

New insights into ILC2 memory

Itziar Martinez-Gonzalez¹  | Fumio Takei² 

¹Molecular, Tumor and Cell biology, Karolinska Institute, Stockholm, Sweden

²Terry Fox Laboratory, British Columbia Cancer Research Institute, Vancouver, British Columbia, Canada

Correspondence

Itziar Martinez-Gonzalez, C7, Biomedicum, Solnavägen 9 171 65 Solna, Sweden.
Email: itziar.martinez.gonzalez@ki.se

Fumio Takei, 601 West 10th Avenue, Vancouver V5Z 1L3, British Columbia, Canada.
Email: ftakei@bccrc.ca

Funding information

Karolinska Institutet; Knut och Alice Wallenbergs Stiftelse, Grant/Award Number: KAW 4-239/2020; Canadian Institute of Health Research, Grant/Award Number: 153304 and 173434; Vetenskapsrådet, Grant/Award Number: 2018-02377

Summary

Group 2 Innate Lymphoid Cells (ILC2s) are innate lymphocytes involved in type 2 immunity. ILC2s are abundant at the barrier tissues and upon allergen exposure, respond to epithelial-derived alarmins by producing type 2 cytokines (e.g., IL-5 and IL-13). Upon activation, some of these activated ILC2s acquire immunological memory and can mount enhanced responses upon further allergen encounters. Here, we review recent findings of the cellular and molecular mechanisms underlying immune memory in ILC2s both in mice and humans and discuss the implications of memory ILC2s in the context of allergic diseases.

KEYWORDS

allergy, ILC2s, immune memory, type 2 inflammation

1 | GROUP 2 INNATE LYMPHOID CELL (ILC2) DEFINITION AND FUNCTION

ILC2s are abundant at the barrier tissues and are the first sensors of changes in the microenvironment of the tissue they reside in. In contrast to their T cell counterparts, ILC2s do not express rearranged receptors and therefore lack antigen recognition. Instead, ILC2s express the receptors for alarmins IL-33, thymic stromal lymphopoietin (TSLP) and IL-25, the receptors for the neuropeptides Neuromedin U (NMU) and vasoactive intestinal polypeptide (VIP), the receptors for the lipid mediators leukotriene D 4 (LTD4) and prostaglandin D 2 (PGD2) and the common gamma chain (γ c) family of cytokine receptors including IL-2R and IL-7R. In response to all these factors, ILC2s proliferate and produce type 2 cytokines, including IL-5 and IL-13 and growth factors such as granulocyte macrophage colony stimulating factor (GM-CSF) and

amphiregulin (AREG).^{1,2} ILC2s regulate tissue homeostasis. For instance, they mediate adipose tissue browning which may prevent the development of metabolic abnormalities.³ Moreover, they are key players during parasite infections as ILC2-derived IL-13 is necessary for helminth expulsion and subsequent tissue repair.⁴ ILC2s also repair the damaged lung epithelium upon virus infection through the release of IL-13 and AREG.⁵⁻⁷

On the contrary, ILC2s can be pathogenic and induce an allergic immune response upon allergen encounter in several tissues including the skin, lung, and intestine. ILC2-derived IL-5 and IL-13 are important mediators of the pathogenesis of several allergic diseases including asthma, atopic dermatitis, and food allergies.⁸⁻¹⁴ ILC2s have been considered the initiators of these allergic responses as they are the first sensors of the tissue-derived alarmins. In a second step, the adaptive immune responses, namely T helper type 2 cells (Th2) cells and IgE, take over the later phases of the allergic responses.¹

2 | DISCOVERY OF MEMORY ILC2s

Lung ILC2s proliferate upon exposure to allergens. The number of ILC2s remain elevated in the lung long after allergen inhalation. This finding led us to the hypothesis that perhaps ILC2s could have a role in subsequent allergen encounters. A single intranasal administration of the alarmin IL-33 a month after the initial exposure to the protease allergen papain, induced a strong expansion and activation of lung ILC2s and eosinophilia in the lung, while little response was observed in naïve mice exposed to a single IL-33 challenge, suggesting that allergen experienced ILC2s are more sensitive to stimuli than naïve ILC2s. This acquired ability to enhance their response to stimuli suggested immunological memory in ILC2s.¹⁵ We demonstrated that the acquisition of immunological memory was a cell intrinsic feature of ILC2s, as transfer of memory ILC2s into naïve mice induced an enhanced response to allergen challenge compared to naïve ILC2 transfer. Moreover, purified memory ILC2s exposed to suboptimal amounts of IL-33+TSLP in vitro resulted in more production of IL-5 and IL-13 than naïve ILC2s. However, we could not exclude a contribution from the environment. One possibility could be that the first exposure to an allergen induces changes in the lung epithelium to constantly release IL-33 and that during the secondary challenge, ILC2s had higher accessibility to IL-33, explaining their enhanced response. However, IL-33 KO mice treated with IL-33 and challenged 1 month later with IL-33 displayed an enhanced ILC2 activation and lung inflammation. Therefore, the maintenance of memory ILC2s could be independent of epithelial-derived IL-33. Whether other changes in the lung environment happening after the first exposure to allergens have a role in the activation of memory ILC2s during the secondary challenge needs to be further explored. One approach may be to administer an allergen into an ILC2 KO mice, which will induce changes in the lung and then challenging 1 month later after transferring memory or naïve ILC2s. An enhanced response in the mice transferred with memory ILC2s would suggest that the lung environment is not as important in this process.

It has been long thought that only adaptive lymphocytes can acquire immunological memory as antigen specificity is thought to be a key characteristic of immunological memory. However, the definition of immunological memory can be revised as the capacity of a cell to remember a previous activation state regardless of the way of activation. This was exactly what we observed in ILC2s.¹⁵⁻¹⁷

Currently, the field has generally accepted that immune memory can also be a characteristic of an innate immune cell. NK cells and other types of ILCs (ILC1s and ILC3s) have been shown to mount memory responses.¹⁸⁻²¹ Indeed, NK cell memory has been extensively investigated and the mechanisms behind it have been elucidated.^{22,23} Moreover, myeloid cells such as macrophages show a similar type of immune memory referred to as "trained immunity".²⁴⁻²⁶

