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IL-9 by INFERence

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Abstract

Despite discovery of the cytokine over 20 years ago, the relevant biological sources of IL-9 have remained a mystery. In this issue of *Immunity*, Licona-Limón et al. (2013) use a newly generated reporter mouse to demonstrate a role for IL-9-secreting T cells in helminthic parasite immunity.

Interleukin-9 (IL-9) was initially identified based on its ability to promote T cell and mast growth (reviewed in (Goswami and Kaplan, 2011)). Consistent with the association of IL-9 in type 2 immune responses, IL-9 promoted allergic inflammation and immunity to helminthic parasites. However, the relevant sources of IL-9 during immune responses are still incompletely defined, at least partly due to a lack of genetic tools to track IL-9-expressing cells. Among the potential sources, reports have documented IL-9 production from mast cells and several T cell subsets including Th2, Th17 and Treg cells. The most potent producers of IL-9 are Th9 cells, T cells that are derived in vitro in the presence of the cytokines TGF-β and IL-4, and innate lymphoid cells (ILCs) (Kaplan, 2013; Wilhelm et al., 2011) (Figure 1). IL-9-producing innate lymphoid cells are observed in protease-induced allergic airway inflammation, and likely contribute to the pro-allergic milieu. Th9 cells are also capable of promoting allergic inflammation in several models. As yet, no comprehensive studies have examined the source of IL-9 during helminthic parasite infection. In this issue of *Immunity*, Licona-Limón et al. describe the generation of new IL-9-deficient and IL-9-reporter mice that make an important contribution to our understanding of the sources of IL-9 during the development of immunity to *Nippostrongylusbrasiliensis*, and the IL-9 target cells that might be important for parasite clearance (Licona-Limón et al., 2013).

Helminthic parasite clearance depends upon the development of Th2 cell-regulated immune responses (Urban et al., 1998) though it is understood that many of the cytokines and transcription factors that promote Th2 cell development are also required for Th9 cell differentiation. The requirement for IL-9 in parasite immunity is less clear. Perhaps owing to redundancy in function among Th2 cell-type cytokines and IL-9, IL-9-deficient mice on a mixed genetic background were able to clear *N. brasiliensis* infection, although transgenic or exogenous IL-9 clearly enhanced immunity to additional parasites (reviewed in (Goswami and Kaplan, 2011)). The reason for differences among these models is unclear, but suggests a more complex role for IL-9 in parasite immunity that might vary with pathogen, genetic background of the host, or other experimental variables.

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In this issue of Immunity, Licona-Limón et al. generate *Il9*$^{-/-}$ mice on a BALB/c genetic background, and a novel IL-9 reporter mouse by placing an IRES-EGFP cassette downstream of the *Il9* gene termed INFER for Interleukin Nine Fluorescent Report (Licona-Limón et al., 2013). Consistent with previous reports on mice with a disrupted *Il9* allele on a mixed genetic background (Townsend et al., 2000), these mice showed no gross abnormalities in development or reproduction. However, in contrast to previous studies using 129×C57BL/6 (F_2)*Il9*$^{-/-}$ mice, the BALB/c *Il9*$^{-/-}$ mice demonstrated diminished clearance of *N. brasiliensis*. The INFER allele reported accurately for IL-9 expression, with GFP observed predominantly in Th9 cell cultures with minimal expression in other Th cellsubset cultures, and about 80% of the IL-9-positive cells also being positive for GFP. This result represents far greater fidelity of gene expression reporting than a previously described *Il9* reporter that identified only about 10% of IL-9-secreting T cells in culture (Wilhelm et al., 2011). Although there are caveats in the use of any reporter allele, mice carrying the INFER allele represent an important reagent going forward in understanding IL-9 biology. Licona-Limón et al (2013) use these new mouse models to add to several important paradigms regarding the mechanisms of IL-9-dependent immune responses.

