

ILC2 memory: Recollection of previous activation

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Summary

Immunological memory, traditionally thought to belong to T and B cells, has now been extended to innate lymphocytes, including NK cells and ILC2s, myeloid cells such as macrophages, also termed "trained immunity" and more recently to epithelial stem cells. In this review, we discuss the mechanisms underlying memory generation on ILC2s and speculate about their potential role in human allergic diseases, such as asthma. Moreover, we examine the relevance of the spontaneous ILC2 activation in the lung during the neonatal period in order to efficiently respond to stimuli later in life. These "training" of neonatal ILC2s may have an impact on the generation of memory ILC2s in the adulthood.

KEYWORDS

allergic diseases, ILC2s, immunological memory, neonates

1 | INTRODUCTION

Immunological memory has been defined by the ability of the immune system to recall previous exposure to pathogens and strongly respond upon re-encountering the same pathogens. Originally described as a

hallmark of adaptive immunity, immunological memory has been focused on T and B cells. When naive T or B lymphocytes expressing specific antigen receptors encounter cognate antigens for the first time, they are activated and differentiated into effector cells as well as memory cells that retain the same antigen receptors. Antigen specificity of immune memory is thought to be essential for preventing auto-immunity. This classical definition of immunological memory has

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recently been challenged by the findings of memory-like properties of some innate immune cells. Natural killer (NK) cells expressing specific receptors for cytomegalovirus (CMV) proteins are activated during CMV infection and acquire memory functions. CMV infection also induces IL-12-mediated activation of NK cells lacking CMV receptors, and the IL-12-activated NK cells become long-lived cells.¹ NK cells activated in vitro by a combination of IL-12, IL-15, and IL-18 also become antigen non-specific memory cells. Therefore, NK cell memory can be both antigen-specific and antigen non-specific, depending on the initial activation pathways of naive NK cells. More recent studies have further expanded antigen non-specific immune memory to other innate immune cells, namely group 2 innate lymphoid cells (ILC2s).² Like NK cells, ILC2s are innate lymphocytes. Similar to cytokine-activated NK cell memory, ILC2s in the lung activated by epithelial-derived cytokines, which are released upon allergen inhalation, persist for many months and become highly responsive to cytokine/allergen challenges, thus displaying antigen non-specific memory functions. Antigen non-specific immune memory has been extended to non-lymphocytes as monocytes and macrophages activated by β -glucan acquire enhanced phagocytic capacity and higher cytokine production and mediate strong protection against certain pathogens. This antigen non-specific memory-like property has been termed "trained immunity".³ Currently, the distinction between immune memory and trained immunity is not clear. More recently, skin epithelial stem cells have been shown to acquire inflammation-induced memory that enables enhanced wound healing in subsequent tissue damage.⁴ Therefore, antigen non-specific memory can be further extended to non-immune cells.

Here, we review antigen non-specific memory of ILC2s in the lung and discuss about the underlying mechanisms and implications of ILC2 memory in asthma development.

2 | ILC2 MEMORY

2.1 | Group 2 innate lymphoid cells (ILC2s)

ILC2s belong to the family of ILCs, which includes NK cells and helper-like ILCs.⁵ NK cells can be seen as innate counterparts of cytotoxic CD8⁺ T cells and are involved in early protection against viral infections and tumor immunosurveillance. They are characterized by the expression of the transcription factors Eomesodermin (EOMES) and T-bet. NK cells are activated by ligation of stimulatory receptors such as NKG2D that recognizes stress-induced molecules on target cells.⁶ They are regulated by combinations of cytokines including IL-2, IL-12, IL-15, and IL-18. Activated NK cells exert cytotoxic functions and secrete interferon- γ (IFN- γ).

Cytokine-producing ILCs can be seen as the innate counterparts of the T-helper cell subsets. They are characterized by the expression of IL-7R α (CD127) and are divided into 3 groups, group 1 (ILC1), group 2 (ILC2), and group 3 (ILC3), based on their expression of transcription factors and cytokines.

ILC1s are important in the protection against intracellular pathogens such as *Toxoplasma gondii* in the intestine. They express T-bet, which is required for their development, and are the innate counterparts

of Th1 cells. These cells are stimulated by IL-12 and are the major producers of IFN- γ among the innate immune cells. IL-15, but not IL-7, is required for the development of these cells. Phenotypically, ILC1s are very similar to NK cells, but they can be distinguished by EOMES and CD49a expression.⁷

ILC2s are considered to be the innate counterparts of Th2 cells based on their expression of the transcription factor GATA3 and the cytokines they produce. Both GATA3 and retinoic-acid-receptor-related orphan nuclear receptor alpha (RORA) are required for their development. ILC2s, upon activation by IL-33 among other cytokines, produce the type 2 cytokines IL-5 and IL-13 as well as the growth factor amphiregulin, which are involved in helminth expulsion, wound healing and tissue repair. Moreover, ILC2s are critical in the initiation of allergic responses.

ILC3s development depends on the transcription factor ROR γ t, similar to Th17 and Th22 cells. They produce IL-17 and IL-22, which are crucial in maintaining intestinal tissue homeostasis and the resistance against fungal infections. These cells include CCR6⁺ lymphoid-tissue-inducer (LTi) and LTi-like cells as well as CCR6⁺ ILC3s. LTi cells develop prenatally and are critical for the development of lymphoid organs. CCR6⁺ ILC3s emerge postnatally during the first few weeks of life and are dependent on aryl hydrocarbon receptor signaling for their expansion. A subset of these cells upregulate the expression of T-bet and natural-cytotoxicity-receptor (NCR) during development and are referred to as NCR⁺ ILC3s. Upregulation of T-bet leads to the downregulation of ROR γ t and adaptation of an ILC1-like phenotype in some of these cells, which are referred to as ex-ILC3s. These cells then acquire the ability to promote tissue inflammation by producing IFN- γ .⁸

Currently, immunological memory has only been described for ILC2s and NK cells. However, it is tempting to speculate that other ILCs could show memory responses as well. In this review, we focus on ILC2s and their ability to acquire memory features. ILC2s are abundant at the barrier tissues including lung, intestine, and skin although they have been found in other tissues such as the liver or pancreas. These cells are identified by a combination of cell surface markers with some tissue-associated differences. Originally, ILC2s were defined as Lineage marker (Lin)[−], CD127 (IL-7R α)⁺, Thy1 (CD90)⁺, ST2 (IL-33R)⁺, and CD25 (IL-2R α)⁺. However, some variation in ST2 expression of naive ILC2s is found depending on the tissue. Skin ILC2s express CD103. On the other hand, intestinal ILC2s are IL-25R⁺ while lung ILC2s express very low levels of this receptor. In most tissues, ILC2s express both inducible T-cell co-stimulator (ICOS) and ICOS-ligand. ICOS:ICOS-ligand signaling is required for the proper expression of cytokines by these cells as well as their survival. Molecules such as cytokines, leukotriens, prostaglandins, and neuropeptides have been shown to activate ILC2s. Activated ILC2s produce large amounts of IL-5 and IL-13 but also some IL-6, granulocyte macrophage colony-stimulating factor (GM-CSF), and amphiregulin. Additionally, they express programmed cell death 1 (PD-1) and KLRG1 that negatively regulate ILC2 activation and proliferation to control the immune response mediated by these cells. IFN α , β , γ and IL-27 as well as Treg-derived IL-10 are also known to inhibit ILC2 activation.