3 | MOLECULAR MECHANISMS OF MEMORY ILC2s

Mouse lung ILC2s express ST2 (IL-33R) and very little IL17RB (IL-25R). Three consecutive intranasal administration of IL-33, but not IL-25, into naïve mice strongly activates lung ILC2s. The majority of lung ILC2s stimulated by intranasal IL-33 or allergens express the proliferation marker Ki67, proliferate and become cytokine-producing effector ILC2s. Effector ILC2s quickly stop proliferating and stop producing cytokine within 2 weeks after the activation, and ILC2 numbers continuously decline over the following 4 weeks while memory ILC2s persist for more than 6 months.¹⁵ Our microarray transcriptome analyses of naïve, effector (4 days after IL-33 treatment) and memory ILC2s (4 months after IL-33 treatment) showed upregulation of a large number of transcripts, mostly those associated with cell activation and proliferation, in effector ILC2s. Most of those activation-associated genes were downregulated to the level of naïve ILC2s in memory ILC2s. However, a small number of those transcripts remained higher in memory ILC2s than naïve ILC2s. Among them is *Il17rb*. Memory, but not naïve, ILC2s in the lung express IL17RB (IL-25R) and strongly react to a single intranasal challenge with IL-25.¹⁵ Preliminary unpublished results from our group show that memory responses of ILC2s are diminished in the IL-25R KO suggesting that this alarmin is important for memory ILC2 responses. In vitro stimulation of purified memory ILC2s with IL-25 does not result in IL-5 and IL-13 production, indicating that activation of memory ILC2s require other co-stimulatory signals. Our unpublished results demonstrate that memory ILC2s need cell contact with dendritic cells and T cell-derived IL-2 in addition to IL-25 to mount full memory responses. These data suggest that memory ILC2s may relocate to specific tissue niches that contribute to their enhanced responsiveness.

Naïve ILC2s express IL-5 and IL-13 mRNA but secrete very little cytokines. Upon activation, ILC2s upregulate those mRNAs and produce high levels of these cytokines. Memory ILC2s, which are in "rest", maintain elevated IL-5 and IL-13 mRNA, but only secrete cytokines when they are activated. This suggests translational regulation of cytokine production in memory ILC2s.

Further analysis of our microarray data of naïve and memory lung ILC2s, revealed that transcriptional regulation mediated by RUNX family of transcription factors may be involved in memory generation (Figure 1A). It has been shown that Runx1 influences effector and memory T CD8+ cell formation.²⁷ Miyamoto et al. showed that during allergic inflammation, the absence of Runx1 impairs the ability of ILC2s to proliferate and produce effector Th2 cytokines and chemokines. Instead, functional deletion of Runx1 induces the expression of exhaustion markers, such as IL-10 and TIGIT, on ILC2s. Thus, suggesting that Runx1 protects ILC2s from exhaustion during allergic inflammation.²⁸ Other pathways that seem important for memory ILC2 formation is the TCF/LEF/ β -catenin pathway and adherent junction (Figure 1B,C). These pathways regulate cell-cell contacts. As mentioned before, memory ILC2s interact with DCs to fully respond to IL-25.

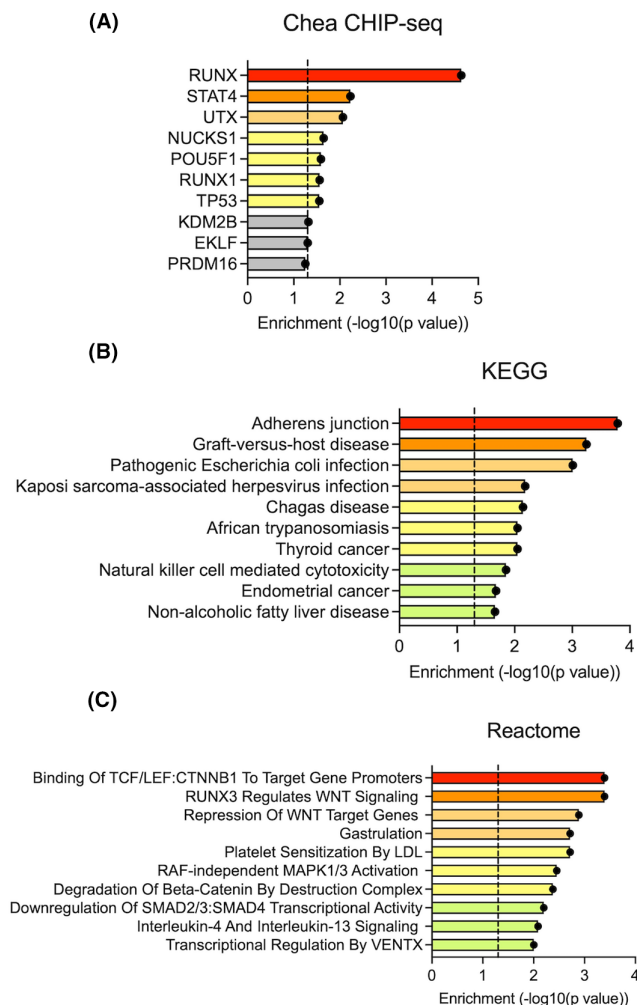


FIGURE 1 Functional enrichment in memory ILC2s. Gene enrichment analysis on differentially expressed genes between memory and naïve ILC2s using Enrichr. (A) Enrichment of RUNX regulated genes in memory ILC2s. (B, C) Pathway enrichment analysis, querying KEGG (B) and Reactome (C) databases, show overrepresentation of cell–cell contact and B-catenin related genes in memory ILC2s compared to naïve ILC2s. Bar graphs represented the significance of the enrichment (p value).

Verma et al.²⁹ have recently reported ILC2 memory in RAG-KO mice. In a chronic asthma model of *Alternaria*, memory ILC2s were responsible for asthma-like symptoms including airway hyperresponsiveness. They further characterized the epigenome landscape and found that Activator Protein 1 (AP-1), a major transcriptional activator, was one of the top DNA-binding motifs of memory ILC2s. They suggest that memory ILC2s express both suppressors' genes such as the transcription factor JunD and "preparedness" genes such as Four-and-a-half LIM domain protein 2 (Flh2) and that after challenge become active and downregulate the repressor genes, allowing ILC2s to strongly respond. Transcriptomic and epigenomic analyses of memory ILC2s have been done with bulk ILC2s and future studies should analyze memory ILC2s at the single cell level. In a follow-up paper, the same authors showed that NFκB1 inhibits

memory formation but supports the effector function of ILC2s in memory-driven asthma.³⁰

