IL-17 Family Cytokines and the Expanding Diversity of Effector T Cell Lineages

Casey T. Weaver,1,2 Robin D. Hatton,1 Paul R. Mangan,2 and Laurie E. Harrington1

1Departments of Pathology and 2Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294; email: cweaver@uab.edu

Abstract

Since its conception two decades ago, the Th1-Th2 paradigm has provided a framework for understanding T cell biology and the interplay of innate and adaptive immunity. Naive T cells differentiate into effector T cells with enhanced functional potential for orchestrating pathogen clearance largely under the guidance of cytokines produced by cells of the innate immune system that have been activated by recognition of those pathogens. This secondary education of post-thymic T cells provides a mechanism for appropriately matching adaptive immunity to frontline cues of the innate immune system. Owing in part to the rapid identification of novel cytokines of the IL-17 and IL-12 families using database searches, the factors that specify differentiation of a new effector T cell lineage—Th17—have now been identified, providing a new arm of adaptive immunity and presenting a unifying model that can explain many heretofore confusing aspects of immune regulation, immune pathogenesis, and host defense.
INTRODUCTION

Adaptive immunity evolved to enhance the eradication of pathogens in vertebrates, layering antigen specificity and memory onto preexistent innate immunity. A hallmark of adaptive immunity is the antigen-driven differentiation of clonally restricted lymphocyte precursors into effector cells of enhanced functional potential. The differentiation of naive T cells into effector T cells is characterized by the acquisition of new profiles of cytokine production and is largely guided by cytokines produced by pathogen-activated cells of the innate immune system. Many of the key advances in our understanding of effector T cell development and function have therefore been tied to advances in cytokine biology. This was so for the discoveries that drove the conception and establishment of the Th1-Th2 paradigm (1) and has been so of recent discoveries leading up to the identification and characterization of a new lineage of effector CD4 T cells, Th17.

The Th17 lineage has emerged from discovery of a new family of cytokines [the interleukin (IL)-17 family], addition of new members of a cytokine family with well-established effector T cell ties (the IL-12 family), and identification of new activities for the pleiotropic cytokines, transforming growth factor (TGF)-β and IL-6. Th17 cells appear to have evolved as an arm of the adaptive immune system specialized for enhanced host protection against extracellular bacteria and some fungi, microbes probably not well covered by Th1 or Th2 immunity, that evolved to enhance clearance of intracellular pathogens and parasitic helminthes, respectively. Although Th17 cells represent the first new class of effector CD4 T cells to be defined since the original description of Th1 and Th2 cells almost 20 years ago, they are not the only effector cells with ties to the IL-17 family. IL-25 (or IL-17E) has links to the Th2 lineage, and other IL-17 family members can be produced by CD8 T cells, γδ T cells, or natural killer (NK) cells, and by granulo-cytes. Members of the IL-17 family are central players in various arms of the adaptive immune response and have become key to an expanded understanding of cytokine networks that coordinate innate and adaptive immunity to certain pathogens. IL-17 cytokines are also key mediators in a diverse range of auto-inflammatory disorders. The identification of Th17 cells as the principal pathogenic effectors in several types of autoimmunity previously thought to be Th1-mediated promises new approaches for therapies of these disorders, as does identification of IL-25 as a potentially important mediator of dysregulated Th2 responses that cause asthma and other allergic disorders. Although our understanding of the links between members of the IL-17 cytokine family and adaptive immunity is relatively new, many features of the landscape and logic of its contribution to innate and adaptive immunity, whether protective or pathogenic, are now in place and are the subject of this review.