2.2 | ILC2 activation

ILC2 activation has been extensively studied in the lung and gut, and more recently also in the skin. These 3 tissues are the main sites for ILC2 localization. ILC2s do not express antigen receptors and instead are activated by cytokines, such as IL-33, IL-25, and thymic stromal lymphopoietin (TSLP). IL-33 belongs to the IL-1 family and is constitutively expressed at high levels in the nuclei of various cell types including endothelial, epithelial, and fibroblast-like cells of almost all organs. IL-33 does not contain a signal sequence to localize it to endoplasmic reticulum or Golgi, and therefore is not secreted like other conventional cytokines. Instead, IL-33 generally functions as a nuclear alarmin that is released after cell injury, although cellular activation through ATP signaling is also able to induce the release of IL-33 in the absence of cell death. Mechanical stress has been also involved in IL-33 release. In accordance to their critical role in wound healing and tissue repair, mouse lung ILC2s express high levels of the IL-33 receptor ST2 and they rapidly and potently respond to IL-33. IL-25 (IL-17E), which belongs to the IL-17 family, induces Th2 and Th9 responses and is involved in helminth expulsion and allergic responses. IL-25 is also released by the epithelial cells upon injury. In the case of helminth infection, a recent report has shown that IL-25 is mainly released by Tuft cells in the intestine and lung.⁹ TSLP is a member of the IL-2 cytokine family and is thought to support B-cell development. During allergic inflammation, the main sources of TSLP are epithelial cells, keratinocytes, and stromal cells although it can be produced by DCs and mast cells as well.¹⁰

As mentioned earlier, there is some phenotypic variability in ILC2s depending on the tissue they reside in and consequently their mechanism of activation also varies. In the lung, mature ILC2s are ST2⁺ and TSLPR⁺ but express low levels of the IL-25 receptor (IL-25R) and as a result intranasal administration of IL-25 does not activate lung ILC2s.² It has been shown that IL-33 is required for ILC2 activation in the lung upon in vivo administration of the protease allergen papain. Moreover, in vitro experiments show that only IL-33 is essential for ILC2 activation while TSLP and IL-25 together with other cytokines such as IL-2 or IL-7 act as enhancers of activation.¹¹ In contrast, it has been reported that ILC2s both in the large and small intestine express IL-25R. In addition, results from our laboratory show that ILC2s in the small intestine are ST2⁺. Several reports have demonstrated a crucial role for IL-25 in ILC2-mediated helminth expulsion.⁹ Therefore, IL-25 appears to play an important role in inducing ILC2-mediated inflammation in the gut, unlike in the lung.

Interestingly, Huang et al¹² have found a subset of lung ILC2s, termed inflammatory ILC2s (iILC2s), that express high levels of IL-25R but are negative for ST2. Intraperitoneal injection of IL-25 but not IL-33 induced the expansion of these iILC2s in the lung and they seem to be the early responders to *N. brasiliensis* infection before ST2⁺ ILC2s are activated. In addition, iILC2s could give rise to ST2⁺ ILC2s both in vivo and in vitro. It is unlikely that this population resides in the naive lung and they probably migrate from other tissues such as the gut. We did not find iILC2 expansion in the lung upon intranasal administration of IL-25, supporting the idea that they are not lung-resident ILC2s.²

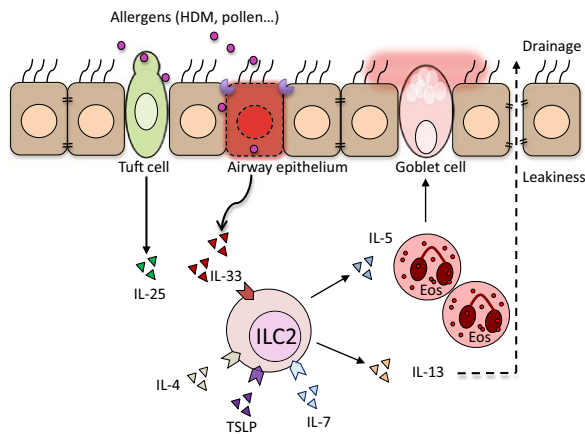
As in the lung, ILC2s are the most abundant ILC population in the skin. Skin ILC2s also express high levels of ST2. In contrast, there are some reports showing that skin ILC2 activation is TSLP-mediated and IL-33-independent.¹³ IL-25 is produced by keratinocytes in the skin upon cell damage. Whether IL-25 is a key cytokine in inducing inflammation or simply acts as an enhancer of TSLP-induced activation remains to be clarified.

In summary, it appears that IL-33 is critical in ILC2-mediated lung inflammation while IL-25 and TSLP seem to be more important for the activation of ILC2s in the gut and in the skin, respectively. It is likely that the local environment within the tissue in which they reside shapes the phenotype of ILC2 and consequently their mechanism of activation.

2.3 | ILC2 memory generation and maintenance

Immunological memory protects us against re-infections with pathogens and is classically defined as the ability of the adaptive immune system to respond faster and more efficiently upon subsequent exposures to the same antigen. Immune memory has been thoroughly studied in T cells, which has led to the development of efficient vaccines that protect us from infections. Classically, an immune memory cell is long-lived and a faster and/or stronger responder to secondary challenges. We have recently shown that mouse lung ILC2s can acquire these characteristics upon activation.² Intranasal administration of allergens (ie, papain or *Aspergillus* protease) or the alarmin IL-33 triggered ILC2 expansion and activation in the lung. The expanded effector ILC2 population produced large amounts of IL-5 and IL-13 inducing eosinophilia in the airways. The expansion phase lasted for a few days and was followed by a contraction phase where the numbers of ILC2s declined and they stopped producing cytokines. It is not known what mechanisms control the contraction of ILC2s. Inhibition of ILC2s by PD1 and KLRG1 pathways together with the decrease in the levels of alarmins may be responsible for the end of the cytokine production. As for the reduction of ILC2 numbers, a possible explanation could be that effector ILC2s become exhausted, as T cells do, and undergo apoptosis and/or migrate to other tissues, including the draining lymph node (LN). By 2 weeks after the allergen exposure, eosinophils were no longer in the lung indicating the resolution of the airway inflammation. However, numbers of ILC2s remained higher than in a naive mouse for up to 1 year, depending on the strength of the stimulus, ie, IL-33 is a more potent ILC2-activator than papain or *Aspergillus*. BrdU-labeling experiments showed that some of these previously activated ILC2s survived for such a long period of time indicating that some ILC2s became long-lived cells, which is a characteristic of immune memory cells. When these mice were re-challenged once with an unrelated allergen or IL-33 months after the first allergen exposure, they showed an enhanced airway inflammation characterized by increased numbers of eosinophils in the lung and higher mucus accumulation in the airways than naive mice receiving the same challenge. This exacerbated inflammatory response was due to an over-activation of the allergen-experienced ILC2s that produced higher amounts of IL-5 and IL-13 compared to naive ILC2s (Figure 1).