4 | ACTIVATION-INDUCED MIGRATION, MEMORY, AND EXHAUSTION OF LUNG ILC2

Upon development in the fetal liver and later in the adult bone marrow, ILC2s seed all the tissues of the organism and remain tissue resident with some self-renewal capacity.^{31–33} The ILC2 turnover rates in adult mice vary between tissues, lung ILC2s having the lowest (30%) and the skin ILC2s the highest rate (80%).³⁴

Two weeks after birth, mouse lung ILC2 numbers increase to a level higher than those in naïve adult mice. It is due to an intrinsic wave of IL-33 production in the lung, which seems to drive functional maturation of lung ILC2s.³⁵ In the skin, ILC2s seem to be less responsive than in other tissues. Topical application of calcipotriol induces atopic dermatitis-like symptoms in the mouse skin, and TSLP is an important mediator in this process.³⁶ Skin ILC2s proliferate as they increase Ki67 expression (unpublished data), but they produce very little IL-5 and IL-13. Even purified skin ILC2s need longer time to respond to stimuli in vitro compared to lung ILC2s. This also happens for ILC2s isolated from the bone marrow. It is possible that skin ILC2s might be dysfunctional due to constant exposure to environmental factors. Alternatively, skin ILC2s might be similar to bone marrow ILC2s and functionally immature. Interestingly, a secondary calcipotriol treatment induces ILC2 activation both in vivo and in vitro, and these ILC2s produce IL-5 and IL-13 (not shown). This observation led us to hypothesize that the high turning over rate of skin ILC2s accounted for their immature state and explains why it takes more than one stimulus to induce functional maturation of ILC2s in the skin.

Tissue ILC2s do not circulate or repopulate empty niches at steady state, however, upon activation, a minor subset of ILC2s enter circulation and may seed other tissues.^{37–39} ILC2 repopulation of empty niches after irradiation was also shown by parabiosis mouse analysis.⁴⁰ We have previously shown that intranasal administration of IL-33 or papain into mice increases ILC2 numbers in blood at day 7 with a subsequent accumulation in the liver.⁴¹ Parabiosis mouse analyses showed that the accumulation of ILC2s in the liver was due to migration of activated ILC2s from the treated lung. The liver is also a reservoir for memory NK cells.⁴² However, we have recently found that these ILC2s accumulated in the liver upon allergen inhalation do not live long and do not respond to i.v. injection of IL-33 more than naïve liver ILC2s, indicating that they are not memory ILC2s (unpublished). Memory generation and migration are not the only fates of activated ILC2s. Based on the rapid expansion and rapid contraction of ILC2 numbers upon allergen inhalation, it is likely that most of the expanded ILC2s undergo cell death, although it has not been well studied. It also seems that some of these activated ILC2s become exhausted.²⁸ Exhausted ILC2s express killer cell lectin-like receptor G1 (KLRG1),

programmed cell death protein 1 (PD-1), and glucocorticoid-induced TNFR-related (GITR) and are unresponsive to subsequent stimuli. However, it should be noted that most effector ILC2s express KLRG1 and PD-1, therefore, they are not specific markers for exhausted ILC2s. In our recent studies, we gave multiple intranasal allergen administrations into mice over 5 weeks and found that lung ILC2s become less responsive to intranasal IL-33 than those stimulated only once, suggesting that ILC2s are exhausted by chronic allergen treatments. Flow cytometry analysis of those dysfunctional ILC2s showed that they are heterogeneous, mostly expressing PD-1, some expressing T Cell Immunoreceptor with Ig and ITIM Domains (TIGIT) and a minor double negative population. When purified and stimulated in vitro with IL-33 plus IL-7, both PD-1⁺ and PD-1⁻ ILC2s were vigorously activated and produced cytokines, whereas TIGIT⁺ ILC2s were not activated, suggesting that they are dysfunctional (unpublished). The relationship between effector, memory, migratory, and exhausted ILC2s remain to be elucidated. Whether memory ILC2s develop from effector cells, or they develop separately remains to be determined (Figure 2). Identifying the molecular mechanisms dictating the fate of an ILC2 after being activated will enable future interventions towards modifying the fate of ILC2s when necessary.

5 | MEMORY ILC2s IN THE LUNG-DRAINING LYMPH NODE

ILC2s reside also in lymphoid organs in very low numbers. Upon intranasal administration of IL-33, ILC2s in the lung-draining mediastinal lymph node (mLN) greatly increase in numbers, and mLN ILC2s incorporate more BrdU than lung ILC2s. Purified ILC2s from the mLN of IL-33-treated mice show an enhanced proliferation rate in vitro compared to lung derived ILC2s (unpublished data). It seems likely that some activated lung ILC2s migrate to the draining mLNs through the lymphatics and proliferate within the mLN. Increased numbers of ILC2s in other tissues draining LNs such as the skin and the gut upon inflammation have also been reported.^{43,44} Role of ILC2s in the LNs is still unknown. ILC2s in mLN can also become memory cells and they differ from lung memory ILC2s both at the transcriptomic and protein levels.¹⁵ In contrast to memory ILC2s in the lung, mLN memory ILC2s maintain high levels of intracellular staining of IL-5 and IL-13 for a long time (more than 6 months). However, it is unlikely that those ILC2s in the mLN secrete IL-5 and IL-13 at this stage as eosinophils are no longer present in the mLN. Purified memory ILC2s from the mLN stimulated with IL-33 plus TSLP produced more IL-5 and IL-13 than memory ILC2s from the lung (unpublished). These findings suggest that memory ILC2s in the lung and mLN are different, reminiscent of central and tissue resident effector memory T cells.⁴⁵ The properties of memory T cells differ depending on the tissue they are in, and it seems that more differentiated memory T cells with less capacity to self-renewal circulate in contrast to the ones located in the LNs which have more stemness and higher potential to mount a strong recall response.⁴⁶⁻⁴⁸