PRINCIPLES OF EFFECCTOR T CELL HETEROGENEITY AND DEVELOPMENT LINKING INNATE AND ADAPTIVE IMMUNITY: THE Th1-Th2 PARADIGM

CD4 T cells play a central role in orchestrating immune responses through their capacity to provide help to other cells of the adaptive or innate immune systems. In early studies of CD4 T cell biology, it became apparent that two classes of CD4 T cells could be defined: those that helped B cells for class switching, or promoted humoral immunity, and those that enhanced macrophage activation, or promoted cell-mediated immunity. With the advent of techniques for cloning T cells (2), investigators found that these activities typically resided in distinct cloned populations (3) and correlated with differential production of factors that promoted or inhibited B cell class switching.
to IgE (4), indicating interclonal functional heterogeneity of CD4 T cells. The activities responsible for these disparate functions were identified as T cell–derived cytokines, initially defined using specific bioassays, and subsequently using cloned cytokines and specific neutralizing antibodies. On the basis of these findings, Mosmann & Coffman (5) proposed the T helper type 1 (Th1)-Th2 hypothesis, which postulated that subsets of CD4 T cells produce reciprocal patterns of immunity through their production of distinct profiles of cytokine secretion—either delayed-type hypersensitivity/cell-mediated immunity (Th1) or allergic/humoral immunity (Th2). Furthermore, each subset promotes its own development and inhibits the development of the other subset, also via their secreted cytokines (6, 7), such that the induction of one type of response suppresses the induction of the other (8). This hypothesis established a new paradigm for understanding immune regulation by CD4 T cells and led to the appreciation that effector CD4 T cells, like class-switched B cells, are functionally heterogeneous.

Th1 cells were defined on the basis of their production of interferon (IFN)-γ, a potent macrophage-activating cytokine important in the clearance of certain intracellular pathogens and a switch factor for induction of IgG2a production by B cells. Th2 cells became defined as producers of IL-4 and IL-5, which promote IgG1 and IgE class switching and eosinophil recruitment. Th2 cells were later shown also to produce IL-13, which participates in IgE class switching and is important for mucosal activation (mucus hypersecretion and increased contractility). Accordingly, the cytokines produced by Th2 cells also became identified as factors involved in the clearance of helminthes (reviewed in 9). The importance of an appropriate Th1 or Th2 response was demonstrated in early studies that examined host protection to challenge by the obligate intracellular protozoal parasite, Leishmania major, by genetically resistant and susceptible strains (10). Protection of a resistant mouse strain, C57Bl/6, correlated with an appropriate Th1-mediated IFN-γ response, whereas susceptibility of BALB/c mice correlated with an inappropriate Th2-mediated IL-4 response (11). Susceptible BALB/c mice could be protected by transfer of a Th1 cell line specific to an immunodominant L. major antigen, whereas transfer of a Th2 cell line exacerbated disease (12). Thus, the Th1-Th2 hypothesis explained the divergence of distinct types of immunity—types 1 and 2, induction of which could determine success or failure of host protection, depending on the type of pathogen.

It was soon established that Th1 and Th2 cells, i.e., effector T cells, were rare in the normal T cell repertoire and required antigen-driven differentiation or clonal expansion of naive T cell precursors for development. However, it was uncertain whether both effector cell types could be generated from clonal precursors of the same antigenic specificity or whether different antigens were somehow linked to the preferential induction or selection of a type 1 or 2 response. With the advent of T cell receptor (TCR) transgenic models, which provided a large source of naive T cell precursors of identical antigenic specificity, definitive studies on the development of Th1 and Th2 cells became possible. Th2 development was soon proven to depend on IL-4, providing the first indication of a positive feedback loop whereby a cytokine produced by effector T cells could induce the differentiation of additional effectors of the same phenotype (13, 14). Thus, Th2 cells may beget Th2 cells, through an IL-4-dependent mechanism.

Discovery of the factors that induce Th1 development followed from studies that directly examined the effects of a Th1-associated pathogen, Listeria monocytogenes, on the differentiation of naive CD4 T cells. Murphy and coworkers (15) found that macrophages activated by heat-killed L. monocytogenes induced strongly polarized Th1 responses and that this effect could be blocked
by neutralization of IFN-γ, although IFN-γ alone could not induce as robust a Th1 response as that of macrophage-derived factors elicited by heat-killed *L. monocytogenes*. This implicated an additional factor acting in concert with IFN-γ to induce Th1 differentiation. IL-12, a heterodimeric cytokine that had been defined as a potent inducer of IFN-γ production by NK cells (16), was soon identified as the Th1-inducing cofactor responsible for Th1 development (17, 18). This provided a mechanism linking pathogen-driven innate immune activation to a directed adaptive T cell response and introduced a new cytokine pathway involved in coordination of innate and adaptive immune responses. Thus, cytokine signals induced by first-line innate responses could guide the adaptive response to enhance pathogen clearance (19).

