IL-12– and IL-23–induced T helper cell subsets: birds of the same feather flock together

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Traditionally, $CD4^+$ T cells have been separated into two different subsets named T helper (Th)1 and Th2. A new IL-23–driven subset of Th cells called Th_{IL-17} has now been described. The data suggest that IL-23 plays an important role in the differentiation of autoreactive pathogenic T cells. Whether these IL-23–induced Th_{IL-17} cells are a unique subset or are related to other Th subsets is discussed.

Th1 and Th2 subsets can be distinguished by their cytokine profiles and effector functions. Th1 cells produce IFN-γ, IL-2, and lymphotoxin, and mediate delayed type hypersensitivity responses and macrophage activation, whereas Th2 cells secrete IL-4, IL-5, IL-10, and IL-13, and contribute to eosinophilic inflammation and allergic reactions (1, 2). IFN-γ and IL-4 antagonize each other and cross-regulate the expansion and functions of Th1 and Th2 cells.

Over the years, considerable effort has been devoted to the identification of cell surface antigens and transcription factors that are unique to Th1 and Th2 cell subsets. Th1 cells preferentially express the IL-12Rβ2 chain, IL-18 receptor, P-selectin glycoprotein ligand-1, Chandra, and the CXCR3 and CXCR5 chemokine receptors (1). Th2 cells express the chemokine receptors CCR3, CCR5, and CCR8, and inducible costimulatory molecule (ICOS) (1). We have recently identified T cell immunoglobulin and mucin containing molecule-3, a cell surface molecule that is differentially expressed on the surface of terminally differentiated Th1 cells (3). The preferential expression of these cell markers is probably a consequence of the cytokines and transcription factors that promote differentiation of these T cell subsets.

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Distinct signaling pathways govern the differentiation of Th1 versus Th2 cells. IL-12 and IFN-γ signals are important for Th1 cell differentiation. The Th1 cytokine IFN-γ signals through signal transducer of activated T cells (STAT-1) which in turn activates the T-box transcription factor T-bet, which is the key inducer of IFN-γ and Th1 cell differentiation (1, 2). The effects of T-bet are so potent that expression of this transcription factor in previously differentiated Th2 cells reverses their phenotype and results in IFN-γ production (4). T-bet appears to act before IL-12 signaling in Th1 cell development, but IL-12–mediated activation of STAT-4 appears to be essential for the stabilization of IFN-γ production and the development of terminally differentiated Th1 cells (1, 2). IL-4 induces STAT-6 activation, which promotes the expression of GATA-3, an essential transcription factor for both IL-4 production and for the development of Th2 cells (1, 2).

A new Th cell subset?

Recently, IL-23 was identified as a new member of the IL-12 family of cytokines (5). IL-23 is a heterodimeric cytokine that comprises the p40 subunit of IL-12 and a specific p19 subunit. The p40–p19 complex is secreted by activated DCs and macrophages (5). In this issue, Langrish et al. (page 233) describe a subset of $CD4^+$ T cells that they call Th_{IL-17} cells, which are generated by isolating T cells from experimental autoimmune encephalomyelitis (EAE)-susceptible SJL mice immunized with an encephalitogenic peptide and culturing them with IL-23 (6). The authors consider this a unique Th subset since these T cells, in contrast to Th1 cells, produce IL-17, IL-17F, IL-6, TNF, and low levels of IFN-γ. These experiments confirm earlier observations by Aggarwal et al. (7), showing that IL-23 could induce T cells that secrete IL-17, no IL-4, and little IFN-γ. Both studies suggested that these Th_{IL-17} cells represent a subset of Th cells distinct from Th1 and Th2 cells (7). It should be noted, however, that IL-23–induced Th_{IL-17} cells continue to produce low levels of IFN-γ, a hallmark cytokine of Th1 subset.

Although the known cytokine profile of Th_{IL-17} cells distinguishes them from previously described Th1 and Th2 cells, their origin remains unclear, and the possibility that they arise from early Th1 or Th2 cells needs to be considered. The work by Langrish et al. (6) and from other groups (7, 8) shows that IL-23R is expressed only on activated and memory cells and that IL-23 does not act on naive T cells to induce Th cell differentiation. In addition, Langrish et al. could detect little or no IL-17–producing cells after primary stimulation of naive TCR transgenic T cells in the presence of IL-23. Therefore, it is unlikely that Th_{IL-17} cells emerge from a unique precursor contained in the pool of naive T cells. Rather, IL-17–producing Th cells could emerge from previously activated Th cells. It is therefore interesting to speculate whether the Th_{IL-17} subset arises from early activated Th1 or Th2 cells before they are terminally differentiated into effector cells by IL-12 or IL-4, respectively (Fig. 1).