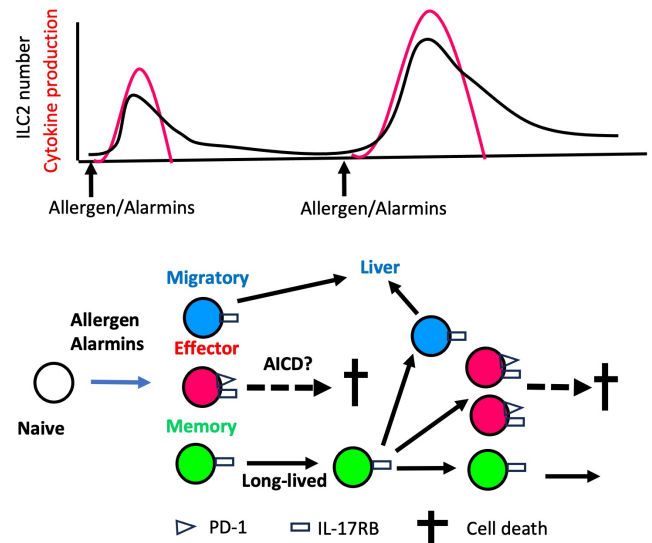


FIGURE 2 Hypothetical scheme of generation of memory ILC2s and relationship between various subsets of activated ILC2s. Lung ILC2s in naïve mice do not express PD-1 and IL-17RB (IL-25R) and remain in the lung as tissue resident lymphocytes. Intranasal administrations of allergens or the alarmin IL-33, but not IL-25, into naïve mice activate lung ILC2s. Most, but not all activated lung ILC2s express PD-1 and IL-17RB, proliferate and become cytokine producing effector ILC2s. A small subset of activated lung ILC2s becomes migratory and exits the lung, circulate and mostly settle in the liver, but they are short-lived in the liver. Effector ILC2s stop producing cytokines within days and slowly decline in numbers, presumably due to activation-induced cell death (AICD). Another small subset of activated PD-1^{lo/-} IL-17RB⁺ ILC2s persist in the lung for months. Weeks after the initial allergen/IL-33 treatment, most lung ILC2s are PD-1^{-/lo} and some IL-17RB⁺ memory ILC2s. Those memory ILC2s are vigorously activated by intranasal administration of allergen, a single dose of IL-33 or IL-25 and become effector and migratory ILC2s while some remain memory ILC2s.

6 | HUMAN MEMORY ILC2s

To study memory in human ILC2s is more complicated as humans are exposed to environmental triggers since birth and therefore it seems unlikely that all ILC2s in healthy individuals are naïve. During inflammation, ILC2 numbers increase in both circulation and tissues from patients with type 2 inflammatory conditions such as asthma, chronic rhinosinusitis with nasal polyps (CRSwNP),⁴⁹⁻⁵¹ and atopic dermatitis.^{12,52,53} These ILC2s have changed their transcriptome and epigenome and have a cell activation signature. In vitro activation of human ILC2s isolated from peripheral blood (PB) evoked substantial H3K4Me2+ epigenome expansion around genes involved in lipid metabolism, cytokine/chemokine signaling, and asthma.⁵⁴ Recently, the human counterparts for the mouse inflammatory ILC2s⁵⁵ have been defined as Lin⁻ CD127⁺ CD45RO⁺ ILC2s in both PB and nasal polyps from CRSwNP patients.⁵⁶ CD45RO⁺ ILC2s can be generated from naïve CD45RA⁺ ILC2s in the presence of IL-33 and TSLP. The CD45RA-CD45RO conversion is tightly linked to Signal transducer and activator of transcription 5 (STAT5) activation and upregulation of the interferon regulatory factor 4 (IRF4)/basic leucine zipper

transcription factor (BATF) transcription factors. Moreover, inflammatory CD45RO⁺ ILC2s are steroid resistant compared to naive CD45RA⁺ ILC2s. In a follow-up study, Golebski et al.⁵⁷ showed that the abundance of CD45RO⁺ ILC2s in PB of patients with CRSwNP is associated with a rapid response to dupilumab, which inhibits ILC2 activation by blocking IL-4/IL-13 alpha receptor. Dupilumab has been shown to be a safe and effective treatment in severe and uncontrolled CRSwNP patients.⁵⁸

ILC2s from PB from healthy individuals are mostly CD45RA⁺ and considered to be naive. We have now described CD127⁺ CD45RO⁺ memory ILC2s.⁵⁹ We purified CD45RA⁺ ILC2s from healthy tissues (PB, skin) and cultured them in two ways, (1) in the presence of IL-2 + IL-7 or (2) IL-2 + IL-7 + IL-33 + TSLP for 5 days. The former maintained CD45RA⁺ ILC2s without activation whereas in the latter, CD45RA⁺ ILC2s were activated and became CD45RA⁻ CD45RO⁺ and maintained CD45RO expression long after they became resting. We then added IL-33 for 24 h into both cultures. The CD45RO⁺ ILC2s produced more IL-5 and IL-13 than the CD45RA⁺ ILC2s.

CD127 (IL7R) is the canonical marker for human ILCs used for identification and isolation of ILC2s from several human tissues. We have observed that activated ILC2s reduced the expression of CD127. We found CD127⁺ CD45RO⁺ ILC2s in healthy tissues including the skin and upon ex vivo stimulation they showed a quicker and more robust response than CD127⁺ CD45RA⁺ ILC2s, characteristics of immunological memory. Memory ILC2s are likely present in healthy skin as is a tissue constantly exposed to the environment. Memory ILC2s expressed many activation-associated genes although they are in a resting state. They were marked by the expression of Nuclear Receptor subfamily 4 group A member 1 (Nr4a1) and genes from the AP-1 pathway such as FosB and JunD. A very recent publication identifies a distinct activation subset of ILC2s in the mouse inflammatory lung that expressed Nr4a1 and shared a gene signature with memory T cells.⁶⁰ Verma et al. transferred FLH2⁺ ILC2s from PB of asthma patients into immunodeficient mice and found that they induced airway hyperreactivity upon alternaria administration, in contrast, FLH2⁻ ILC2s had no effect. Whether memory ILC2s in humans can be identified by the expression of FLH2 needs to be further validated. ILC2s from adult PB express cMaf whereas cord blood ILC2s are cMaf negative.^{61,62} In mice, naive ILC2s are cMaf⁻ and it is induced upon activation and remains highly expressed in memory ILC2s. The deletion of cMaf in memory ILC2s inhibits their enhanced response, suggesting that this transcription factor may be important in memory ILC2s.