Following establishment of the cytokines that polarize Th1 or Th2 differentiation, delineation of key signaling pathways followed (Figure 1) (see 20 for review). Notably, each of the Th1- or Th2-polarizing cytokines are members of the type I cytokine superfamily, receptors for which are heterodimers that signal via JAK/STAT complexes (21). Th1 differentiation is initiated by coordinate signaling through the TCR and STAT1-associated cytokine receptors (Figure 1a). Both type I and type II IFNs activate STAT1, as can the IL-12 family member IL-27, receptors for each of which are expressed on naïve T cells (22–24). STAT1 signaling induces the transcription factor, T-bet (Tbx-21), which is a master regulator of Th1 differentiation (25, 26). T-bet potentiates expression of the *Ifng* gene and upregulates the inducible chain of the IL-12 receptor (IL-12Rβ2), expression of which enables IL-12 signaling through STAT4. Activation of the IL-12 receptor further potentiates IFN-γ production and induces expression of IL-18Ra, thereby conferring responsiveness to IL-18 by mature Th1 cells. The IL-12-driven component of Th1 development, which is downstream of STAT1-induced early differentiation, results in mature effector cells that can produce IFN-γ through either TCR-dependent or -independent (IL-12 plus IL-18) pathways (27, 28). Thus, IL-12, elicited from innate immune cells activated by pathogen recognition, may affect Th1 development through multiple mechanisms; it can act early, by recruiting IFN-γ production from NK cells, and late, by driving STAT4-dependent IFN-γ production in concert with TCR or IL-18 signaling.

Th2 differentiation is initiated by TCR signaling in concert with IL-4 receptor signaling via STAT6 (Figure 1b). Signals that emanate from the TCR and IL-4 receptors act cooperatively to upregulate low-level expression of GATA3, master regulator of Th2 differentiation (29–31). GATA3 autoactivates its own expression and drives epigenetic changes that enable expression of the Th2 cytokine cluster (*Il4*, *Il5*, and *Il13* genes), while suppressing factors critical to the Th1 pathway, such as STAT4 and the IL-12Rβ2 chain (32–34). Thus, early IL-4 signaling rapidly initiates positive and negative feedback loops that serve to reinforce early commitment to Th2 development while extinguishing Th1 development.

The cellular sources of the polarizing cytokines responsible for Th1 and Th2 development in vivo have been the subject of considerable debate. Although Th1 and Th2 cells can themselves provide IFN-γ or IL-4 for the recruitment of Th1 or Th2 differentiation, respectively, investigators have not yet definitively determined which cells initiate effector T cell differentiation in primary versus secondary responses. Plasmacytoid dendritic cells (DCs), NK cells, or NKT cells appear to be involved in early production of type I and II IFNs for induction of Th1 cells, but which cells initiate Th2 development is less clear. Basophils, eosinophils, mast cells, and NKT cells are sources of IL-4 that may be important for Th2 differentiation (35–39), and each of these cell populations may be important for initiating Th2 responses to distinct pathogens or in distinct settings.
Figure 1
Models for (a) Th1 and (b) Th2 differentiation. See text, and references therein, for details.
**THE IL-17 CYTOKINE FAMILY**

**Discovery, Family Relationships, and Origins**

Recent discovery of the IL-17 cytokine family has provided a new pathway for crosstalk between, and coordination of, adaptive and innate immunity. The family now includes six members (Table 1), at least three of which are produced by T cells and have potent proinflammatory properties. A cDNA encoding IL-17 was isolated from a murine cytotoxic T lymphocyte (CTL) hybridoma cDNA library in a screen to identify inducible CTL-associated transcripts, and was originally called CTLA-8 (cytotoxic T lymphocyte-associated-8) (39a). Subsequently, the homologous T lymphotrophic herpesvirus Saimiri gene 13 was cloned and used to isolate a cDNA encoding a receptor that bound CTLA-8 (40). The mammalian and viral homologs were renamed IL-17 and vIL-17, respectively, and the receptor was named IL-17R. Five homologous cytokines were later identified through database searches and degenerative PCR strategies (see 41 for review). IL-17 has been designated IL-17A to indicate that it is the founding member of this extended, six-member cytokine family, which now includes IL-17A–F. IL-17E was independently identified and named IL-25 (42), a nomenclature that we use here.