Activation of naïve ILC2s



Activation of memory ILC2s

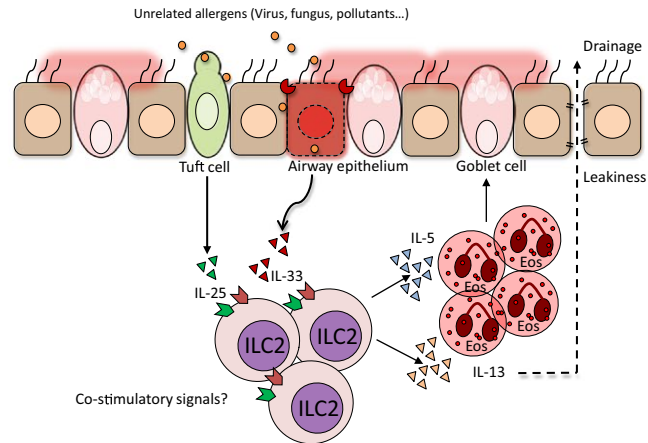


FIGURE 1 Activation of memory ILC2s. A, Upon allergen encounter, the lung epithelium is damaged and releases the alarmins IL-25 and IL-33. Naïve lung ILC2s express very low levels of the IL-25 receptor and are activated by the released IL-33. These cells proliferate, produce type 2 cytokines and acquire memory characteristics. B, Upon later unrelated allergen exposure, memory ILC2s that express IL-25 receptor show a potent response to IL-25 and IL-33 and produce more cytokines than naïve ILC2s. IL-25 alone is not sufficient to activate memory ILC2s and co-stimulatory signals are required. These cells mediate an enhanced type 2 response resulting in increased eosinophil recruitment and mucus production

The ability to mount a more potent response in subsequent stimulations is also a characteristic typically associated with memory cells, suggesting that these allergen-experienced ILC2s have acquired immune memory features. Therefore, similar to NK and T cells, ILC2s go through 5 phases after allergen exposure: expansion, contraction, memory generation, memory maintenance, and recollection.

Allergen-experienced ILC2s present many of the immunological memory characteristics of adaptive immune cells, except antigen specificity as they recognize cytokines rather than antigens. NK cells, which also belong to the innate immune system, can become antigen-specific memory cells after recognizing viral antigens from CMV through the Ly49H receptor.¹⁴ However, cytokine-activated NK cells can also mount enhanced responses to viruses compared to naïve NK cells in an antigen-independent manner, similarly to memory ILC2s.¹⁵ Moreover, recently it has been shown that not only lymphocytes but also myeloid cells can mount enhanced responses once they have been primed. Bacille Calmette-Guerin (BCG)-activated monocytes seem to respond stronger during subsequent stimulation with *C. albicans*.¹⁶ This phenomenon has been called “trained immunity.” Interestingly, Naik et al⁴ just showed that epithelial stem cells can remember previous inflammation and induce faster epithelial regeneration upon subsequent insults. We have recently suggested that the definition of immunological memory should be revised to include any cells that are able to remember previous activation, regardless of the mode of the initial activation, including both antigen and cytokine recognition.¹⁷

The mechanisms leading to memory ILC2 generation in the lung are still mostly unknown. It seems ILC2s need to be activated, and IL-33 is required as IL-33-deficient mice do not mount ILC2-mediated allergic responses. However, IL-33 alone does not activate naïve ILC2s in vitro, suggesting some other factors are also required. The

combination of IL-33 with other cytokines such as IL-25, IL-2, IL-7, or TSLP stimulates ILC2s in vitro. As mentioned earlier, some allergens trigger the release of TSLP and IL-25, but IL-25- or TSLP-deficient mice showed only partial reduction of ILC2 activation, indicating that IL-25 and TSLP are not essential. There might be some constitutive IL-2 and IL-7 production in the lung, but the source is not clear and it has not been proven that these cytokines are responsible for ILC2 activation. It has also been shown that basophil-derived IL-4 can activate ILC2s. However, IL-4KO mice can mount Th2 responses to papain, which are ILC2-dependent. Therefore, IL-4 may not be required for ILC2 activation in this context.¹⁸ It is possible that apart from IL-33, small amounts of TSLP, IL-25, IL-7, IL-2, and/or IL-4 are necessary or that another factor yet to be found is required. Very recently, a connection between nerves and ILC2 activation has been demonstrated.¹⁹ The neuropeptide NMu, produced upon parasite infection by neurons located in the small intestine, strongly and specifically activated intestinal ILC2s. Interestingly, intranasal administration of NMu activated lung ILC2s as well. It remains to be determined if NMu-mediated activation of ILC2s is alarmin-independent. Further study of how ILC2s are activated will allow us to understand the mechanisms of memory ILC2 generation.

It is still unknown whether there is a subset of naïve ILC2s committed to acquire memory. Recent studies suggest that in the case of memory T cells, the cells that divide the most after activation become exhausted and die while the progenies of T cells that undergo less rounds of division acquire a memory phenotype. Regarding NK cell memory generation, there is some evidence suggesting that the subset that transiently expressed *Rag1* during development acquires memory features as *Rag1* deficiency resulted in impaired expansion and persistence of memory NK cells following MCMV infection.²⁰

Interestingly, fate mapping reporter mice have shown that some ILC2s also expressed *Rag1* during development.²¹ Whether this subset has the potential to become memory ILC2s needs to be addressed. Preliminary results of our laboratory show that long-term memory ILC2 responses are impaired in *Rag1*-deficient mice. This could suggest that previous expression of *Rag1* in ILC2s is required for memory ILC2 generation. However, it is also possible that memory ILC2s require T cells to respond to a secondary challenge (I. Martinez-Gonzalez, M. Ghaedi, F. Takei, unpublished results).

Survival and maintenance of memory T cells have been extensively studied. Although they do not need the presence of the antigen, an initial strong TCR activation is important to secure long-term maintenance of the memory T-cell pool. Similarly, IL-33 stimulation in vivo maintains higher numbers of memory ILC2s for a longer period of time compared to papain administration, suggesting that the stronger the primary activation, the longer memory ILC2s are maintained. Moreover, IL-7 and IL-2 signaling are also key in the homeostasis of memory T cells. In the case of memory NK cells, IL-15 seems to be critical for their survival. It has been suggested that IL-2 also supports survival in ILC2s. However, the role of IL-2 in memory ILC2 maintenance has yet to be clarified.