Th subsets in autoimmunity

Several studies have supported the notion that Th1 cells that produce proin-

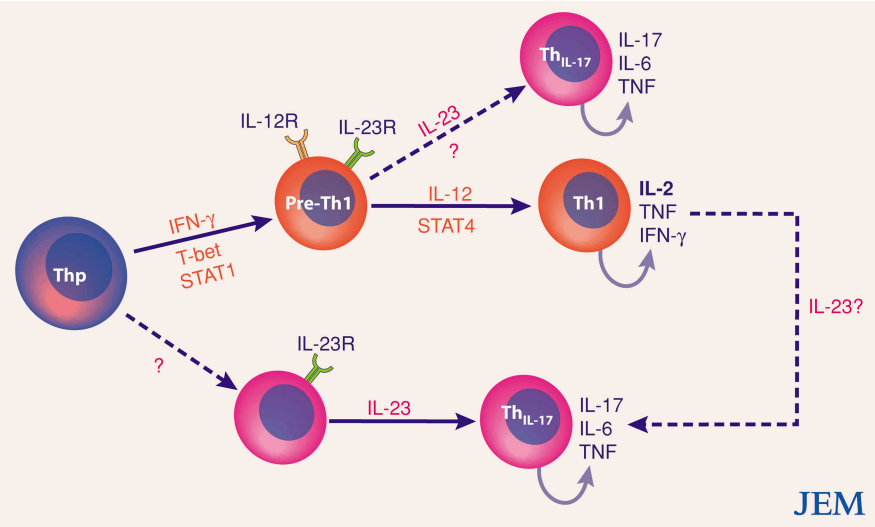

Figure 1. Hypothetical model of Th$_{IL-17}$ cell development. IL-23–driven TH$_{IL-17}$ cells may represent a unique subset of Th cells that arises from a novel Th precursor cell. Alternatively, TH$_{IL-17}$ cells may develop from pre–Th1 cells that differentiate into Th1 cells in the presence of IL-12, but TH$_{IL-17}$ cells in the presence of IL-23. Dashed arrows indicate hypothetical pathways of Th$_{IL-17}$ subset development.

flammatory cytokines promote the development of inflammation and autoimmunity. In mouse models of autoimmune diseases, such as type 1 diabetes and EAE, CD4$^+$ T cells that produce large amounts of IFN-γ and little IL-4 induce disease and tissue destruction upon adoptive transfer (9, 10). Th1 cytokines are present in the inflammatory EAE lesions in the central nervous system, and Th2 cells are absent, strongly suggesting that Th1 cytokines play a role in the pathogenesis of the disease (11, 12). Furthermore, T cells from resistant B10.S mice could transfer EAE when activated in the presence of IL-12 before transfer, suggesting that there is a defect in the generation of Th1 cells in the resistant mice and once differentiated to Th1 cells in vitro, the cells can mediate their effector function and transfer disease (13, 14). These findings, along with the observation that Th1 clones with specificity for myelin antigens can transfer EAE, strongly suggests that Th1 cells are crucial for the induction of EAE (15, 16). But there are several reports demonstrating that mice deficient for IFN-γ, IFN-γ receptor, or STAT-1 are highly susceptible to EAE, which calls into question the role of Th1 cells in inducing disease (15–19). However, T-bet is essential for the induction of EAE. T-bet$^{-/-}$ mice are highly resistant to EAE, and antisense oligonucleotides that suppress the induction of T-bet and IFN-γ completely prevent development of disease (19, 20).

Langrish et al. show that IL-23–driven T cells that produce IL-17 are highly pathogenic in contrast to Th1 cells, which in their hands cannot induce disease (6). Can the new findings of Langrish et al. help resolve the conflicting data regarding the role of Th cells in EAE?

IL-17 in autoimmunity

IL-17 is the prototypic member of a new cytokine family that is involved in the proliferation, maturation, and chemotaxis of neutrophils. IL-17 has pleitropic activities including the induction of proinflammatory cytokines TNF, IL-1, IL-6, IL-8, granulocyte colony–stimulating factor, and monocyte chemoattractant protein (MCP-1) on various cell types and the promotion of stem cell factor– and granulocyte colony–stimulating factor–mediated granulopoiesis. IL-17 also acts on T cells as a costimulatory factor and enhances allorejection via promotion of DC maturation (21, 22). IL-17 has also been detected in the sera and diseased tissues of patients with rheumatoid and Lyme arthritis, multiple sclerosis, systemic lupus erythematous, and asthma, suggesting its involvement in the development of various human autoimmune diseases (21, 22).

