

Helper T cell differentiation enters a new era: Le Roi est mort; vive le Roi!

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In the dark ages of T cell biology, we considered two fates for differentiated CD4⁺ T cells: T helper (Th)1 and Th2 cells. Now we know that the reality is much more complex and interesting. The newest Th cell subset produces the cytokine IL-17. New evidence shows that the IL-17-related cytokine IL-25 is essential for Th2 responses in two infectious disease models.

In this commentary, we will consider the new information pertaining to IL-25 in the context of the emerging understanding of the regulation and action of other IL-17 family members. We will also discuss how these new insights change our concept of Th cell differentiation and immunoregulation in host defense and immune-mediated disease.

Cytokines driving Th cell differentiation

The Th1/Th2 cell dichotomy and the essential role of the cytokine environment in driving their differentiation are well accepted. Interleukin (IL)-12, acting via the transcription factors Stat4 and T-bet, drives the differentiation of naive CD4⁺ T cells into interferon (IFN)- γ -producing Th1 cells, whereas IL-4, acting via Stat6 and GATA3, drives the differentiation of IL-4-producing Th2 cells. Numerous infectious models support the notion that Th1 and Th2 cells act independently. Th1 cells are responsible for the cell-mediated elimination of intracellular pathogens, whereas eradication of helminthic infestations is dependent on adequate Th2 responses. However, the immunopathogenesis of autoimmune disease does not fit quite so neatly into this dichotomy. CD4⁺ T cells can also differentiate into cells that produce the immunosuppressive cytokines IL-10 and TGF β , so-called

Th3 cells, and can become "adaptive" regulatory T cells. Both cell types appear to protect against autoimmunity. Studies of experimental autoimmune encephalomyelitis and adjuvant-induced arthritis have pointed to the importance of yet another Th cell subset, which produces IL-17 and is now termed Th17 cells. Although this population of T cells has been implicated in the exacerbation of autoimmune pathology (1–3), the role of Th17 cells in host defense remains to be elucidated.

The IL-17 family

The subject of two papers in the issue (Owyang et al., p. 843 [4] and Fallon et al., p. 1105 [5]) is the Th2-like cytokine, IL-25, which as a member of the IL-17 family is also known as IL-17E. Structurally, this family of six cytokines (IL-17A–F) is thought to form cystine knots and in this respect is related to other better known cytokines such as TGF β and platelet-derived growth factor. IL-17 (IL-17A) was originally reported to be produced mainly by effector and memory CD4⁺ T cell subsets, but has more recently been suggested to be more widely expressed (6, 7). IL-17F is located adjacent to IL-17 on mouse chromosome 1 (human chromosome 6), and although it seems to be regulated in a similar manner, it may be more widely expressed than IL-17. Less well studied are IL-17B, IL-17C, and IL-17D, which are thought to be expressed in a variety of nonhematopoietic tissues, although IL-17D is reported to be produced by CD4⁺ T cells (8). Five IL-17 receptors have been identified, but only the receptors for IL-17/

IL-17F and IL-25 have been characterized. The IL-17 receptor is structurally distinct from other cytokine receptors, and engagement of the receptor activates MAP kinases and NF- κ B. Signaling via IL-25 is reported to be dependent on the adaptor molecule TRAF6 (9).

With respect to their biological actions, IL-17 and IL-17F are most intensively studied. These cytokines evoke inflammation largely by inducing the production of chemokines, as well as granulocyte colony-stimulating factor and granulocyte macrophage colony-stimulating factor, with the subsequent recruitment of polymorphonuclear leukocytes. In some settings, however, IL-17- or IL-17F-induced inflammation is dominated by macrophages (10). The induction of matrix metalloproteinase production by epithelial cells may be another important proinflammatory function of IL-17 (11). Abundant data point to pathogenic roles of IL-17 in models of immune-mediated disease and in human autoimmune disorders. Less well defined are the beneficial roles of IL-17 family cytokines in host defense, although recent data indicate IL-17 is important for resistance against *Klebsiella pneumoniae* and *Mycobacterium tuberculosis* (12–14).

Unlike IL-17 and IL-17F, IL-25 is produced by Th2 cells and mast cells (15). Administration of recombinant IL-25 (rIL-25) to mice has been shown to evoke an inflammatory response characterized by the overproduction of Th2 cytokines, hyperproduction of immunoglobulins IgA and IgE, overproduction of mucus, epithelial cell hyperplasia, and eosinophilia. Furthermore, the observed pathology was dependent on the classical Th2 cytokines IL-4 and IL-13, as shown by the lack of effect of administering rIL-25 to mice that lack these cytokines or their cognate receptor.