2.4 | Activation of memory ILC2s

As previously mentioned, naive ILC2s in the mouse lung, in contrast to other tissues such as the gut, express very low levels of the receptor for the alarmin IL-25 and therefore IL-25 stimulation both in vitro and in vivo does not activate ILC2s in the lung. Upon allergen or IL-33 stimulation in vivo, effector ILC2s upregulate IL-25R and memory ILC2s maintain IL-25R expression for a long time. Accordingly, in contrast to naive ILC2s, memory ILC2s show a potent response to IL-25 intranasal administration in vivo (Figure 1). Preliminary data from our laboratory show that IL-25 alone does not stimulate purified memory ILC2s and that secondary signals involving interactions with other cells are required. The need for co-stimulatory signals is a phenomenon very common for other memory lymphocytes such as T or NK cells. Indeed, it has been recently shown that the assistance provided by CD4⁺ T cells to cytokine-induced memory NK cells is dependent on the presence of macrophages.²²

ILC2 memory is similar to cytokine-induced memory of NK cells since both of them are generated by cytokines rather than antigens. However, in contrast to in vivo allergen-induced memory ILC2s, cytokine-induced memory NK cells were generated in vitro by stimulation with a combination of cytokines including IL-12, IL-15, and IL-18.²² Interestingly, Lanier's group has shown that CMV infection in vivo not only generated antigen-specific Ly49H⁺ memory NK cells but also IL-12-induced Ly49H⁻ memory NK cells.¹ Although these in vivo-generated cytokine-induced memory NK cells showed enhanced response to ex vivo cross-linking of activating receptors, it was much lower than the one mounted by antigen-specific memory NK cells. Moreover, the cytokine-induced memory NK cells did not show increased IFN- γ production upon cytokine stimulation and more importantly they showed diminished response to *L. monocytogenes* infection compared to naive NK cells. In contrast, in vivo-generated memory

ILC2s show enhanced response upon both cytokine re-stimulation ex vivo and to unrelated allergen challenge in vivo.

Preliminary results using the IL-25R-deficient mouse suggest that memory responses of ILC2s are explained by their enhanced response to IL-25. The fact that memory ILC2s acquire the capacity to respond to IL-25 may explain why they show enhanced responses compared to naive ILC2s, since it is known that IL-25 synergizes with IL-33 to activate ILC2s. Interestingly, memory ILC2s not only respond more intensely but are more sensitive to activation than naive ILC2s.² A single dose of intranasal administration of papain is not sufficient to activate naive ILC2s nor induce allergic inflammation. In contrast, it induces the production of large amounts of IL-5 and IL-13 by memory ILC2s and consequently eosinophils are recruited into the lung of these mice. Therefore, memory ILC2s have a lower threshold of activation by alarmins.

Although in vitro and transplantation experiments showed that memory responses are intrinsic to ILC2s, we cannot exclude the possible contribution of the tissue environment. Allergen-sensitized lung epithelium might also remember and produce higher amounts of alarmins in subsequent encounters. Although our analyses of IL-33 KO mice showed that IL-33 is not required for maintenance and activation of memory ILC2s, we cannot rule out the possibility that higher production of IL-7 or other factors such as leukotrienes and prostaglandins could be contributing to the enhanced activation of memory ILC2s.

2.5 | Memory ILC2s in other tissues

Memory ILC2s are found in the lung-draining mediastinal LN. While only a few hundred ILC2s colonize the naive LN, after intranasal administration of an allergen or IL-33, there is a 100- to a 1000-fold increase in ILC2 numbers, and they produce higher amounts of IL-5 and IL-13 than lung ILC2s as shown by higher MFI of intracellular cytokine staining and higher levels of cytokines measured by ELISA after in vitro culture² (I. Martinez-Gonzalez, M. Ghaedi, F. Takei, unpublished results). It remains to be determined whether ILC2s proliferate in the LN, migrate from the lung or a combination of both. Although the exact role for ILC2s in the LN is not known, their activation coincides with an influx of eosinophils into the LN. Following the expansion of the ILC2 population by intranasal injections of IL-33 or papain, the LN ILC2 population contracts more quickly than lung ILC2s and the eosinophils in LN disappear within a few days whereas they persist in the lung for a few weeks. Whether LN ILC2s undergo apoptosis, migration or both need to be investigated. Interestingly, LN ILC2s remain positive for intracellular IL-5 and IL-13 for a long time unlike lung ILC2s. However, they are unlikely secreting these cytokines as eosinophils are no longer present in the LN. These long-lived LN ILC2s also acquire memory responses as a secondary challenge enhances their response compared to naive LN ILC2s. Moreover, LN memory ILC2s also upregulate IL-25R to higher levels than lung memory ILC2s. Accordingly, LN memory ILC2s are efficiently activated by IL-25 both in vivo and ex vivo. However, IL-25 alone does not stimulate purified memory ILC2s isolated from LN and lung, indicating that some other

secondary signals are required (I. Martinez-Gonzalez, M. Ghaedi, F. Takei, unpublished results).

Memory ILC2s in the LN showed higher proliferation and activation than in the lung, and we could speculate that, in a similar manner to memory T cells, upon re-challenge memory ILC2s migrate from the LN to the lung and promote the enhanced lung inflammation. However, *Rorc*-deficient mice, which do not have LNs, showed greater memory responses upon re-challenge than Wildtype mice, suggesting that the LN memory ILC2s are not required for memory responses (I. Martinez-Gonzalez, L. Mathae, Takei, unpublished). However, it is also possible that memory ILC2s that were supposed to colonize the LN have been retained in tertiary LN formed within the lung, and therefore mounting enhanced responses.

The fact that memory ILC2s are present not only in the lung but also in the LN could mean that there might be different subsets of memory ILC2s, similar to memory T cells. However, memory ILC2s in the LN differ from the LN central memory T cells since the former remain positive for intracellular cytokines and therefore ready to respond, while the latter have little or no effector function.

Parabiosis experiments suggest that naive ILC2s are tissue-resident and do not circulate.²³ Nothing is known yet regarding migration and circulation of memory ILC2s, but we have found an increase in ILC2 numbers in the blood and in the liver a few weeks after activation, which implies that some memory ILC2s circulate and may colonize other tissues. Interestingly, some studies have shown that the liver is a reservoir for memory NK cells.²⁴ Whether there is a memory ILC2 reservoir outside of the lung or lung draining LN remains to be elucidated.

The finding of memory ILC2s in the LN raises the question whether they can be generated in other tissues. In the case of the skin, ILC2s are involved in allergic responses and play an important role in atopic dermatitis (AD), another chronic inflammatory disease. The same alarmins that activate lung ILC2s are shown to activate skin ILC2s, therefore it is likely that some of these effector ILC2s may acquire memory features and respond more intensely during subsequent damage of the skin. Interestingly, the upregulation of the IL-25R has been reported in ILC2s isolated from the skin of patients with AD.²⁵ Moreover, the disease is clinically characterized by exacerbations and remissions of skin inflammation and susceptibility for cutaneous bacterial and mycotic infections. The recurrence, chronicity, and difficulty in the management of the disease suggests that antigen non-specific responses may take place.