Langrish et al. tested whether IL-17 production could contribute directly to EAE severity by injecting mice with neutralizing antibodies against IL-17. Anti–IL-17 antibody treatment resulted in partial protection from EAE (6). These results are in accord with previous results which showed that IL-17–deficient mice are resistant to the development of collagen-induced arthritis and EAE and suggest a pathogenic role of IL-17 in EAE (23). However, it is important to note that in the current study the anti–IL-17 antibody treatment could only confer partial protection and that the severe disease observed with the transfer of Th$_{IL-17}$ cells could not be blocked by anti–IL-17 antibody treatment. These observations suggest that either the IL-17 could not be completely neutralized by the antibody or that other factors besides IL-17 are also critical in the pathogenicity of Th$_{IL-17}$ cells in EAE.

Origin of Th$_{IL-17}$ cells?

The lack of pathogenicity of Th1 cells in the experiments described by Langrish et al. (6), although surprising, could explain previous data that showed that IFN-γ (and potentially Th1 cells) is not crucial for the induction of disease and that IFN-γ$^{-/-}$ and IFN-γR$^{-/-}$ mice are still susceptible to EAE (15–18). It is IL-23–driven Th$_{IL-17}$ cells that are crucial for inducing EAE! As discussed by the authors, it is possible that myelin-specific IFN-γ–producing short-term T cell lines described previously as inducers of EAE, contained IL-17–producing cells that were highly pathogenic. However, given that IFN-γ and IL-17 production are almost mutually exclusive in long-term culture, residual IL-17 production in fully differentiated Th1 cells or clones produced in the presence of IL-12 is unlikely to account for the previously well-described pathogenicity of Th1 cells. In addition, these data do not explain why T-bet–deficient mice, generated by two dif-

ferent technologies (19, 20), are resistant to the development of EAE.

One possible mechanism to explain and integrate all these results into a cohesive schema is that IL-23 acts on previously activated, and possibly differentiated, T cells to generate Th_{IL-17} effector T cells. IL-12–driven Th1 cells and IL-23–driven Th_{IL-17} cells may be related and arise from the same T-bet–expressing precursor, a "pre–Th1 cell" (Fig. 1). This model would predict that when T-bet is activated to induce Th1 differentiation, these cells express IL-12R and IL-23R on their surface. But depending on the cytokine available in the milieu they become further differentiated; they become bonafide IFN-γ–producing Th1 cells if they come in contact with IL-12, but become Th_{IL-17} cells if further activated by IL-23. This model might explain why T-bet (19), IL-12/IL-23 p40– (24) and IL-23p19–deficient mice (25) are resistant to EAE and why IFN-γ–, IFN-γR– (14–17), and IL-12p35–deficient mice (24, 26) are still susceptible to disease. Based on this schema, T-bet would play a central role in the generation of Th1 precursors that can further differentiate into either Th1 cells, which are crucial for resistance against intracellular pathogens, or Th_{IL-17} cells, which may be essential for mediating tissue destruction and inducing autoimmunity.