IL-17F has the highest homology with IL-17A, as they are 50% identical at the protein level. IL-17B–D have lower homology, and IL-25 (IL-17E) is the least related, sharing only 16% identity at the primary amino acid sequence in humans. IL-17A and IL-17F are syntenic, tightly linked in all species examined to date; the remaining family members each map to different chromosomes. IL-17 shares no sequence homology with other known mammalian proteins and therefore constitutes a distinct cytokine family.

Recombinatorial systems for generation of diverse antigenic receptors in lymphocytes appear at the dawn of vertebrate evolution and define the emergence of the adaptive immune system approximately 500 mya (43). The emergence of most cytokines coincided with vertebrate evolution and, with the exception of IL-1 receptor–related molecules (including TLRs) and TGF-β, they have not been identified in invertebrates. Homologs of the IL-17 family have been identified in all vertebrates examined to date. As with most other cytokine families, the IL-17 family appears to have arisen prior to the divergence of teleosts (bony fishes) and tetrapods (land-dwelling vertebrates). Five genes with homology to mammalian IL-17 members have been identified in zebrafish, suggesting that gene duplications that gave rise to the IL-17 family occurred very early in vertebrate evolution (44). Three of the zebrafish genes show homology to both IL-17A and IL-17F, and, although two are linked, they do not show synteny with human IL-17A or IL-17F. IL-17D is the most evolutionarily conserved IL-17 family member, with four divergent fish species sharing significant sequence identity and synteny with the human and mouse IL-17D genes. Interestingly, an IL-17R homolog has been identified in lamprey, an ancient jawless fish, suggesting that the IL-17 family may have been one of the first cytokine families to emerge (45). Because IL-17 expression can be regulated by TGF-β and IL-1 family members, both more primitive components of the innate immune system, IL-17 may have evolved to bridge innate and adaptive immune functions. Th2 cytokines (IL-4, IL-5, and IL-13) have only been identified in mammals, indicating emergence much later in evolution, and have recently been identified as targets for induction by IL-25 (42).

**Structure and Function**

The crystal structure of a single IL-17 family member, IL-17F, has been solved (Figure 2) (46). It reveals that IL-17F is a structural homolog of the cysteine knot family of proteins, so named for their unusual pattern of intra-chain disulfide bonds. A similar structural motif is found in growth factors such as TGF-β,
Table 1  The mouse IL-17 cytokine family

<table>
<thead>
<tr>
<th>Family member</th>
<th>Alternate names</th>
<th>Gene name</th>
<th>Chromosomal location</th>
<th>Predicted molecular weight (kDa)</th>
<th>% Homology with IL-17A</th>
<th>% Homology with human</th>
<th>Cellular sources</th>
<th>Receptor (alternative name)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-17A</td>
<td>IL-17 CTLA-8</td>
<td>Il17a</td>
<td>1A4</td>
<td>35</td>
<td>100</td>
<td>62</td>
<td>Th17 cells, CD8 T cells, NK cells, γδ T cells, neutrophils</td>
<td>IL-17RA (IL-17R)</td>
</tr>
<tr>
<td>IL-17B</td>
<td>CX1 NERF</td>
<td>Il17b</td>
<td>18D3</td>
<td>41</td>
<td>21</td>
<td>88</td>
<td>?</td>
<td>IL-17RB (IL-17RH1) (IL-25R)</td>
</tr>
<tr>
<td>IL-17C</td>
<td>CX2</td>
<td>Il17c</td>
<td>8E1</td>
<td>43</td>
<td>24</td>
<td>75</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>IL-17D</td>
<td>IL-27</td>
<td>Il17d</td>
<td>14C3</td>
<td>45</td>
<td>16</td>
<td>82</td>
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<td>IL-17RB (IL-17RH1) (IL-25R)</td>
</tr>
<tr>
<td>IL-17E</td>
<td>IL-25</td>
<td>H25</td>
<td>14C2</td>
<td>38</td>
<td>16</td>
<td>76</td>
<td>Th17 cells, CD8 T cells, NK cells, γδ T cells, neutrophils</td>
<td>IL-17RA (IL-17RL)</td>
</tr>
<tr>
<td>IL-17F</td>
<td>ML-1</td>
<td>Il17f</td>
<td>1A4</td>
<td>34</td>
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<td>Th17 cells, CD8 T cells, NK cells, γδ T cells, neutrophils</td>
<td>IL-17RA (IL-17RL)</td>
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</tbody>
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nerve growth factor (NGF), bone morphogenetic proteins, and platelet-derived growth factor-BB, except that in these other growth factors the cysteine knot is formed with six cysteines rather than four. The four cysteines that form the cysteine knot structure in IL-17F are conserved in all IL-17 family members and across species.