In the skin, the majority of CD45RO⁺ ILC2s do not express CD127 and CD45RA⁺ ILC2s are CD127⁺. CD127⁺ CD45RO⁺ skin ILC2s cultured with IL-2 acquired CD127 expression while maintaining CD45RO expression. Nasal polyps of CRSwNP patients contained CD127⁺ CD45RO⁺ inflammatory ILC2s and CD127⁺ CD45RO⁺ ILC2s in addition to CD127⁺ CD45RA⁺ naive ILC2s. There was an inverse correlation between CD45RO and CD127 expression. Moreover, upon in vitro stimulation, CD127⁺ CD45RO⁺ ILC2s produced more cytokines than CD127⁺ CD45RO⁺ ILC2s. These findings indicate dynamic changes in CD127 expression that

may reflect a continuum of activation and memory states of ILC2s. This phenomenon has been recently shown for memory T cells.⁶³ CXC chemokine receptor 3 (CXCR3) expression in CD8⁺ T cells correlates with the differentiation degree after encountering an antigen. The higher the expression CXCR3 is, the more differentiated and less responsive T cells are. CXCR3 low T cells show enhanced capacity to respond to stimuli, suggesting that they are memory cells.

It has been suggested that upon allergen challenge in humans, ILC2s from the circulation are recruited into the airways and fill the alveolar space.⁶⁴ These ILC2s in the bronchoalveolar lavage (BAL) had a gene activation profile linked to lower expression of *IL7R*, suggesting they could be memory ILC2s, which were found elevated in the BAL after the allergen challenge.

7 | IMPLICATIONS OF MEMORY ILC2s IN DISEASES

We have previously shown that priming mice airways with the protease allergen papain and challenging with the fungus *Aspergillus* induced a stronger memory ILC2 activation and allergic lung inflammation than in the mice receiving *Aspergillus* challenge without papain priming.¹⁵ Asthmatic patients usually react to many different allergens. Diagnosis is done using skin prick tests, where skin is exposed to suspected allergy-causing substances and is then observed for signs of an allergic reaction. There are many false negatives, which are explained by the test's lack of sensitivity. However, these tests are based on antigen-specific reactions as there will be allergic reactions only if the individual presents with the specific IgE. It may be possible that antigen nonspecific memory ILC2s are responsible for the IgE-independent allergy.

ILC2s also play a role in allergic reactions in other tissues, such as the skin and the intestine. Skin biopsies from patients with atopic dermatitis are enriched with ILC2s and mouse models of atopic dermatitis demonstrate a role for ILC2s in the pathogenesis. In food allergy, the role of ILC2s is less clear but it has been suggested that ILC2-derived IL-4 suppresses regulatory T cells and activates mast cells in the gut facilitating allergic reactions in the intestine in a mouse model of food allergy.⁶⁵

The allergy journey starts in childhood with the appearance of atopic dermatitis followed by food allergy and continues with respiratory allergies such as rhinitis and asthma. This clinical observation is referred to as "atopic march" and the immune mechanisms behind it are not known.⁶⁶ As the allergens that cause the reactions at the different tissues are usually not the same, it is hard to place T cells as the main responsible factor. On the contrary, previously activated ILC2s which enter the circulation may seed other tissues or be recruited by them when they encounter an allergen. Elucidating the role for memory ILC2s in the atopic march process will unveil novel targets for an early intervention to stop more serious allergic diseases such as asthma from developing later in life. Emerging evidence shows that memory T cells can also mount antigen non-specific responses.^{67,68} Upon differentiation, Th2 cells express ST2

(IL-33R) and therefore become responsive to this alarmin. However, in our mouse models of allergic lung inflammation involving unrelated allergens, the contribution of T cells to the overall IL-5 and IL-13 production in the lung was minimal and the majority of IL-5 and IL-13 positive cells were ILC2s. Memory ILC2s produced more cytokines per cell than Th2 cells. We have previously shown that memory ILC2s enhanced Th2 responses,¹⁵ suggesting that memory ILC2s are not only important for the allergen nonspecific inflammation but they also potentiate Th2 cell responses.

Whether memory ILC2 numbers reflect disease conditions in allergic patients is not known. It is also unknown whether memory ILC2s are steroid resistant and sensitive to dupilumab like inflammatory ILC2s. Correlation between the abundance of memory ILC2s and response to treatment needs to be determined. It is possible that memory ILC2s could be used as a biomarker to guide a more personalized therapeutic approach.

8 | CONCLUDING REMARKS

Growing evidence indicates that the five classical subtypes of ILCs (NK cells, ILC1s, ILC2s, ILC3s, and LTi) are not as well-defined as we originally thought. Plasticity is a common phenomenon in ILCs and almost every ILC can transition into other ILC subtype in the appropriate tissue microenvironmental conditions. Furthermore, upon activation, ILCs can acquire several cell states, that is, inflammatory, memory, regulatory etc. Indeed, in some contexts, single cell transcriptomic analysis cluster ILCs based on their cellular state rather than their lineage origin. Acknowledging these blurry boundaries in mature ILCs may be important when approaching future studies in the field.

The finding of immunological memory on ILCs has opened a new field of research with many questions. What are the implications of having memory ILCs in our organism? Would memory ILCs protect us better to unrelated infections? Would they become faster in repairing our tissues with accumulating episodes of tissue damage? Given their antigen nonspecific nature and their enhanced sensitivity to stimuli, would memory ILCs connect apparently unrelated diseases? These compelling questions warrant future studies.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Sergio Martinez-Hoyer for data and figure support. IMG is financially supported by Wallenberg Academy Fellow (KAW 4-239/2020), Swedish Research Council and Karolinska Institute (2018-02377). FT is supported by grants from the Canadian Institute of Health Research (Grant numbers: 153304 and 173434).

CONFLICT OF INTEREST STATEMENT

Authors declare no conflicts.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ORCID