REFERENCES

1. Ho, I.C., and L.H. Glimcher. 2002. Transcription: tantalizing times for T cells. *Cell.* 109:S109–S120.
2. Murphy, K.M., and S.L. Reiner. 2002. The lineage decisions of helper T cells. *Nat. Rev. Immunol.* 2:933–944.
3. Monney, L., C.A. Sabatos, J.L. Gaglia, A. Ryu, H. Waldner, T. Chernova, S. Manning, E.A. Greenfield, A.J. Coyle, R.A. Sobel, G.J. Freeman, and V.K. Kuchroo. 2002. Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. *Nature.* 415:536–541.
4. Szabo, S.J., S.T. Kim, G.L. Costa, X. Zhang, C.G. Fathman, and L.H. Glimcher. 2000. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell.* 100: 655–669.
5. Oppmann, B., R. Lesley, B. Blom, J.C. Timans, Y. Xu, B. Hunte, F. Vega, N. Yu, J. Wang, K. Singh, et al. 2000. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity.* 13: 715–725.
6. Langrish, C.L., Y. Chen, W.M. Blumenschein, J. Mattson, B. Basham, J.D. Sedgwick, T. McClanahan, R.A. Kastelein, and D.J. Cua. 2005. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* 201:233–240.
7. Aggarwal, S., N. Ghilardi, M.H. Xie, F.J. de Sauvage, and A.L. Gurney. 2003. Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. *J. Biol. Chem.* 278:1910–1914.
8. Parham, C., M. Chirica, J. Timans, E. Vaisberg, M. Travis, J. Cheung, S. Pflanz, R. Zhang, K.P. Singh, F. Vega, et al. 2002. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rβ1 and a novel cytokine receptor subunit, IL-23R. *J. Immunol.* 168:5699–5708.
9. Kuchroo, V.K., C.A. Martin, J.M. Greer, S.T. Ju, R.A. Sobel, and M.E. Dorf. 1993. Cytokines and adhesion molecules contribute to the ability of myelin proteolipid protein-specific T cell clones to mediate experimental allergic encephalomyelitis. *J. Immunol.* 151:4371–4382.
10. Baron, J.L., J.A. Madri, N.H. Ruddle, G. Hashim, and C.A. Janeway, Jr. 1993. Surface expression of alpha 4 integrin by CD4 T cells is required for their entry into brain parenchyma. *J. Exp. Med.* 177:57–68.
11. Khoury, S.J., W.W. Hancock, and H.L. Weiner. 1992. Oral tolerance to myelin basic protein and natural recovery from experimental autoimmune encephalomyelitis are associated with downregulation of inflammatory cytokines and differential upregulation of transforming growth factor beta, interleukin 4, and prostaglandin E expression in the brain. *J. Exp. Med.* 176:1355–1364.
12. Merrill, J.E., D.H. Kono, J. Clayton, D.G. Ando, D.R. Hinton, and F.M. Hofman. 1992. Inflammatory leukocytes and cytokines in the peptide-induced disease of experimental allergic encephalomyelitis in SJL and B10.PL mice. *Proc. Natl. Acad. Sci. USA.* 89:574–578.
13. Segal, B.M., and E.M. Shevach. 1996. IL-12 unmasks latent autoimmune disease in resistant mice. *J. Exp. Med.* 184:771–775.
14. Segal, B.M., B.K. Dwyer, and E.M. Shevach. 1998. An interleukin (IL)-10/IL-12 immunoregulatory circuit controls susceptibility to autoimmune disease. *J. Exp. Med.* 187:537–546.
15. Ferber, I.A., S. Brocke, C. Taylor-Edwards, W. Ridgway, C. Dinisco, L. Steinman, D. Dalton, and C.G. Fathman. 1996. Mice with a disrupted IFN-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). *J. Immunol.* 156:5–7.
16. Tran, E.H., E.N. Prince, and T. Owens. 2000. IFN-gamma shapes immune invasion of the central nervous system via regulation of chemokines. *J. Immunol.* 164:2759–2768.
17. Willenborg, D.O., S. Fordham, C.C. Bernard, W.B. Cowden, and I.A. Ramshaw. 1996. IFN-gamma plays a critical down-regulatory role in the induction and effector phase of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. *J. Immunol.* 157:3223–3227.
18. Willenborg, D.O., S.A. Fordham, M.A. Staykova, I.A. Ramshaw, and W.B. Cowden. 1999. IFN-gamma is critical to the control of murine autoimmune encephalomyelitis and regulates both in the periphery and in the target tissue: a possible role for nitric oxide. *J. Immunol.* 163:5278–5286.
19. Bettelli, E., B. Sullivan, S.J. Szabo, R.A. Sobel, L.H. Glimcher, and V.K. Kuchroo. 2004. Loss of T-bet, but not STAT1, prevents the development of experimental autoimmune encephalomyelitis. *J. Exp. Med.* 200:79–87.
20. Lovett-Racke, A.E., A.E. Rocchini, J. Choy, S.C. Northrop, R.Z. Hussain, R.B. Ratts, D. Sikder, and M.K. Racke. 2004. Silencing T-bet defines a critical role in the differentiation of autoreactive T lymphocytes. *Immunity.* 21:719–731.
21. Nakae, S., Y. Komiyama, A. Nambu, K. Sudo, M. Iwase, I. Homma, K. Sekikawa, M. Asano, and Y. Iwakura. 2002. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity.* 17:375–387.
22. Kolls, J.K., and A. Linden. 2004. Interleukin-17 family members and inflammation. *Immunity.* 21:467–476.
23. Nakae, S., A. Nambu, K. Sudo, and Y. Iwakura. 2003. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. *J. Immunol.* 171:6173–6177.
24. Becher, B., B.G. Durell, and R.J. Noelle. 2002. Experimental autoimmune encephalitis and inflammation in the absence of interleukin-12. *J. Clin. Invest.* 110:493–497.
25. Cua, D.J., J. Sherlock, Y. Chen, C.A. Murphy, B. Joyce, B. Seymour, L. Lucian, W. To, S. Kwan, T. Churakova, et al. 2003. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature.* 421:744–748.
26. Gran, B., G.X. Zhang, S. Yu, J. Li, X.H. Chen, E.S. Ventura, M. Kamoun, and A. Rostami. 2002. IL-12p35-deficient mice are susceptible to experimental autoimmune encephalomyelitis: evidence for redundancy in the IL-12 system in the induction of central nervous system autoimmune demyelination. *J. Immunol.* 169:7104–7110.