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NEWS AND COMMENTARY

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IL-21: a new player in Th17-cell differentiation

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In recent years, a new population of effector cells, Th17 cells, has emerged that has been implicated in the pathogenesis of various autoimmune conditions. Consequently, much interest has focussed on the molecular requirements governing Th17 cell development and function. While TGF-β, together with interleukin (IL)-6, and IL-23 appear to be required for the generation and maintenance, respectively, of Th17 cells from naïve $CD4^+$ T cells,[1–5] several new reports published in *Nature*[6,7] and *Nature Immunology*[8] now reveal an important role for IL-21 in the generation of Th17 cells.

$CD4^+$ T cells have traditionally been categorized as Th1 or Th2 cells.[9] These subsets have important roles in regulating protective immune responses against infection with intracellular pathogens (viruses, bacteria) or extracellular pathogens and parasites, respectively. Th1 cells also mediate cell-mediated/delayed-type hypersensitivity responses, while Th2 have been associated with allergic responses (Figure 1).[9] The function of Th1 and Th2 cells lies in their ability to produce reciprocal sets of cytokines: interferon (IFN)-γ by the former and IL-4, IL-5 and IL-13 by the latter. The molecular requirements for the development of these lineages of effector $CD4^+$ T cells include the cytokine milieu present during their activation, as well as specific sets of transcription factors. Thus, IL-12 induces the generation of Th1 cells by activating STAT4, which results in the sequential induction of the master regulator of Th1 lineage differentiation T-bet, and subsequently IFN-γ. On the other hand, induction of Th2 cells is initiated by IL-4-mediated activation of STAT6, which induces expression of the transcription factor GATA3 that imprints naïve $CD4^+$ T cells with the ability to produce IL-4, IL-5 and IL-13 via epigenetic modification of the locus encoding this cluster of cytokine genes. The generation of specific $CD4^+$ T-cell immune responses is reinforced by the ability of both IFN-γ and IL-4 to act in regulatory feedback loops such that they promote further expansion of Th1- and Th2-type cells, respectively, in an autocrine manner, while concomitantly antagonizing the induction of the alternative effector Th cell type (Figure 1).[10,11]

Several diseases have also been associated with dysregulated behaviour or function of these $CD4^+$ T-cell subsets. For instance, Th1 cells have been implicated in the development and pathogenesis of animal models of inflammatory bowel disease, multiple sclerosis (experimental autoimmune encephalomyelitis) and arthritis (collagen-induced arthritis), while pathogenic Th2 cells are likely to contribute to allergy, atopy and asthma.

During the past few years, it has become apparent that IL-12, STAT4 and IFN-γ, and therefore Th1 cells, are not the primary instigators of model inflammatory diseases.[12] Rather, the pathogenic cells in these models