ILCs may play a role in systemic autoimmune diseases such as systemic sclerosis, characterized by fibrosis of the skin and other organs and vascular dysfunction.²⁶ In these patients, higher numbers of skin and circulating ILC2s have been found compared to healthy individuals. Moreover, skin ILC2s in these patients seem to upregulate IL-25R expression. Therefore, it may be possible that memory ILC2s are generated and involved in the severity of these diseases.

In the steady state, ILC2s in the small intestine already express IL-25R similar to memory ILC2s in the lung. However, they do not express ST2. Therefore, if memory can be generated in gut-resident ILC2s, the mechanisms may differ.

As mentioned earlier, preliminary results from our laboratory have shown that memory ILC2s accumulate in the liver. Some studies have reported critical roles of IL-33 and liver-resident ILC2s in mouse models of hepatic inflammation and fibrosis and there have been some clinical cases that associate liver diseases such as cirrhosis or steatohepatitis with asthma but the causes are not clear.²⁷ The potential role of memory ILC2s in liver diseases is intriguing.

3 | DEVELOPMENT AND ACTIVATION OF NEONATAL ILC2S

3.1 | Development of ILC2s in the neonatal period

ILC2s are considered to be tissue-resident lymphocytes that develop early in life.²⁴ The studies on the developmental pathways of ILCs have identified progenitors in adult bone marrow (BM). It is thought that all lymphocytes including ILCs develop from common lymphoid progenitors (CLPs) in the BM. Common progenitors for NK cells and helper-like ILCs that express the transcription factor *Tcf7* have been found in adult mouse BM and termed early ILC progenitors (EILPs).²⁸ Downstream of EILPs, common progenitors for helper-like ILCs termed CHILPs that express transcriptional regulator inhibitor of DNA binding 2 (Id2) have also been found in adult mouse BM and fetal liver.^{29,30} CHILPs can differentiate into ILC1s (but not NK cells), ILC2s, and ILC3s (including LTi cells). Downstream of CHILPs, committed ILC precursors (ILCPs) express the transcriptional repressor Promyelocytic Leukemia Zinc Finger (PLZF).³¹ ILCPs can differentiate into ILC1s, ILC2s, and ILC3s but not NK cells and LTi cells. ILC2-committed progenitors that efficiently engraft in the lung of irradiated hosts have also been found in adult mouse BM.³² However, studies with parabiotic mice showed that ILCs in steady state do not circulate and very few, if any, seem to develop from the BM progenitors in adult mice at the steady state.²⁴ Instead, ILCs likely develop early in life, migrate into tissues and persist as tissue-resident lymphocytes in adults. Indeed, lung ILC2s labeled by BrdU in the neonatal period can be detected in adult lungs, indicating that neonatal ILC2s persist in the lung of adult mice.²¹ Therefore, the neonatal period is likely critical for the development of ILC2s.

ILC2s are almost undetectable in the lung of newborn mice but they rapidly increase in numbers in the following 10 days after birth.²¹ Meanwhile, hematopoietic progenitors are found in the spleen, but not BM or liver of newborn mice. It appears that during the transition of hematopoiesis from fetal liver to BM after birth, hematopoiesis transiently takes place in the spleen. A subset of the lymphoid-primed multipotent progenitors (LMPPs) expressing IL-7R α and much smaller numbers of CLPs as well as CHILPs and ILC2Ps are found in the spleen but not in the BM of newborn mice. The numbers of those progenitors in the spleen decline as they increase in the BM while ILC2 numbers in the lung increase in the first 5 days after birth. IL-7R α ⁺ LMPPs are more abundant than CLPs during this period and they are highly efficient in developing into ILCs and T cells in transplantation assays, suggesting that CHILPs might develop from the IL-7R α ⁺ LMPPs in neonatal mice.

3.2 | Stimulation of neonatal lung ILC2s by endogenous IL-33

The number of lung ILC2s gradually increase reaching adult levels around day 7 after birth.³⁴ After a week, the ILC2 population keeps expanding beyond the number of adult ILC2s and reaches a peak around day 10 which lasts up to 2 weeks postnatally. At this point, the population contracts to adult numbers around weaning (day 20). These ILC2s appear to be activated as they have higher amounts of intracellular IL-5 and IL-13 compared to naive adult ILC2s. In accordance with the role of ILC2s in recruiting eosinophils, there is a peak of eosinophil number around 2 weeks after birth coinciding with the increased numbers of ILC2s. This eosinophil recruitment to neonatal lungs is impaired in the ILC2-deficient *Rora*^{sg/sg} mice. Moreover, ST2-deficient mice³³ and IL-33-deficient mice³⁴ have much reduced numbers of ILC2s and eosinophils in the lung during the peak as compared to Wildtype littermates. Whole lung homogenate and immunohistochemistry staining confirmed higher amounts of IL-33 in neonatal lungs than adult lungs.^{33,34} Therefore, neonatal lung ILC2s are likely stimulated by a release of IL-33 in the lung, without exogenous stimuli, resulting in ILC2 proliferation. There are many potential reasons for this release of IL-33. Saluzzo et al³³ proposed that the mechanical stress of the first breath triggers IL-33 induction by alveolar epithelial type II cells. Steer et al³⁴ referred to evidence of oxidative stress in healthy infants after birth as a potential reason. de Kleer et al³⁵ suggested that the alveolization that is occurring at this period results in increased production of IL-33 in the process of lung development.³⁶ However, it must be noted that IL-33-deficient adult mice do not seem to present any defects in lung development.^{33,35} Regardless, IL-33 does not seem to be required for the development of ILC2s as the ILC2 numbers in IL-33KO adult mice are comparable to those in Wildtype mice.³⁴

The amounts of IL-33 in neonatal lungs are much lower than those in the lung of mice treated with protease allergen papain, and neonatal lungs do not show signs of type 2 inflammation evidenced by a lack of mucus production. Indeed, the amount of intracellular IL-5

and IL-13 staining and the actual numbers of ILC2s do not compare to allergen stimulated adult lung ILC2s, indicating that the small amount of endogenous IL-33 released in neonatal lungs does not strongly activate lung ILC2s. Nevertheless, the weak activation of lung ILC2s has significant effects on the development of type 2 responses later in life.