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The Th17 lineage

Th17 cells are now thought to be a separate lineage of effector Th cells, but the optimal recipe to differentiate Th17 cells in vitro remains somewhat unclear (10, 16–18), and the in vivo requirements for their differentiation have yet to be firmly established. Several groups have pointed to the importance of IL-23 as a critical ingredient for the generation and/or maintenance of this lineage (10, 13, 17–20). It should be kept in mind that because of its structural similarity to IL-12 (p35/p40) (21–24) IL-23 (p19/p40) also has the potential to induce a Th1-like response. Recently, another group has come up with a different recipe to make Th17 cells that involves a milieu of cytokines produced by lipopolysaccharide-activated dendritic cells (namely IL-1, IL-6, and TNF- α) and the presence of TGF β (25). These findings not only illustrate that there is overlap in the groups of cytokines that induce different populations of CD4⁺ T cells, but also emphasize that a particular cytokine, in this case TGF β , may have both proinflammatory (Th17-inducing) and antiinflammatory (Th3-inducing) roles, depending on the circumstances.

Roles for IL-25 in host defense and controlling inflammation

In this issue, two new studies by Owyang et al. (4) and Fallon et al. (5) show that *IL-25*^{-/-} mice have increased susceptibility to the parasitic helminthes, *Trichuris muris*, and *Nippostrongylus brasiliensis*. Both groups found that the antihelminth activity of IL-25 depends on its ability to induce the Th2-associated cytokines IL-4, IL-5, and IL-13. In the case of *T. muris* infection, the susceptibility of *IL-25*^{-/-} mice was associated with impaired IL-4 and IL-13 production and reduced *Trichuris*-specific IgG1 and total IgE. In addition, chronically infected *IL-25*^{-/-} mice had exacerbated pathology, which was characterized by severe inflammation and disruption of intestinal epithelial structure, and was associated with increased production of IFN- γ , IL-17, and total IgG2a. Thus, in addition to an essential role in driving antihelminth

Th2 responses, this study implies an antiinflammatory role for IL-25 in suppressing Th1 and Th17 responses. In contrast, Fallon et al. (5) found that IL-25 was not absolutely required for the generation of Th2 responses in *N. brasiliensis* infection, but that IL-25 was important for the timely resolution of the infection. When *IL-25*^{-/-} mice were challenged with *N. brasiliensis*, the polarization of the Th2 response, manifested as enhanced IL-4, IL-5, and IL-13 production, was delayed. This resulted in slower elimination of worms and delayed IgE production. Interestingly, blockade of Th1 responses with anti-IL-12 and anti-IFN- γ antibodies reduced the worm burden and enhanced Th2 responses in *Trichuris*-infected *IL-25*^{-/-} mice, as well as in the naturally susceptible AKR mouse strain. Thus, IL-25 does not appear to be absolutely essential for Th2 responses to some helminthes, and may promote host defense by inhibiting expression of type 1 cytokines.

Which cell types produce IL-25 and which cell types respond to it? Using mice that have the reporter *lacZ* knocked into the *IL-25* locus, Owyang et al. (4) found that IL-25 expression is, in fact, very limited. It was constitutively expressed by CD4⁺ and CD8⁺ T cells in the gut but was not expressed in a variety of innate immune cells. These data hint at the possible existence of effector T cells that selectively produce IL-25 (a putative Th25 lineage), which may play a specific role in mucosal immunity. In their study, Fallon et al. (5) report that the target of IL-25 is a nonlymphoid c-kit⁺ cell present in the draining lymph node. The finding that the IL-25-mediated expulsion of *N. brasiliensis* was T and B cell independent emphasizes the importance of this nonlymphoid population. Using mice in which the reporter *eGFP* was knocked into the *IL-4* locus, Fallon et al. (5) show that nonlymphoid c-kit⁺ cells are major producers of IL-4 during infection and their appearance in the draining lymph node precedes the expansion of IL-4-producing CD4⁺ cells. These findings are consistent with previous work using rIL-25, which indicated that this cytokine does not have

direct effects on differentiating T cells, but rather that its action requires nonlymphoid cells.

New findings, new questions

As all good studies should, these two papers raise more questions than they answer (4, 5). Together they suggest a new conceptual framework for Th2 cell differentiation. The findings are of considerable interest because, compared to the seemingly clear cut mechanisms involved in Th1 cell differentiation, the factors that drive Th2 cell differentiation have been more difficult to understand. Both groups convincingly show that IL-25 is an important regulator of Th2 responses and host defense. Whether IL-25 is produced by T cells for T cells or by APCs for nonlymphoid effector populations it indisputably serves to enhance IL-4 production resulting in amplification of Th2-mediated immunity. These reports also indicate that IL-25 controls inflammation during helminth infection and dampens counterproductive Th1 responses, independent of IL-25's effects on Th2 cell differentiation. The duality of IL-25 activity mirrors that of the well-known immunosuppressive cytokine TGF β , which, as mentioned, induces the polarization of proinflammatory Th17 cells (25).