During *N. brasiliensis* infection, *Il9* reporter activity is observed in both CD4$^+$ T cell and ILC populations. Although the experiments presented in this report do not distinguish between these sources of IL-9 in terms of their relative contribution to immunity, results suggest that GFP$^+$ ILC outnumber GFP$^+$ CD4$^+$T cells in the lung, but GFP$^+$ CD4$^+$T cells predominate soon after infection in the mediastinal lymph node. The differences in abundance in separate locations might indicate distinct roles for each IL-9-secreting population. However, the authors demonstrate that transfer of GFP$^+$ Th9 cells to an *Il9*$^{-/-}$ recipient results in decreased worm burden. Thus, Th9 cells, as the only source of IL-9 in vivo, are sufficient to mediate parasite immunity, and IL-9 from ILC is clearly not required to mediate IL-9-dependent clearance of *N. brasiliensis*.

The authors add support to the paradigm that IL-9 appears as an early cytokine during type 2 immune responses, specifically demonstrating this during *N. brasiliensis* infection. Licona-Limón et al observe *Il9* mRNA peaking 3–5 days earlier in infected target organs and peripheral lymphoid tissue than peaks of mRNA encoding the Th2 cell-type cytokines IL-4, IL-5 and IL-13. This is consistent with observations in a house dust mite model of airway inflammation where IL-9 preceded Th2 cell cytokines, and in atopic infants where IL-9 is significantly increased in serum early in life, before Th2 cell cytokines, but does not stay elevated as the atopic infants age (Jones et al., 2012; Yao et al., 2013). The early production of IL-9 suggests a role for IL-9 in the induction of Th2 cell cytokines, consistent with a requirement for Th2 cell cytokines in IL-9-induced allergic inflammation (Temann et al., 2002). The mechanisms behind this sequential induction are not clear. Since IL-4 is required for the differentiation of both Th9 and Th2 cells these results suggest either that TGF-β predominates during the induction of type 2 responses and represses IL-4-induced Th2 cell development, or that early Th9 cell development might arise through IL-4-independent mechanisms. Further experiments will be able to distinguish these possibilities.

Among the still unanswered questions in IL-9 biology is which IL-9-responsive cells mediate cytokine effects during immune responses. The authors demonstrate that transferred GFP$^+$Th9 cells promote increased mast cell numbers, consistent with previous reports (Jones et al., 2012), but also increased basophil cell numbers in infected tissues and peripheral lymphoid organs. Moreover, the authors demonstrate that basophil accumulation in Th9 cell recipients is IL-9-dependent. Among myeloid cells, *Il9ra* is expressed in mast cells and basophils in far higher amounts than in eosinophils and neutrophils, suggesting that both populations are important IL-9 targets. Yet, it is still not clear which if either of these cell types is the critical responders in IL-9-mediated parasite clearance (Figure 1).

Licona-Limón et al also demonstrate a functional difference between Th9 and Th2 cells, an important observation given the extensive functional overlap between the two subsets. Transfer of Th2 or Th9 cells into infected *Rag2*$^{-/-}$ recipients results in a decrease of parasite burden only in Th9 cell recipients. Decreased parasite burden in Th9 cell recipients correlated with the ability of Th9 cells, but not Th2 cells, to increase mast cell and basophil numbers in the lung and spleen. In contrast, Th2, but not Th9 cells induced a modest increase in eosinophil numbers in draining lymph nodes. This suggests that Th2 and Th9 cells do have distinct or at least specialized functions as they cooperate in the regulation of immunity.

The report by Licona-Limón et al strongly supports a functional role for IL-9-secreting T cells during immune responses, a role that had been questioned from work using the previous *Il9* reporter allele (Wilhelm et al., 2011). How Th9 cells contribute to immunity and inflammation is still not clear, but the identification of basophils, mast cells, and Th2 cell cytokine production as targets of IL-9 provides both insight to the process, and avenues for further exploration (Figure 1). The reasons for the differences observed in the contribution of IL-9 to *N. brasiliensis* infection between Licona-Limón et al (2013) and Townsend et al (2000) is not clear. The differing genetic background of the mice might suggest that mixed genetic background mice are less reliant upon IL-9-dependent mechanisms that include growth and recruitment of mast cells and basophils, or lung and intestinal goblet cell metaplasia, in parasite immune responses. Based on these studies, the new tools generated by Licona-Limón et al will allow researchers to INFERfurther insight on the role of IL-9 in immunity and inflammation.