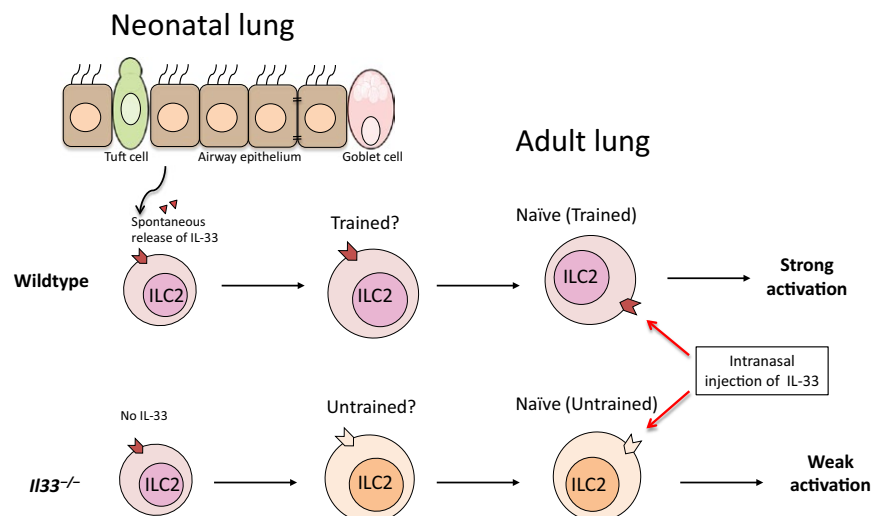
The observation of the skewing of the lung environment toward type 2 immunity is not new, although it was previously thought to be driven by Th2 cells. Naive CD4⁺ T cells are intrinsically biased toward a type 2 phenotype. However, new data below indicate that this bias is also influenced by ILC2s. ILC2-deficient pups show impaired Th2 cell responses compared to Wildtype pups, demonstrating that ILC2s are the drivers of neonatal Th2 cell bias. Moreover, the neonatally sensitized mice showed stronger Th2 responses upon challenges in adulthood compared to adult sensitized mice.^{34,35}

3.3 | Trained immunity of neonatal ILC2s?

Whereas strong activation of ILC2s by allergens or exogenous IL-33 in adult mice leads to the development of memory ILC2s as discussed above, the weak activation of neonatal lung ILC2s by small amounts (~0.10 pg/mg protein³³) of endogenous IL-33 unlikely results in the generation of memory ILC2s (Figure 2). Similar to activated adult mouse lung ILC2s, neonatal lung ILC2s exposed to endogenous IL-33 express the IL-25R. However, unlike adult memory ILC2s, neonatal ILC2s do not maintain the IL-25R expression as mice mature. Nevertheless, the exposure to endogenous IL-33 in the neonatal period may have significant effects on the functions of ILC2s later in life. Preliminary results showed that IL-33KO mice have the same number of lung ILC2s as Wildtype while intranasal administration of IL-33 induced weaker ILC2 activation in IL-33KO adult mice than Wildtype mice. Moreover, IL-33 injections into IL-33 KO pups in the neonatal period increase the responsiveness of lung ILC2s in adults³⁴ (C.S. Steer, L. Mathae, F. Takei, unpublished observation).

As discussed above, neonatal ILC2s persist in the lung of adult mice. Intranasal administration of BrdU in adult mice labeled approximately

FIGURE 2 “Trained” neonatal ILC2s. Neonatal ILC2s are exposed to endogenous low levels of IL-33 in the neonatal period which may have implications on the functions of these cells later in life. The neonatal exposure of ILC2s to IL-33 provides tonic signals that result in functional maturation and fitness of these cells. In IL-33-deficient mice, neonatal ILC2s are not exposed to IL-33. Adult naive ILC2s in these mice respond less intensely to exogenous stimulation with IL-33 than Wildtype “trained” naive ILC2s



20–30% of lung ILC2s,² suggesting that only some ILC2s seem to turn over in the lung while the majority of ILC2s derive from the neonatal period. It remains to be determined whether neonatal ILC2s persisting in adult lungs are more responsive to allergens/IL-33 than those turning over in adult lungs. Nevertheless, these observations suggest that the neonatal IL-33 “experience” may make ILC2s better “trained” to respond to allergens later in life, similar to trained immunity of monocytes/macrophages.³⁶

4 | IMPLICATIONS OF MEMORY ILC2S IN ASTHMA

4.1 | ILC2-mediated airway inflammation in asthma phenotypes

Airway inflammation can be induced by many different triggers, such as viruses, bacteria, fungi, allergens, pollutants, or trauma. In genetically susceptible individuals, most of these triggers can induce asthmatic responses with symptoms that include wheezing, shortness of breath, and chest tightness. More than 300 million people suffer from asthma and its prevalence and morbidity is rising particularly in the developed countries. Currently, there is no cure for this disease and only palliative treatments such as inhaled corticosteroids are available but not always effective. The lack of effective treatment is responsible for the associated high economic and social burden.

Asthma is no longer considered a disease but a chronic and heterogeneous pulmonary syndrome characterized by recurrent episodes of airway obstruction and airway hyperresponsiveness (AHR) to both specific and non-specific stimuli, mucus hyperproduction, and airway remodeling. Asthma is traditionally defined as a type-2-mediated allergic inflammatory disease with a defined role for the Th2 cytokines IL-4, IL-5, IL-9, and IL-13. For instance, IL-5 is responsible for eosinophilia and together with IL-13, induces mucus production and airway remodeling, both of which are hallmarks of allergic asthma. Although other innate cells such as mast cells or eosinophils can also produce IL-5, ILC2s are the initial and major source at this early stage of the inflammation. Th2 cell-derived IL-4 promotes IgE production from B cells in response to a particular allergen. The allergen-specific IgE antibodies then bind to the FcεR1 on basophils and mast cells, which induces degranulation and release of inflammatory mediators that promote AHR. Based on the presence or absence of antigen-specific IgE in the serum, asthma is broadly divided in allergic and non-allergic asthma, respectively.

Extensive amount of data generated from mouse models of allergic inflammation and supported by observational studies in patients undergoing different allergic diseases have shown that lung-resident ILC2s are major intermediaries between the allergen and the allergic responses. Inhalation of some allergens such as the protease papain, chitin or house dust mite extract has been shown to induce allergic responses in the lung of mice that lack adaptive immune cells but not ILC2s such as *Rag1*^{−/−}. However, the same allergens did not induce any inflammation in *Rag2*^{−/−}*Il2rg*^{−/−} mice which also lack ILC2s.

Moreover, allergic inflammation in *Rag2*^{−/−}*Il2rg*^{−/−} mice was restored by engraftment of ILC2s, suggesting a role for ILC2s in allergic lung inflammation in a T cell-independent manner.³⁷ This is supported by studies in asthmatic patients, which show an increased frequency of ILC2s in peripheral blood as well as sputum and bronchoalveolar lavage fluid compared to healthy individuals.^{38,39} As mentioned previously, ILC2s do not directly recognize allergens but are highly sensitive to the alarmins released by both allergen-activated epithelial and myeloid cells. Epithelial cells and some myeloid cells such as DCs can recognize allergens through pattern recognition receptors such as TLR4, proteases receptors, or C-type lectin receptors.⁴⁰ Moreover, the protease activity of many allergens including house dust mite, papain, or cockroach extract, cleaves tight junction proteins on epithelial cells and disrupts their barrier function allowing the allergen to enter the airways, allowing myeloid cells to have more access to them. There are many innate immune cells other than ILC2s that are activated by the epithelial-derived signals, such as basophils, mast cells, eosinophils, DCs, macrophages, or epithelial cells themselves. However, ILC2s are the main effector cells in inducing allergic inflammation at this early stage. In addition to their response to epithelial-derived alarmins, ILC2s interact with some of these cell types. For instance, activated basophils have been shown to become an early and local source of IL-4 that support ILC2 activation by other alarmins.⁴¹ Mast cells produce the lipid mediator LTD₄ that can also activate ILC2s.⁴² Moreover, activated mast cells secrete IL-2 that supports ILC2 survival.³⁷ However, IL-2 also activates Tregs, which have been shown to suppress ILC2 activation by the release of the anti-inflammatory cytokine IL-10.⁴³ Therefore, ILC2 activation is regulated by interactions with other immune cells.

