JEM Journal of Experimental Medicine

INSIGHTS

Emerging in vivo tools for ILC2 research

Timotheus Y.F. Halim[1]

ILC2 are critical regulators of inflammation and tissue homeostasis in diverse anatomical sites. ILC2-targeted mouse models have underpinned this emerging field of research. In this issue of *JEM*, (Kabil et al. https://doi.org/10.1084/jem.20241671) developed a novel *Il17rb*$^{CreERT2.eGFP}$ mouse to study the role of *Rora* in mature ILC2.

Group 2 innate lymphoid cells (ILC2) are increasingly recognized as important tissue-resident regulators of type-2 immune processes in both inflammatory and homeostatic settings (Schuijs and Halim, 2018). Their pleiotropic effects are highly dependent on anatomical location and disease state, which are capturing the attention of an increasing breadth of research themes. Studying ILC2 was difficult because of a sparsity of reagents that specifically target these cells in mouse studies. Immunologists have worked toward developing better ILC2-targeted mouse lines, with recently published models providing the community with more options. Here, Kabil et al. (2025) report a new *Il17rb*$^{CreERT2.eGFP}$ mouse line, targeting the IL-25R heterodimer, which is highly expressed by ILC2. The authors use this mouse to investigate the balance between ILC2, ILC3, and type-3 immunity in different inflammatory contexts, although I will primarily discuss this new ILC2-targeted CreERT2 mouse among an evolving selection of other in vivo models.

The original discovery of ILC2 benefitted from genetically engineered mice (GEM); type-2 cytokine reporter mice aided flow cytometry and immunofluorescence microscopy studies that identified an innate non-T cell source of type-2 cytokines (Moro et al., 2010; Neill et al., 2010; Price et al., 2010). These studies were further supported by GEMs that broadly lacked both innate and/or adaptive lymphocytes (i.e., *Rag1/2*$^{-/-}$, *Rag1/2*$^{-/-}$*Il2rg*$^{-/-}$, *Il7*$^{-/-}$, *Id2*-targeted mice, etc.), or the ability to deplete ILC2 somewhat specifically using antibodies. Similarly, whole body type-2 cytokine-deficient mice or neutralizing antibodies can also be used to study the roles of ILC2, although other potential cellular sources cannot be easily excluded. The more widespread availability of these mice has supported many previous and current studies and remains a reasonable starting point for many groups embarking on ILC2 research.

Nevertheless, there are some major limitations of these first generation models, including nonspecific deletion/depletion of other lymphoid subsets. Early transcriptomic profiling studies identified transcription factors that were important for ILC2 development, such as *Rora* and *Gata3*, which resulted in the generation of more specific ILC2-deficient mice, including *Il7r*$^{Cre/+}$*Rora*$^{fl/fl}$ animals or bone marrow chimeras derived from *Rora*$^{sg/sg}$ donors (Halim et al., 2012; Hoyler et al., 2012; Wong et al., 2012). Caveats of these models include the requirement for more complicated controls that consider the possible side effects of deleting *Rora* in other lymphoid lineages (i.e., CD4 T cells). However, these mice remain among the most reliable and well-characterized constitutive ILC2-deficient GEMs.

Extensive efforts to generate refined ILC2-targeted GEMs (such as ILC2-specific inducible depletion or expression of Cre-recombinase) led to the development of several new lines, including *Il5*$^{tdTom.iCre}$ (Red5) mice, which is a highly versatile mouse line that allows temporal ILC2-depletion when crossed to *Rosa26*$^{lsl\text{-}DTR}$ mice, for example (Nussbaum et al., 2013).

Timotheus Y.F. Halim.