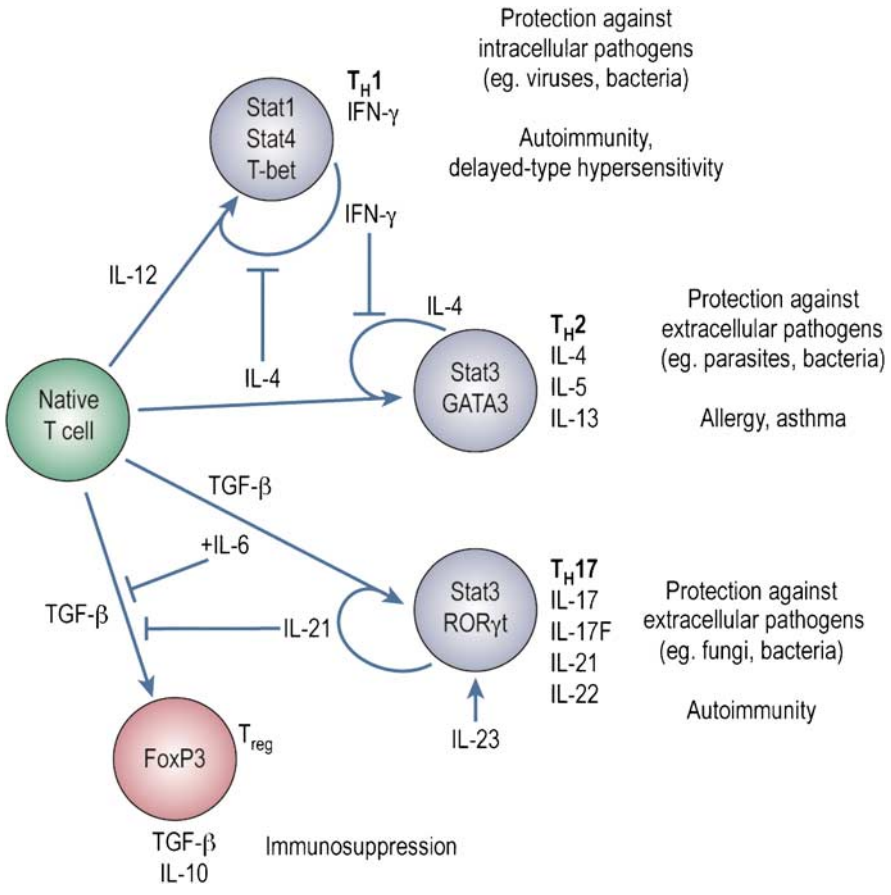

Figure 1 Molecular requirements for Th-cell differentiation.

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of disease are induced in response to signals provided *in vivo* by the IL-12-like cytokine IL-23, and are characterized by the production of a suite of cytokines, chemokines, effector molecules and transcription factors distinct from that of Th1 cells.[4,5,11,12] One of the genes most highly expressed in these effector cells is the pro-inflammatory cytokine IL-17.[5] Consequently, they have been termed Th17 cells. Additional features of Th17 cells include the production of IL-22 and expression of IL-23R.[11,12] These IL-17-producing $CD4^+$ T cells have been recognized as key mediators of inflammation and tissue damage in several animal models of human diseases.[4,5,11,12]

Following the characterization of Th17 cells, interest turned to identifying the factors responsible for their generation. IL-23 was initially proposed as a key mediator of Th17-cell generation, because IL-23-deficient mice displayed severely decreased numbers of IL-17 producing $CD4^+$ T cells compared to wild-type mice.[4] Later work, however, revealed that IL-23 was not required for the generation of Th17 cells from naïve T cells *per se*, but rather acted later on cells that were already committed to the Th17 lineage.[1–3] This was consistent with the original finding that IL-23 promoted proliferation of activated/memory, but not resting/naïve $CD4^+$ T cells.[13] Subsequently, it was shown that IL-17 production by naïve $CD4^+$ T cells could be driven by TGF-β and IL-6.[1–3] These cytokines act in a STAT3-dependent manner to induce expression of the orphan nuclear receptor RORγt, which subsequently increases production of IL-17.[14,15] Thus, analogous to the central roles played by T-bet and GATA3 in generating Th1 and Th2 cells, respectively,[10] RORγt is considered to be the transcription factor responsible for guiding the development of Th17 cells.[15] The role of TGF-β in the development of these cells is particularly interesting, as TGF-β is also important for driving the generation of regulatory T cells (Treg; Figure 1). Thus, TGF-β will promote the differentiation of inhibitory Treg, however, in the presence of additional inflammatory signals such as IL-6, Th17 cells will be generated. Thus, IL-6 represented a crucial switch for controlling the differentiation of $CD4^+$ T cells to the Th17 or Treg lineages. It remained unclear, though, whether other cytokines were also capable of controlling this switch.