The paradigm of Th1 and Th2 responses is firmly supported by an immense amount of in vitro data but, more importantly, is substantiated in many infectious disease models and some autoimmune disease models. As the papers in this issue exemplify (4, 5), the generation of other T cell subsets in models of host defense is less clear cut (Fig. 1). IFN- γ promotes Th1 cell differentiation by inducing T-bet, but inhibits both Th17 cell and Th2 cell differentiation (26, 27). Similarly, IL-4, by inducing GATA-3, inhibits Th17 cell and Th1 cell differentiation. Thus, it would appear that the generation of Th1, Th2, and Th17 cells is carefully balanced. In contrast to the well-described positive effects of IFN- γ and IL-4 on their respective lineages, no data have been provided indicating that IL-17 directly regulates Th cell differentiation,

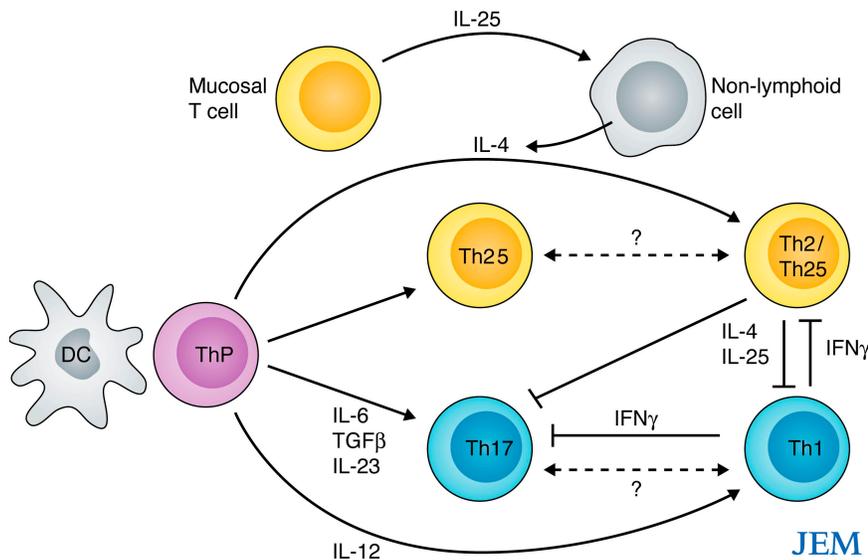


Figure 1. Th cell differentiation: how many flavors? On pathogen challenge, CD4⁺ T cells will differentiate into either IFN- γ -producing Th1 cells or IL-4-producing Th2 effector cells, which directly cross-regulate each other. Th17 cells have recently been added to the list of Th effector cells. Two new reports now suggest that the IL-17 family member IL-25—possibly produced by a population of T cells in the gut mucosa—induced Th2 cell differentiation during helminth infection. The target of IL-25 may be a nonlymphoid c-kit⁺ population, which produces IL-4 and in turn enhances Th2 cell differentiation by CD4⁺ T cells. In addition, IL-25 may have a direct role in inhibiting both Th1- and Th17-producing cells during helminth infection in order to limit the inflammatory response. The extent to which IL-25-producing T cells are a distinct Th lineage (Th25) or if IL-25 production is a feature of many Th2 cells will need further investigation.

either positively or negatively (27, 28). Furthermore, will we find that like Th1 and Th17 cells, IL-25-secreting cells are really a distinct lineage from Th2 cells and subject to different modes of regulation? To this end, the question of whether the putative Th25 cell population expresses lineage-specific transcription factors that determine its cytokine production profile will likely be a focus of future studies. Further studies are also needed to understand and define the functional role of Th17 and Th25 cells as either primary effectors similar to Th1 and Th2 cells, or sentinel populations that promote or enhance more specific cell-mediated immunity in mucosal tissues. One might envision a scenario in which pleiotropic cytokines such as IL-17, TGF β , and IL-25 not only potentiate early Th responses and recruitment of cells to lymph nodes and tissues, but also regulate aberrant inflammatory responses during the resolution of infection. The ability of IL-25 to limit

Th1-induced inflammation might be an example of the latter. How all of this relates to human health and disease is less clear, and with future studies refined concepts of T cell differentiation will come to bear.

In conclusion, this is a very exciting time for T cell biologists. In the age of monarchies, the expression “Le roi est mort; vive le roi” marked the passing of eras, ushering out one regime while introducing another. Analogously, it might be said, “T cell differentiation is dead; long live T cell differentiation.” Suddenly, understanding the molecular regulation of T cell differentiation has become much more complicated. The simple notion of a dualistic view of Th1/Th2 cell differentiation is moribund, but the era of new complexities of immunoregulation promises to provide better understanding of mechanisms of host defense and immune-mediated disease.

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