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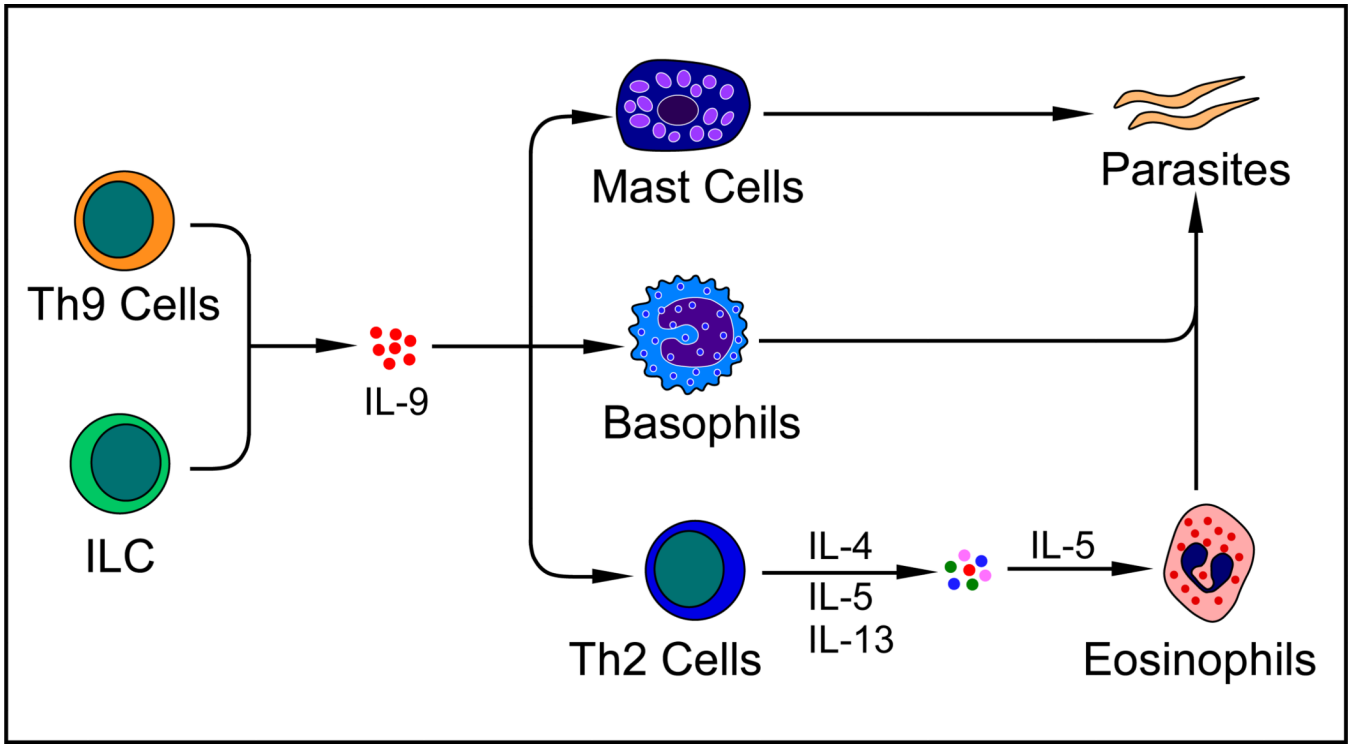

Figure 1.
IL-9-dependent immunity. The schematic identifies IL-9-producing T cells (Th9 cells) and innate lymphoid cells (ILCs) as major sources of IL-9. IL-9 promotes growth of mast cells and basophils, and Th2 cytokine production, that each can contribute to clearance of gastrointestinal worms.