Itziar Martinez-Gonzalez  <https://orcid.org/0000-0002-1695-5691>

Fumio Takei  <https://orcid.org/0000-0002-3620-5046>

REFERENCES

- Martinez-Gonzalez I, Steer CA, Takei F. Lung ILC2s link innate and adaptive responses in allergic inflammation. *Trends Immunol.* 2015;36(3):189-195. doi:10.1016/j.it.2015.01.005
- Klose CSN, Artis D. Innate lymphoid cells as regulators of immunity, inflammation and tissue homeostasis. *Nat Immunol.* 2016;17(7):765-774. doi:10.1038/ni.3489
- Lee MW, Odegaard JI, Mukundan L, et al. Activated type 2 innate lymphoid cells regulate beige fat biogenesis. *Cell.* 2015;160(1-2):74-87. doi:10.1016/j.cell.2014.12.011
- Campbell L, Hepworth MR, Whittingham-Dowd J, et al. ILC2s mediate systemic innate protection by priming mucus production at distal mucosal sites. *J Exp Med.* 2019;216(12):2714-2723. doi:10.1084/jem.20180610
- Stier MT, Bloodworth MH, Toki S, et al. Respiratory syncytial virus infection activates IL-13-producing group 2 innate lymphoid cells through thymic stromal lymphopoietin. *J Allergy Clin Immunol.* 2016;138(3):814-824.e11. doi:10.1016/j.jaci.2016.01.050
- Wu X, Kasmani MY, Zheng S, et al. BATF promotes group 2 innate lymphoid cell-mediated lung tissue protection during acute respiratory virus infection. *Sci Immunol.* 2022;7(67):eabc9934. doi:10.1126/sciimmunol.abc9934
- Monticelli LA, Sonnenberg GF, Abt MC, et al. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat Immunol.* 2011;12(11):1045-1054. doi:10.1031/ni.2131
- Matsuyama T, Matsuyama H, Dotake Y, Takagi K, Machida K, Inoue H. The therapeutic potential for targeting group 2 innate lymphoid cells in asthma. *Front Immunol.* 2022;13:930862. doi:10.3389/fimmu.2022.930862
- Chen R, Smith SG, Salter B, et al. Allergen-induced increases in sputum levels of group 2 innate lymphoid cells in subjects with asthma. *Am J Respir Crit Care Med.* 2017;196(6):700-712. doi:10.1164/rccm.201612-2427OC
- Akdis CA, Arkwright PD, Brüggemann MC, et al. Type 2 immunity in the skin and lungs. *Allergy.* 2020;75(7):1582-1605. doi:10.1111/all.14318
- van der Ploeg EK, Carreras Mascaro A, Huylebroeck D, Hendriks RW, Stadhouders R. Group 2 innate lymphoid cells in human respiratory disorders. *J Innate Immun.* 2020;12(1):47-62. doi:10.1159/000496212
- Salimi M, Barlow JL, Saunders SP, et al. A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *J Exp Med.* 2013;210(13):2939-2950. doi:10.1084/jem.20130351
- Imai Y, Yasuda K, Sakaguchi Y, et al. Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice. *Proc Natl Acad Sci USA.* 2013;110(34):13921-13926. doi:10.1073/pnas.1307321110
- Mashiko S, Mehta H, Bissonnette R, Sarfati M. Increased frequencies of basophils, type 2 innate lymphoid cells and Th2 cells in skin of patients with atopic dermatitis but not psoriasis. *J Dermatol Sci.* 2017;88(2):167-174. doi:10.1016/j.jdermsci.2017.07.003
- Martinez-Gonzalez I, Mathä L, Steer CA, Ghaedi M, Poon GFT, Takei F. Allergen-experienced group 2 innate lymphoid cells acquire memory-like properties and enhance allergic lung inflammation. *Immunity.* 2016;45(1):198-208. doi:10.1016/j.immuni.2016.06.017
- Martinez-Gonzalez I, Mathä L, Steer CA, Takei F. Immunological memory of group 2 innate lymphoid cells. *Trends Immunol.* 2017;38(6):423-431. doi:10.1016/j.it.2017.03.005