A consideration of these mice is that other *Il5*-expressing cells, such as type-2 helper T (Th2) cells, are often targeted too. Moreover, the relatively low baseline expression of *Il5* limits the Cre efficiency and may require breeding homozygous Red5 mice (which are *Il5* deficient) or administration of reagents to promote Il5-tdTom.iCre expression by ILC2 and Th2 cells. Indeed, the co-expression of many genes by both ILC2 and Th2 cells made it difficult to genetically target one over the other without using more complicated experiments, such as adoptive transfers or mixed bone marrow chimeras. This was a major limitation when addressing questions about the relative importance or role of ILC2 or Th2 cells in earlier models (reviewed in Cording et al., 2018) and stimulated the creation of innovative new GEMs, which broadly fit into two categories: GEMs that exploit an emerging understanding of ILC2 biology to target single genes or genetic loci that are believed

[1]Cancer Research UK – Cambridge Institute, Li Ka Shing Centre, University of Cambridge, Cambridge, UK.

Correspondence to Timotheus Y.F. Halim: tim.halim@cruk.cam.ac.uk.

to be more exclusive to ILC2, or GEMs that use Boolean logic and genetically target multiple loci to achieve better ILC2 specificity.

In terms of the latter, *CD4*$^{Cre/+}$*Icos*$^{loxP\text{-}DTR\text{-}loxP}$ (iCOS-T) mice incorporated a human diphtheria toxin receptor (DTR) cassette flanked by loxP sites that targeted the *Icos* gene, which is expressed by most ILC2 and some T cells; however, when crossed to *Cd4*^{Cre} mice (which delete the DTR cassette in T cells, but not ILC2), the DTR becomes more specific to ILC2, allowing their temporal deletion (Oliphant et al., 2014). A similar approach was used more recently in Boolean-ILC2-Cre (BIC) mice, where three loci targeting *Cd28*, *Icos*, and *Il13* using diverse DNA recombinases enable the selective expression of Cre in ILC2 (Szeto et al., 2024). This approach was used to study transcription factor utilization by ILC2 and/or Th2 cells in isolation. Despite its elegance, this line has some limitations, including the cost of maintaining complex GEMs, and the constitutive expression of Cre recombinase.

The other approach mentioned aims to identify highly ILC2-specific genes to create refined GEMs. Extending on the use of type-2 cytokine-targeted GEMs, several groups discovered that ILC2 could respond to neuropeptide neuromedin U (*Nmu*) via the receptor Nmur1, which is largely ILC2 specific among leukocytes in naïve mice (reviewed in Schuijs and Halim [2018]). *Nmur1*$^{iCre.eGFP}$ mice (Tsou et al., 2022) are now commercially available and have been used in many recent ILC2 studies; nevertheless, these mice also have some potential caveats, including constitutive Cre expression and reported *Nmur1* expression by nonimmune cells, activated Th2 cells (Szeto et al., 2024), as well as eosinophils (Li et al., 2023). Alongside the quest for more ILC2-specific genes, recent advances indicate that specific regulatory elements confer cell specificity of co-expressed genes. For instance, while *Gata3* is highly expressed by both ILC2 and Th2 cells, a certain enhancer of *Gata3* is more important for ILC2 development and function and drove stronger, but not exclusive, expression of a fluorescent reporter (Kasal et al., 2021). Hence, we do not yet have a single-gene GEM that meets key requirements of selective and universal selectivity of ILC2, temporal induction of a Cre recombinase, and high efficiency of recombinase activity in ILC2.

Now the McNagny lab introduces a new tool to genetically target ILC2 in mice, leveraging a rather surprising gene, namely *Il17rb*, which encodes the IL-17RB receptor that together with IL-17RA (*Il17ra*) comprises the IL-25 receptor complex (Kabil et al., 2025). While ILC2 are known to respond to IL-25, the expression of IL-25R was believed to be more restricted to a subset of ILC2 more commonly found in the gut, while ILC2 in other sites expressed less IL-25R at steady state (reviewed in Schuijs and Halim [2018]). The authors inserted a polycistronic cassette at the end of the endogenous *Il17rb* gene, driving the expression of IL-17RB, CreERT2, and eGFP at stoichiometric ratios. Notably, endogenous IL-25R expression is unaffected by the transgene insertion. Moreover, the authors report high eGFP expression in almost 100% of ILC2 from many different anatomical sites at rest, including organs where IL-25R expression is believed to be lower. This may be because of other regulatory mechanisms that govern surface expression of the IL-17RB/IL-17RA heterodimer. Moreover, the authors profile other immune cells and find that eGFP is not expressed by other ILC lineages (ILC1, ILC3, and NK cells), or mature adaptive lymphocytes in most organs. Profiling of embryonic and adult lymphocyte progenitors reveals that ILC2 progenitors start to express Il17rb-eGFP, while some expression of eGFP is also noted on thymic NKT cells, ILC progenitors, and T cell precursors, which is a consideration for extended tamoxifen-dosing studies. Moreover, the authors show that acute IL-33–driven type-2 inflammation does not result in significant Il17rb-eGFP expression by CD4 T cells, although it will be important to investigate chronic Th2 cell–driven models in the future.

