priming phase or during the effector response[52,70]. Indeed, it has been observed that cells derived from MPP^{type2} cells support T cell responses both *in vitro* and *in vivo*; furthermore, tissue–derived ILC2 cells derived from a lymphoid source also express MHC class II under certain conditions, which raises the possibility that 'canonical' ILC2 cells may also be able to present antigen to T cells[70]. Another mechanism of 'instruction' could be the secretion of 'instructive' cytokines such as IL-6, which is known to be critical for the activation of naive T cells[48,71]. In addition to their potential interactions with T cells, ILC2 cells also may be involved in the 'instruction' of B cell responses, in particular through their production of IL-5, IL-6 and IL-13. Because IL-5 can support IgA responses and IL-6 is a critical B cell growth factor, it might be expected that ILC2 cells would influence B cell responses. Indeed, ILC2 cells from fat-associated lymphoid clusters can enhance IgA production and support the self-renewal of B-1 cells[48]. Furthermore, ILC2 cells express the inducible costimulator ICOS, which may bind to its ligand ICOSL on B cells and potentially regulate germinal-center responses[22]. Finally, an ILC2 population that can enhance production of IgE from B cells *in vitro* has been described, which raises the possibility that ILC2 cells may also regulate IgE-dependent allergic sensitization[72].

In addition to 'instructing' adaptive responses, ILC2 cells also seem to respond to a variety of signals produced by T cells and B cells, which suggests that the adaptive response also feeds back onto ILCs. ILCs express the receptors for IL-2 and IL-9, which raises the possibility that T cell–derived cytokines may influence the development, proliferation, survival and/or activation of ILC2 cells[22,49]. RAG-deficient mice

have fewer ILC2 cells than do wild-type mice during worm infection, which suggests a dependence on the adaptive immune system for their maintenance[22]. Notably, IL-2 can support the proliferation of ILC2 cells and can potentiate the secretion of type 2 cytokines induced by IL-25 and IL-33 (ref. 48). Furthermore, in certain cases, IL-2 is essential for costimulating the activation of ILC2 cells in response to IL-33 (ref. 62). These data suggest that ILC2 cells may have important roles both before and after the initiation of adaptive immunity (**Fig. 3**).

Many questions remain to be answered about the potential for interactions between ILC2 cells and the adaptive immune system. Important aspects of ILC2 cell biology that remain to be determined include where and how are these cells activated, as well as their chemotactic ability and homing properties, which ultimately would affect the ongoing inflammatory response and possible interactions with other cell types. In particular, it will be important to determine how location correlates with the expression of molecules that facilitate interactions with the adaptive immune system. Interestingly, a study has found that CCR9 deficiency considerably impairs the homing of precursors of ILC2 cells to the intestinal lamina propria but is dispensable for their homing to other compartments[53]. More detailed spatio-temporal analysis of the induction of ILC2 cells, together with that of T cells and B cells, will be necessary for full appreciation of the extent of interactions between ILC2 cells and adaptive immunity during type 2 responses.

Beneficial roles for ILCs in response to allergens

In this Review we have focused on the role of ILCs in allergic pathologies. However, ILC2 cells responses can also be beneficial—for example, in response to helminth infection. Furthermore, ILC2 cells have been found to participate in tissue repair in the lungs after infection with influenza virus through the production of amphiregulin, a member of the epidermal growth factor family that is expressed by T_H2 cells during helminth infection[73,74]. The ability of ILC2 cells to participate in tissue repair fits well with the hypothesis that type 2 responses may have evolved to mediate wound repair[75], as well as the hypothesis that type 2 responses evolved to protect against noxious xenobiotics, such as venoms[2]. One intriguing possibility is that ILC2 cells act to maintain the mucosal barrier in the presence of continuous exposure to environmental allergens by initiating constitutive, low, innate type 2 responses, including the production of mucus and induction of tissue repair. From this perspective, one can imagine that ILC2 might protect against the development of true, adaptive-dependent allergic responses by acting as a first line of defense against noxious allergens; if this first line of defense is successful, it may prevent the initiation of 'true' allergic responses. Indeed, in type 1 responses, the removal of innate defenses leads to compensatory activation of the adaptive immune response[76]. In addition, given the intrinsic characteristics of ILC2 cells and their location at mucosal sites, it will be interesting to explore the role of these cells in maintaining tissue homeostasis under steady-state conditions. In this context, a study has demonstrated that ILC2 cells participate in the regulation of metabolic homeostasis by maintaining visceral adipose tissue eosinophils and alternatively activated macrophages[77].

Concluding remarks

Advances in the understanding of the mechanisms by which type 2 responses are initiated have shown the presence of a previously unappreciated group of innate lymphoid cells that produce classic type 2 cytokines in response to helminth infection and allergens. Although much remains to be learned about the biology of these cells, their integral role in the orchestration of type 2 responses suggests that studies of these cells may identify new therapeutic avenues in the treatment of asthma and allergies.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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