17. Martinez-Gonzalez I, Ghaedi M, Steer CA, Mathä L, Vivier E, Takei F. ILC2 memory: recollection of previous activation. *Immunol Rev*. 2018;283(1):41-53. doi:[10.1111/imr.12643](https://doi.org/10.1111/imr.12643)
18. Wang X, Peng H, Cong J, et al. Memory formation and long-term maintenance of IL-7R α + ILC1s via a lymph node-liver axis. *Nat Commun*. 2018;9(1):4854. doi:[10.1038/s41467-018-07405-5](https://doi.org/10.1038/s41467-018-07405-5)
19. Weizman OE, Song E, Adams NM, et al. Mouse cytomegalovirus-experienced ILC1s acquire a memory response dependent on the viral glycoprotein m12. *Nat Immunol*. 2019;20(8):1004-1011. doi:[10.1038/s41590-019-0430-1](https://doi.org/10.1038/s41590-019-0430-1)
20. Ham J, Kim J, Sohn KH, et al. Cigarette smoke aggravates asthma by inducing memory-like type 3 innate lymphoid cells. *Nat Commun*. 2022;13(1):3852. doi:[10.1038/s41467-022-31491-1](https://doi.org/10.1038/s41467-022-31491-1)
21. Serafini N, Jarade A, Surace L, et al. Trained ILC3 responses promote intestinal defense. *Science*. 2022;375(6583):859-863. doi:[10.1126/science.aaz8777](https://doi.org/10.1126/science.aaz8777)
22. Geary CD, Sun JC. Memory responses of natural killer cells. *Semin Immunol*. 2017;31:11-19. doi:[10.1016/j.smim.2017.08.012](https://doi.org/10.1016/j.smim.2017.08.012)
23. Lau CM, Wiedemann GM, Sun JC. Epigenetic regulation of natural killer cell memory. *Immunol Rev*. 2022;305(1):90-110. doi:[10.1111/imr.13031](https://doi.org/10.1111/imr.13031)
24. Zahalka S, Starkl P, Watzenboeck ML, et al. Trained immunity of alveolar macrophages requires metabolic rewiring and type 1 interferon signaling. *Mucosal Immunol*. 2022;15(5):896-907. doi:[10.1038/s41385-022-00528-5](https://doi.org/10.1038/s41385-022-00528-5)
25. Jeyanathan M, Vaseghi-Shanjani M, Afkhami S, et al. Parenteral BCG vaccine induces lung-resident memory macrophages and trained immunity via the gut-lung axis. *Nat Immunol*. 2022;23(12):1687-1702. doi:[10.1038/s41590-022-01354-4](https://doi.org/10.1038/s41590-022-01354-4)
26. Bekkering S, Domínguez-Andrés J, Joosten LAB, Riksen NP, Netea MG. Trained immunity: reprogramming innate immunity in health and disease. *Annu Rev Immunol*. 2021;39:667-693. doi:[10.1146/annurev-immunol-102119-073855](https://doi.org/10.1146/annurev-immunol-102119-073855)
27. Pipkin ME. Runx proteins and transcriptional mechanisms that govern memory CD8 T cell development. *Immunol Rev*. 2021;300(1):100-124. doi:[10.1111/imr.12954](https://doi.org/10.1111/imr.12954)
28. Miyamoto C, Kojo S, Yamashita M, et al. Runx/Cbfb complexes protect group 2 innate lymphoid cells from exhausted-like hyporesponsiveness during allergic airway inflammation. *Nat Commun*. 2019;10(1):447. doi:[10.1038/s41467-019-08365-0](https://doi.org/10.1038/s41467-019-08365-0)
29. Verma M, Michalec L, Sripada A, et al. The molecular and epigenetic mechanisms of innate lymphoid cell (ILC) memory and its relevance for asthma. *J Exp Med*. 2021;218(7):e20201354. doi:[10.1084/jem.20201354](https://doi.org/10.1084/jem.20201354)
30. Verma M, Verma D, Sripada AS, et al. NF κ B1 inhibits memory formation and supports effector function of ILC2s in memory-driven asthma. *Front Immunol*. 2023;14:1217776. doi:[10.3389/fimmu.2023.1217776](https://doi.org/10.3389/fimmu.2023.1217776)
31. Harly C, Cam M, Kaye J, Bhandoola A. Development and differentiation of early innate lymphoid progenitors. *J Exp Med*. 2018;215(1):249-262. doi:[10.1084/jem.20170832](https://doi.org/10.1084/jem.20170832)
32. Gasteiger G, Fan X, Dikiy S, Lee SY, Rudensky AY. Tissue residency of innate lymphoid cells in lymphoid and nonlymphoid organs. *Science*. 2015;350(6263):981-985. doi:[10.1126/science.aac9593](https://doi.org/10.1126/science.aac9593)
33. Ghaedi M, Takei F. Innate lymphoid cell development. *J Allergy Clin Immunol*. 2021;147(5):1549-1560. doi:[10.1016/j.jaci.2021.03.009](https://doi.org/10.1016/j.jaci.2021.03.009)
34. Schneider C, Lee J, Koga S, et al. Tissue-resident group 2 innate lymphoid cells differentiate by layered ontogeny and in situ perinatal priming. *Immunity*. 2019;50(6):1425-1438.e5. doi:[10.1016/j.immuni.2019.04.019](https://doi.org/10.1016/j.immuni.2019.04.019)
35. Steer CA, Martinez-Gonzalez I, Ghaedi M, Allinger P, Mathä L, Takei F. Group 2 innate lymphoid cell activation in the neonatal lung drives type 2 immunity and allergen sensitization. *J Allergy Clin Immunol*. 2017;140(2):593-595.e3. doi:[10.1016/j.jaci.2016.12.984](https://doi.org/10.1016/j.jaci.2016.12.984)
36. Li M, Hener P, Zhang Z, Kato S, Metzger D, Chambon P. Topical vitamin D3 and low-calcemic analogs induce thymic stromal lymphopoietin in mouse keratinocytes and trigger an atopic dermatitis. *Proc Natl Acad Sci USA*. 2006;103(31):11736-11741. doi:[10.1073/pnas.0604575103](https://doi.org/10.1073/pnas.0604575103)
37. Ricardo-Gonzalez RR, Schneider C, Liao C, Lee J, Liang HE, Locksley RM. Tissue-specific pathways extrude activated ILC2s to disseminate type 2 immunity. *J Exp Med*. 2020;217(4):e20191172. doi:[10.1084/jem.20191172](https://doi.org/10.1084/jem.20191172)
38. Huang Y, Mao K, Chen X, et al. S1P-dependent interorgan trafficking of group 2 innate SD. *Science*. 2018;359(6371):114-119. doi:[10.1126/SCIENCE.AAM5809](https://doi.org/10.1126/SCIENCE.AAM5809)
39. Mathä L, Takei F, Martinez-Gonzalez I. Tissue resident and migratory group 2 innate lymphoid cells. *Front Immunol*. 2022;13:877005. doi:[10.3389/fimmu.2022.877005](https://doi.org/10.3389/fimmu.2022.877005)
40. Stier MT, Zhang J, Goleniewska K, et al. IL-33 promotes the egress of group 2 innate lymphoid cells from the bone marrow. *J Exp Med*. 2018;215(1):263-281. doi:[10.1084/jem.20170449](https://doi.org/10.1084/jem.20170449)
41. Mathä L, Romera-Hernández M, Steer CA, et al. Migration of lung resident group 2 innate lymphoid cells link allergic lung inflammation and liver immunity. *Front Immunol*. 2021;12:679509. doi:[10.3389/fimmu.2021.679509](https://doi.org/10.3389/fimmu.2021.679509)
42. Jiang X, Chen Y, Peng H, Tian Z. Memory NK cells: why do they reside in the liver? *Cell Mol Immunol*. 2013;10(3):196-201. doi:[10.1038/cmi.2013.8](https://doi.org/10.1038/cmi.2013.8)
43. Huang Y, Paul WE. Inflammatory group 2 innate lymphoid cells. *Int Immunol*. 2016;28(1):23-28. doi:[10.1093/intimm/dxv044](https://doi.org/10.1093/intimm/dxv044)
44. Leyva-Castillo JM, Geha RS. Cutaneous type 2 innate lymphoid cells come in distinct flavors. *JID Innov*. 2021;1(3):100059. doi:[10.1016/j.xjidi.2021.100059](https://doi.org/10.1016/j.xjidi.2021.100059)
45. Beura LK, Wijeyesinghe S, Thompson EA, et al. T cells in nonlymphoid tissues give rise to lymph-node-resident memory T cells. *Immunity*. 2018;48(2):327-338.e5. doi:[10.1016/j.immuni.2018.01.015](https://doi.org/10.1016/j.immuni.2018.01.015)
46. Gaide O, Emerson RO, Jiang X, et al. Common clonal origin of central and resident memory T cells following skin immunization. *Nat Med*. 2015;21(6):647-653. doi:[10.1038/nm.3860](https://doi.org/10.1038/nm.3860)
47. Sung JH, Zhang H, Moseman EA, et al. Chemokine guidance of central memory T cells is critical for antiviral recall responses in lymph nodes. *Cell*. 2012;150(6):1249-1263. doi:[10.1016/j.cell.2012.08.015](https://doi.org/10.1016/j.cell.2012.08.015)
48. Miron N, Kumar BV, Meng W, et al. Human lymph nodes maintain TCF-1hi memory T cells with high functional potential and clonal diversity throughout life. *J Immunol*. 2018;201(7):2132-2140. doi:[10.4049/jimmunol.1800716](https://doi.org/10.4049/jimmunol.1800716)
49. Walford HH, Lund SJ, Baum RE, et al. Increased ILC2s in the eosinophilic nasal polyp endotype are associated with corticosteroid responsiveness. *Clin Immunol*. 2014;155(1):126-135. doi:[10.1016/j.clim.2014.09.007](https://doi.org/10.1016/j.clim.2014.09.007)
50. Bal SM, Bernink JH, Nagasawa M, et al. IL-1 β , IL-4 and IL-12 control the fate of group 2 innate lymphoid cells in human airway inflammation in the lungs. *Nat Immunol*. 2016;17(6):636-645. doi:[10.1038/ni.3444](https://doi.org/10.1038/ni.3444)
51. Stevens WW, Kato A. Group 2 innate lymphoid cells in nasal polyposis. *Ann Allergy Asthma Immunol*. 2021;126(2):110-117. doi:[10.1016/j.anai.2020.08.001](https://doi.org/10.1016/j.anai.2020.08.001)
52. Imai Y. ILC2s in skin disorders. *Allergol Int*. 2023;72(2):201-206. doi:[10.1016/j.alit.2023.01.002](https://doi.org/10.1016/j.alit.2023.01.002)
53. Roediger B, Kyle R, Le Gros G, Weninger W. Dermal group 2 innate lymphoid cells in atopic dermatitis and allergy. *Curr Opin Immunol*. 2014;31:108-114. doi:[10.1016/j.coi.2014.10.008](https://doi.org/10.1016/j.coi.2014.10.008)
54. Stadhouders R, Li BWS, de Bruijn MJW, et al. Epigenome analysis links gene regulatory elements in group 2 innate lymphocytes to asthma susceptibility. *J Allergy Clin Immunol*. 2018;142(6):1793-1807. doi:[10.1016/j.jaci.2017.12.1006](https://doi.org/10.1016/j.jaci.2017.12.1006)
55. Huang Y, Guo L, Qiu J, et al. IL-25-responsive, lineage-negative KLRG1(hi) cells are multipotential "inflammatory" type 2 innate lymphoid cells. *Nat Immunol*. 2015;16(2):161-169. doi:[10.1038/ni.3078](https://doi.org/10.1038/ni.3078)