Recent studies from the Kuchroo,[6] Dong[7] and Littman[8] labs approached this question from different perspectives, but all reached the same conclusion that IL-21, a member of the IL-2 family of cytokines,[16] also controls the generation of Th17 cells. They showed that IL-21, in combination with TGF-β, induces IL-17 production from naïve $CD4^+$ T cells. This was accompanied by the acquired expression of RORγt and IL-23R. Conversely, IL-21 inhibited the generation of $FoxP3^+$ Treg induced by TGF-β. Furthermore, analysis of IL-6-deficient mice revealed that the ability of IL-21 to generate Th17 cells from $CD4^+$ T cells was independent of IL-6. Thus, IL-21, like IL-6, can dictate the generation of Th17 versus Treg. For both IL-21 and IL-6, this switch seems to be mediated by STAT3 and RORγt.[6–8] These papers also demonstrated that IL-6 or IL-21 could induce Th17 cells themselves to produce IL-21. Such endogenous production of IL-21 by Th17 cells appeared to be biologically significant, because the number of IL-17-producing cells generated by TGF-β and IL-6 was reduced in the absence of IL-21/IL-21R signalling.[6,7] Thus, IL-6 can elicit IL-21 production by $CD4^+$ T cells, which then functions in an autocrine loop to amplify the Th17 response in a similar way to IL-4 for Th2 and IFN-γ for Th1 cells (Figure 1). Furthermore, both IL-21 and IL-6 upregulated IL-23R, thereby priming Th17 cells to the amplifying and stabilizing effects of IL-23.[1–4,13]

Targeting molecules involved in the generation, maintenance and effector function of Th17 cells, such as TGF-β, IL-6, IL-23 and IL-17, have been proposed as novel therapeutics for human inflammatory disorders. These findings on the role of IL-21 in the biology of Th17 cells[6–8] add IL-21 to this list of potential targets. In particular, since IL-21 functions in a self-amplifying loop (Figure 1) on established Th17 cells, IL-21 may be a better target for inhibiting the pathogenic inflammatory response than IL-6. Recent reports showing that neutralizing IL-21 in murine models of lupus or rheumatoid arthritis ameliorated disease severity[17,18] demonstrate the potential utility of IL-21-targeted therapies for human autoimmune conditions.

These findings also raise some interesting questions that will no doubt be addressed in future studies. First, Th17 cells are not the only population of $CD4^+$ T cells capable of producing IL-21. Indeed, $CXCR5^+$ T follicular helper (T_{FH}) cells are also a rich source of this cytokine.[19] Interestingly, T_{FH} cells have also been found to be overrepresented, and assumed to be disease-causing, in murine lupus.[19] Humans deficient in ICOS have a reduction in the frequency of circulating T_{FH} cells, and activated $CD4^+$ T cells from these patients exhibit reduced production of IL-17.[20] The reduction in IL-17 secretion may be a direct result of the deficiency of circulating T_{FH} cells in ICOS-deficient patients. Thus, it is tempting to speculate that T_{FH} cells are also capable of producing IL-17. For these reasons, in addition to the finding that both T_{FH} and Th17 cells can provide B-cell help for Ab production,[19,21] it will be important to establish the relationship between Th17 cells and T_{FH} cells to determine whether they have a common developmental programme. It will also be important to determine whether production of IL-21 by T_{FH} cells contributes to the generation of Th17 cells, or whether the IL-21 required for this process is strictly derived in an autocrine manner.

Second, it will be important to determine if other signature Th17 mediators, such as IL-22, are differentially regulated by IL-6 and IL-21 and whether the abilities of both IL-6 and IL-21 to induce Th17 cells represent redundant systems, or if they favour the development of Th17 cells for responses against specific pathogens. For instance, it has been found that animal models of inflammatory dermatitis can develop independently of IL-17[22] and that production of IL-17 and IL-22 by Th17 cells is differentially regulated in such conditions.[23] Thus, it is possible that Th17 cells induced by TGF-β and either IL-6 or IL-21 make qualitatively different contributions to the development of inflammation depending on the pathogen and affected tissue. Notwithstanding these uncertainties, these recent findings[6–8] expand our understanding of Th17 cells and highlight potential new strategies for effectively treating human inflammatory diseases.

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