Structural features of IL-17 family members deduced from the crystal structure of IL-17F suggest that, similar to many cytokines, each of the family members is likely produced as a homodimer, although structural similarities do not exclude the possibility that heterodimers may exist. With the exception of IL-17B, the homodimers are stabilized by interchain disulfide bridges. IL-17F dimerizes in a parallel fashion to that of NGF and other neurotrophins, with each monomer forming a relatively large, flat interface that is stabilized by interchain disulfide links. Along the edges of contact faces of the IL-17F homodimer are two large cavities positioned similarly to sites where NGF binds its high-affinity receptor, TrkA, suggesting parallels between IL-17 family members and neurotrophins with respect to receptor recognition. Interestingly, modeling of other IL-17 family members on the basis of the IL-17F structure suggests that this surface feature is conserved, with the lateral cavities lined by variable residues that may confer receptor binding specificity. By extrapolation to the binding of NGF with its high-affinity receptor, researchers propose that IL-17 family members likely interact with their cognate receptors through interactions in these surface cavities (46).

Like the IL-17 cytokine family, the IL-17 receptors form a unique family (reviewed in 47). There are five family members, including the founding member, IL-17R (or IL-17RA), and four additional members have been identified through sequence homology searches. Individual family members share only limited sequence similarity with each other and do not contain domains found in other proteins; they therefore constitute a novel family of cytokine receptors. All are predicted to

Figure 2
Structure of IL-17F. (a) Ribbon diagram of the IL-17F monomer, highlighting sulfur atoms of cysteines involved in disulfide bonds (yellow balls). The inset shows a representation of the canonical cystine knot fold. (b) Ribbon diagram of the IL-17F homodimer, with the disulfides (inter- and intrachain) highlighted as in (a). (c and d) Orthogonal surface views of the side (c) and front (d) of the IL-17F homodimer, with acidic residues indicated in red, basic residues in blue, and neutral residues in white. The positions of the potential receptor binding cavities are indicated by broken circles. Used with permission from Reference 46.
be single-pass transmembrane proteins with an extracellular amino terminus and large intracellular tails. Four of the IL-17 receptor family members map to human chromosome 3 in two tightly linked clusters: IL-17RB (also called IL-17RH1 or IL-25R) with IL-17RD (also called SEF or IL-17RLM), and IL-17RC (also IL-17RL) with IL-17RE. There is similar clustering of these pairs in the mouse. Evidence suggests that, with the notable exception of IL-17RA, each of these receptors has an alternative splice variant. Alternative splicing of IL-17RB and IL-17RC creates frameshifts and introduces stop codons that result in secreted soluble proteins (48, 49). Presumably these soluble receptors retain their ligand-binding properties and act as decoys, although this has not been demonstrated.

Detailed functional studies of the IL-17 receptor family have not yet been reported. The best-studied member to date, IL-17RA, binds both IL-17A and IL-17F, although IL-17A binds with more than tenfold higher affinity (46). Expression of IL-17RA appears to be ubiquitous, hence the broad tissue responsiveness to IL-17A. Mice with targeted deletion of Il17ra have profound defects in host protection (50), consistent with a critical role for IL-17A and IL-17F in host defense. Of the four remaining family members, only IL-17RH1 has been shown to bind IL-17 cytokines, namely IL-17B and IL-25 (51, 52); IL-17RL, IL-17RD, and IL-17RE have only been identified by sequence similarities to IL-17R, and their ligand specificities are not yet reported.