56. van der Ploeg EK, Golebski K, van Nimwegen M, et al. Steroid-resistant human inflammatory ILC2s are marked by CD45RO and elevated in type 2 respiratory diseases. *Sci Immunol*. 2021;6(55):eabd3489. doi:[10.1126/sciimmunol.abd3489](https://doi.org/10.1126/sciimmunol.abd3489)
57. Golebski K, van der Lans RJL, van Egmond D, et al. Inflammatory innate lymphoid cells predict response speed to dupilumab in chronic rhinosinusitis with nasal polyps. *Allergy*. 2023;14:3265-3268. doi:[10.1111/all.15877](https://doi.org/10.1111/all.15877)
58. Bachert C, Han JK, Desrosiers M, et al. Efficacy and safety of dupilumab in patients with severe chronic rhinosinusitis with nasal polyps (LIBERTY NP SINUS-24 and LIBERTY NP SINUS-52): results from two multicentre, randomised, double-blind, placebo-controlled, parallel-group phase 3 trials. *Lancet*. 2019;394(10209):1638-1650. doi:[10.1016/S0140-6736\(19\)31881-1](https://doi.org/10.1016/S0140-6736(19)31881-1)
59. Matha L, Krabbendam L, Martinez-Hoyer S, et al. Human CD127 negative ILC2s show immunological memory. *bioRxiv*. 2023. doi:[10.1101/2023.10.29.564625](https://doi.org/10.1101/2023.10.29.564625)
60. Xu S, Zhang Y, Liu X, et al. Nr4a1 marks a distinctive ILC2 activation subset in the mouse inflammatory lung. *BMC Biol*. 2023;21(1):218. doi:[10.1186/s12915-023-01690-3](https://doi.org/10.1186/s12915-023-01690-3)
61. Trabanelli S, Ercolano G, Wyss T, et al. c-Maf enforces cytokine production and promotes memory-like responses in mouse and human type 2 innate lymphoid cells. *EMBO J*. 2022;41(12):e109300. doi:[10.15252/embj.2021109300](https://doi.org/10.15252/embj.2021109300)
62. Björklund AK, Forkel M, Picelli S, et al. The heterogeneity of human CD127+ innate lymphoid cells revealed by single-cell RNA sequencing. *Nat Immunol*. 2016;17(4):451-460. doi:[10.1038/ni.3368](https://doi.org/10.1038/ni.3368)
63. Zwijnenburg AJ, Pokharel J, Varnaitė R, et al. Graded expression of the chemokine receptor CX3CR1 marks differentiation states of human and murine T cells and enables cross-species interpretation. *Immunity*. 2023;56(8):1955-1974.e10. doi:[10.1016/j.immuni.2023.06.025](https://doi.org/10.1016/j.immuni.2023.06.025)
64. Winkler C, Hochdörfer T, Israelsson E, et al. Activation of group 2 innate lymphoid cells after allergen challenge in asthmatic patients. *J Allergy Clin Immunol*. 2019;144(1):61-69.e7. doi:[10.1016/j.jaci.2019.01.027](https://doi.org/10.1016/j.jaci.2019.01.027)
65. Noval Rivas M, Burton OT, Oettgen HC, Chatila T. IL-4 production by group 2 innate lymphoid cells promotes food allergy by blocking regulatory T-cell function. *J Allergy Clin Immunol*. 2016;138(3):801-811.e9. doi:[10.1016/j.jaci.2016.02.030](https://doi.org/10.1016/j.jaci.2016.02.030)
66. Yang L, Fu J, Zhou Y. Research progress in atopic march. *Front Immunol*. 2020;11:1907. doi:[10.3389/fimmu.2020.01907](https://doi.org/10.3389/fimmu.2020.01907)
67. Strutt TM, McKinstry KK, Dibble JP, et al. Memory CD4+ T cells induce innate responses independently of pathogen. *Nat Med*. 2010;16(5):558-564. doi:[10.1038/nm.2142](https://doi.org/10.1038/nm.2142)
68. Chapman TJ, Castrucci MR, Padrick RC, Bradley LM, Topham DJ. Antigen-specific and non-specific CD4+ T cell recruitment and proliferation during influenza infection. *Virology*. 2005;340(2):296-306. doi:[10.1016/j.virol.2005.06.023](https://doi.org/10.1016/j.virol.2005.06.023)

How to cite this article: Martinez-Gonzalez I, Takei F. New insights into ILC2 memory. *Immunol Rev*. 2024;00:1-8. doi:[10.1111/imr.13323](https://doi.org/10.1111/imr.13323)