Importantly, ILC2s not only play a role during the initiation of allergic responses but they are also involved in the initiation of the adaptive immunity,⁴⁴ which enhances the allergic inflammation in the lung. In a mouse model of allergic inflammation induced by intranasal administration of papain, ILC2-derived IL-13 promoted the migration of activated antigen-presenting MHCII^{hi} DCs into the lung draining LN where they polarized naive CD4⁺ T cells into Th2 cells in an IL-4-independent manner.¹⁸ Moreover, it has been reported that the expression of MHCII on ILC2s has direct effect on Th2 cell activation in vitro.⁴⁵ In addition to Th2 responses, growing evidence suggests that Th17 immune responses are also important in the pathogenesis of asthma.⁴⁶ IL-17 cytokine levels are elevated in patients with severe asthma and steroid resistance.⁴⁷ Mouse experimental studies have shown that IL-17 is involved in the induction of neutrophilic but not eosinophilic inflammation and AHR.⁴⁸ Neutrophilic asthma is considered non-allergic asthma since IL-4-dependent IgE is not involved. While both innate and adaptive responses are involved in allergic asthma, it seems that innate cells such as ILC2s, basophils, and eosinophils play a more important role in non-allergic asthma, where the triggers may be allergen proteases or other non-allergic stimuli (pollutants, cigarette smoke, virus, and fungus infections). Furthermore, exercise and obesity have been shown to play a role in inducing asthmatic responses and ILC2s and ILC3s are active players in this type of airway inflammation.^{49,50}

4.2 | Memory ILC2s in allergic asthma

Individuals become sensitized to allergens in early childhood or in adulthood. It has been thought that allergen-specific memory Th2 cells generated during the sensitization are the major drivers of allergic inflammation in subsequent allergen exposures. Chronic allergic inflammation of the airway results in asthma development. Asthma patients often suffer from episodes of the exacerbation of symptoms, which can be induced by allergic or non-allergic triggers. The recent findings discussed above suggest that memory ILC2s are also generated during the first allergen sensitization and they might play an important role in asthma. Memory ILC2s in sensitized individuals can be activated by IL-33 and IL-25 released by damaged epithelium, and they produce large amounts of IL-5 and IL-13, likely contributing to asthma exacerbation. When allergen-sensitized individuals encounter the same allergen, not only memory Th2 cells but also memory ILC2s are activated. We have shown that in papain-treated and re-challenged lungs, cytokine-producing cells were equally distributed between memory ILC2s and T cells. Moreover, ILC2-derived IL-13 is important for Th2 cell activation.¹⁸ Memory ILC2s produce more IL-13 than naive ILC2s, and memory ILC2s enhance memory Th2 cell activation.² In conclusion, memory ILC2s seem to contribute to allergic responses by directly producing type 2 cytokines and also enhancing allergen-specific Th2 responses.

Allergen-sensitized individuals sometimes react to unrelated stimuli,⁵¹ and memory ILC2 would be the major responders in such allergen non-specific inflammation. Although memory Th2 cells have also been reported to respond directly to IL-33 as they upregulate ST2,⁵² we have shown that 90% of the cytokine-producing cells were memory ILC2s rather than Th2 cells when fungal protease-sensitized mice were challenged by papain.² Moreover, memory ILC2s produced more IL-5 and IL-13 per cell basis as compared to Th2 cells. In a recent work by Christianson et al⁵³, repeated allergen challenge with house dust mite, aspergillus, and ragweed showed that IL33-ILC2 feedback but not T cells were important for the maintenance of AHR and lung inflammation. These results support the role of memory ILC2s in allergen non-specific exacerbation of airway inflammation.

Airway infections can also exacerbate asthmatic responses in adulthood. Fungi infections are associated with late-onset allergic asthma characterized by eosinophilic/neutrophilic-mixed airway inflammation.⁵⁴ ILC2s respond to the IL-33 as well as CysLT released upon fungi infection by producing IL-5 and IL-13. We and others have shown ILC2-mediated lung inflammation upon intranasal administration of the fungus *Aspergillus* or *Alternaria alternata*.^{2,55} The Locksley laboratory has shown that ILC2s are key during allergic responses to chitin, a key component of fungi that induces the release of alarmins by the epithelium.⁵⁶ Moreover, fungal infection can also exacerbate the allergic response in asthmatic patients and it has been associated to a more severe and steroid-resistant asthma. ILC2s have been shown to be sensitive to steroids.⁵⁷ However, higher numbers of ILC2s have been found in these patients compared to steroid-sensitive asthmatic patients.³⁹ It is not known whether memory ILC2s are steroid-resistant and responsible for these more severe asthmatic phenotypes.

Virus infection can both promote the development of airway inflammation and exacerbation in asthmatic patients. Respiratory syncytial virus (RSV) infection is common in children under 2 years old and is associated with asthma development later in life.⁵⁸ RSV infection was shown to induce IL-13-producing ILC2s in neonatal but not adult mice through epithelial-derived IL-25 production, resulting in mucus production and airway responsiveness.⁵⁹ This could be mediated by IL-25-responding neonatal ILC2s, since neonatal ILC2s, in contrast to naive adult ILC2s, express high levels of IL-25R. In this sense, neonatal and memory ILC2s share the ability to respond to IL-25. Human rhinovirus and influenza are more associated with asthma exacerbations in adults. In healthy individuals, ILC2s respond to virus infections, which trigger the release of alarmins.⁶⁰ Activated ILC2s produce the epidermal growth factor amphiregulin that contributes to the regeneration of the tissue.⁶¹ However, these activated ILC2s will also produce IL-5 and IL-13, thus triggering allergic reactions. Therefore, it is likely that ILC2s and particularly memory ILC2s may be responsible for the virus-induced exacerbation of the asthmatic responses. Rhinovirus-induced exacerbation of asthma was shown to be dependent on IL-25.⁶² The fact that memory ILC2s are particularly highly responsive to this alarmin suggests a potential role for memory ILC2s in virus-induced exacerbations in asthma.

There is a growing concern about the failure of asthma vaccines and the large number of patients that are underdiagnosed.⁶³ Allergen non-specific reactions mediated by memory ILC2s in these patients may be responsible. Moreover, memory ILC2s may be the underlying cause of most of the exacerbations occurring in asthmatic patients. Therefore, memory ILC2s appear as a new target for the development of new treatments and diagnosis techniques.