Next, the author assessed the efficiency and specificity of tamoxifen-induced Cre activity of the *Il17rb*$^{CreERT2.eGFP}$ mouse using a *Rosa26*$^{lsl\text{-}RFP}$ fate map line. A 3- to 5-day course of tamoxifen resulted in very efficient (>90%) labelling of lineage-negative cells, which were identified as ILC2 in most tissues. Notably, the authors found some RFP+ cells that downregulated Il17rb-eGFP in the small intestine and assumed an eosinophil-like phenotype; they noted that eosinophil progenitors are known for *Il17rb* expression, which is interesting given the fact that *Nmur1* can also be expressed by eosinophils.

The authors subsequently generated *Il17rb*$^{CreERT2.eGFP}$*Rosa26*$^{lsl\text{-}RFP}$*Rora*$^{fl/fl}$ mice to selectively and temporally delete *Rora* in ILC2, resulting in reduced ILC2 numbers within weeks after tamoxifen treatment. However, while *Rora* is essential for ILC2 development, the authors observe that only a subset of mature ILC2 are reliant on *Rora* for their survival. Functionally, depletion of Rora in adult ILC2 resulted in reduced intestinal IL-10^{+} ILC2, with effects on downstream adaptive immunity. These studies support the idea that ILC2 can influence local adaptive immunity, and the *Il17rb*$^{CreERT2.eGFP}$ mice will be useful to temporally and efficiently investigate ILC2-derived factors, such as IL-10, in these regulatory networks. Overall, the development of new ILC2-targeted GEMs will greatly benefit future investigations into their roles in health and disease.

Acknowledgments

T.Y.F. Halim is supported by Cancer Research UK (A24995) and Wellcome Trust (301533/Z/23/Z) funding.

Author contributions: T.Y.F. Halim: conceptualization and writing—original draft, review, and editing.

Disclosures: The author declares no competing interests exist.

References

Cording, S., et al. 2018. *Eur. J. Immunol.* https://doi.org/10.1002/eji.201747388
Halim, T.Y.F., et al. 2012. *Immunity.* https://doi.org/10.1016/j.immuni.2012.06.012
Hoyler, T., et al. 2012. *Immunity.* https://doi.org/10.1016/j.immuni.2012.06.020
Kabil, et al. 2025. *J. Exp. Med.* https://doi.org/10.1084/jem.20241671
Kasal, D.N., et al. 2021. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.2106311118
Li, Y., et al. 2023. *Science.* https://doi.org/10.1126/science.ade4177
Moro, K., et al. 2010. *Nature.* https://doi.org/10.1038/nature08636
Neill, D.R., et al. 2010. *Nature.* https://doi.org/10.1038/nature08900
Nussbaum, J.C., et al. 2013. *Nature.* https://doi.org/10.1038/nature12526
Oliphant, C.J., et al. 2014. *Immunity.* https://doi.org/10.1016/j.immuni.2014.06.016
Price, A.E., et al. 2010. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.1003988107
Schuijs, M.J., and T.Y.F. Halim. 2018. *Ann. N. Y. Acad. Sci.* https://doi.org/10.1111/nyas.13604
Szeto, A.C.H., et al. 2024. *Science.* https://doi.org/10.1126/science.adl0370
Tsou, A.M., et al. 2022. *Nature.* https://doi.org/10.1038/s41586-022-05297-6
Wong, S.H., et al. 2012. *Nat. Immunol.* https://doi.org/10.1038/ni.2208