4.3 | Memory ILC2s in non-allergic asthma

The role of ILC2s in non-allergic asthma, which includes neutrophilic asthma and obesity-related asthma is not as well studied as allergic asthma. As mentioned earlier, ILC3s has been shown to be involved in obesity-related AHR since these patients present elevated numbers of ILC3s in the lung. The same is observed in high-fat diet-induced AHR mouse models. This AHR has been shown to be dependent of IL-17 but independent of adaptive Th17 cells suggesting a role for IL-17-producing ILC3s. Activated macrophages in obese mice produce IL-1 β , which activates ILC3s to secrete IL-17.⁴⁹ Recently, it has been shown that ILC2s are also increased in numbers in house dust mite-challenged obese mice and IL-1 β can also stimulate ILC2s to produce Th2 cytokines in this context.⁵⁰ Although it is not clear if ILC2s can differentiate into ILC3s yet, some reports suggest that indeed in some contexts, ILC2s can produce both IL-13 and IL-17 at the same time.^{12,64} On the other hand, ILC2s may be involved in inducing obesity since IL-33-activated ILC2s regulate metabolic homeostasis and induce beiging of the white adipose tissue. Therefore, there might be a role for ILC2s and consequently for memory ILC2s in this type of asthma.

Neutrophilic asthma is commonly present in steroid-resistant severe asthmatic patients, occupational asthma and in some cases in

mixed phenotypes where Th2 and Th17 cytokines play a role. Among ILCs, IL-17-producing ILC3s are important in this subtype of asthma, however, ILC3s are a very rare population in the mouse lung, while ILC2s are abundant. If ILC2 transdifferentiation into ILC3s is confirmed, it will raise the question of where the increased numbers of ILC3s in the neutrophilic asthma models are coming from. A combination of ILC3 recruitment from other tissues, local proliferation of ILC3s and ILC2 plasticity into ILC3s may be occurring at the same time. A subset or a state of ILC2s that produce IL-5 and IL-13 at the same time as IL-17 can contribute to the severe asthma phenotype characterized with a mixture of eosinophil/neutrophilic inflammation. Further investigation will elucidate whether memory ILC2s can be plastic and become ILC3s, which would be more potent effector cells.

Exposure to air pollutants such as cigarette smoke or ozone and some viral respiratory infections trigger a Th1 response in the lung by inducing the release of IL-1 β and IL-12 from the airway epithelium.⁶⁵ It has been recently shown that ILC2s are plastic and under IL-1 β and IL-12 stimulation can differentiate into ILC1s and therefore contribute to the response to viruses.^{66–68} Interestingly, IL-1 β primes ILC2s to become more responsive to IL-33, which is also produced by the damaged epithelium upon pollutants or viruses encounters. For instance, ozone exposure, a common oxidant air pollutant in the photochemical smog, can induce ILC2-dependent airway inflammation mediated by IL-33. Ozone can increase AHR in obese mice compared with lean mice and this was also mediated by IL-13-producing ILC2s.⁶⁹ Interestingly, these irritants can adversely affect people already suffering from asthma, who presumably have memory ILC2s in their lungs asthmatic. Therefore, although plasticity has not been addressed in memory ILC2s, it is likely that memory ILC2s contribute to these type 1-induced exacerbations.

There are other airway diseases apart from asthma where ILC2s are shown to be involved such as COPD or lung fibrosis^{67,70} and it remains to be determined whether memory ILC2s can worsen these diseases. ILC2s have been reported to induce lung inflammation and fibrosis in a bleomycin-induced mouse model.⁷¹ ILC2-derived IL-13 contributed to fibrotic processes by promoting collagen release from macrophages and fibroblasts. Other models involve IL-25 in fibrotic processes which points out a possible role for memory ILC2s. Accordingly, BALF from patients with idiopathic pulmonary fibrosis contained more ILC2s than their respective controls.⁷⁰

5 | CONCLUDING REMARKS AND FUTURE DIRECTIONS

Antigen non-specific memory ILC2s have been characterized in mouse lungs. Memory ILC2s are more responsive to allergen/cytokine challenges and produce more cytokines than naive ILC2s. The molecular mechanisms by which memory ILC2s more vigorously respond than naive ILC2s upon allergen/cytokine challenges are only partially understood. Although IL-25 and IL-25R play an important role in memory ILC2s, it is highly likely that other cytokines and/or

cells are also involved, because IL-25 alone fails to activate purified memory ILC2s *in vitro*. Epigenetic modifications such as demethylation in the conserved non-coding sequence 1 of the *IFNG* locus have been suggested to drive HCMV-dependent memory NK generation.⁷² Trained immunity of macrophages as well as memory of epithelial stem cells can also be explained by epigenetic modifications.⁷³ Whether memory ILC2 phenotype involves epigenetics modifications needs to be addressed. It is worth noting that mRNA levels of IL-5 and IL-13 do not change between naive and memory ILC2s, suggesting that post-transcriptional rather than epigenetic changes occur in these genes. It is still plausible that epigenetic changes in other genes such as the gene encoding IL-25R underlie the memory ILC2 phenotype.

We have shown a pathological role for memory ILC2s, however memory ILC2s may also play a beneficial role by improving tissue repair in the airway after viral infection. Whether memory ILC2s produce higher levels of amphiregulin than naive ILC2s contributing to a more efficient healing of the previously damaged epithelium is not known.

It is likely that memory ILC2s play an important role in allergic lung diseases including asthma, and they are a potential therapeutic target. However, there remains a number of questions to be answered in order to gain a better understanding of memory ILC2s. While ILC2s are broadly distributed in mucosal tissues, memory ILC2s have only been characterized in mouse lungs and lung-draining lymph node thus far. Whether memory cells can be generated from ILC2s in other tissues and whether activation of ILC1s and ILC3s also generates memory cells still remain to be answered. It is possible that ILC2s in the lung are unique as they are exposed to endogenous IL-33 in the neonatal period. Whereas neonatal lung ILC2s that experience endogenous IL-33 persist in adult lungs as tissue-resident cells, the relationship between neonatal ILC2s exposed to IL-33 and memory ILC2s generated by intranasal injection of IL-33 into adult mice is still to be elucidated.

In humans, allergic airway inflammation can be divided into early and late-onset based on whether the symptoms start before or after 12 years of age. ILC2s may play an important role in early life asthma development as we discussed earlier in this review but also have been involved in the late-onset airway inflammation that usually is linked to nasal polyposis. Indeed, ILC2s are increased in the nasal polyps of these patients.⁷⁴ Accumulating data show that not only asthmatic patients but also patients with other allergic diseases such as chronic rhinosinusitis have elevated numbers of ILC2s in the blood, BALF and sputum compared to healthy adults.⁶⁶ These findings agree with our experimental data that show that numbers of memory ILC2s remain high for a long time in allergen-treated mice. It will be important to determine whether memory ILC2s are present in allergic patients. Phenotypic characterization of human memory ILC2s is complicated, because the ILC2 population in healthy humans likely comprises of both naive and memory ILC2s as humans are exposed to the environment through their whole life. Nevertheless, future efforts should be focused on identifying and characterizing memory ILC2s in human patients of allergic diseases.

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CONFLICT OF INTEREST

All other authors have no conflict of interest.

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