Like all other known cytokine receptors, IL-17RA appears to be expressed as a multimer, existing as a preformed complex prior to ligand binding (53). Interestingly, the IL-17RA complex undergoes a conformational change upon binding of IL-17A or IL-17F, which leads to dissociation of the intracellular domains. Whether IL-17R family members can interact with other, nonfamily member components for signal transduction is unknown, although the cytoplasmic domains do not appear to contain identifiable catalytic motifs (40, 54). In this regard, it is notable that the neurotrophins, with which the IL-17R ligands have structural homology, not only bind specific Trk receptors, but also bind simultaneously to p75NTR, a common second receptor component. Although not yet proven, the IL-17 receptors may be multicomponent signal transducers analogous to neurotrophin receptors (46). A single report has identified the participation of a second IL-17R family member, IL-17RC, as a component of a heteromeric IL-17R that includes IL-17RA (54a), suggesting greater complexity in the IL-17R family than previously appreciated.

Relatively little is known regarding signal cascades generated by ligand binding of IL-17 family members. Current studies indicate that IL-17Rs may signal through MAP kinases or NF-κB, the latter suggesting similarities to the tumor necrosis factor (TNF), IL-1, and TLR families. In this regard, IL-17R signaling is impaired by deficiency of TNF-receptor-associated factor 6 (TRAF-6) (55). However, there is no identifiable homology of the IL-17R intracellular domain with those of the TNF, IL-1, or TLR families. Thus, neither the intracellular nor extracellular domains of IL-17 receptors resemble other receptors, establishing their unique evolutionary origins and function.

**Cellular Sources and Targets**

The cellular sources for members of the IL-17 family are distinct. IL-17A and IL-17F were originally associated with memory CD4 T cells and have more recently been specifically linked to the Th17 lineage (reviewed in 41, 56). However, IL-17A and IL-17F do not appear to be limited to CD4 effector T cells. CD8 T cells, γδ T cells, and NK cells have also been identified as sources of these two cytokines, as have neutrophils (57–59; L. Harrington & C. Weaver, unpublished observations). To date, there are no reports of discordant expression of IL-17A and IL-17F by immune cells, consistent with their tightly...
clustered genomic organization and, perhaps, coordinate regulation during effector T cell development. These two genes therefore appear to comprise a Th17 gene cluster analogous to the Th2 gene cluster composed of the Il4, Il5, and Il13 genes. IL-17D may be expressed by resting CD4 T cells and at low levels from B cells, although its function is not yet well defined (60). IL-17B and IL-17C do not appear to be products of lymphocytes, but they may be recruited indirectly by IL-17A and IL-17F and have a number of overlapping activities with these family members (47, 51, 61, 62). IL-25 has been linked to Th2-type responses and is produced by in vitro–derived Th2 lines (42), although whether IL-25 is expressed by classical Th2 cells in vivo is unclear (see below).

Both IL-17A and IL-17F can induce the expression of diverse proinflammatory cytokines and chemokines from a large variety of cells, as expected from the broad tissue expression of IL-17RA (reviewed in 41). In the initial characterizations of IL-17A, it was found to be a potent inducer of IL-6 and IL-8 (CXCL8) by fibroblasts (40, 63). Subsequent studies have demonstrated proinflammatory effects on a broad range of cellular targets, including epithelial and endothelial cells, fibroblasts, osteoblasts, and monocyte/macrophages. Depending on the target cell population, principal activities of IL-17A and IL-17F include the induction of expression of colony-stimulating factors (e.g., GM-CSF and G-CSF), CXC chemokines (e.g., CXCL8, CXCL1, and CXCL10), metalloproteinases, and IL-6. Accordingly, IL-17A and IL-17F have potent actions to mobilize, recruit, and activate neutrophils. Indeed, the capacity of T cell–derived IL-17A and IL-17F to expand and recruit the neutrophil pool is a defining feature of these family members and provides a novel mechanism by which T cells coordinate adaptive and innate immunity (see 41, 64 for review). Although there appears to be considerable overlap in the expression and functions of IL-17A and IL-17F, there are also likely to be nonredundant features; the substantially higher affinity of IL-17R for IL-17 over IL-17F suggests that IL-17F may preferentially bind a distinct member of the IL-17R family (46). Consistent with this, mice with a targeted deletion of the Il17a gene alone have a profound phenotype that does not appear to be compensated by intact Il17f expression (65, 66).

Interestingly, some actions of IL-17A and IL-17F are potentiated by other inflammatory cytokines, particularly TNF-α and IL-1β. In this regard, it is notable that IL-17B and IL-17C are potent activators of TNF-α and IL-1β expression by certain macrophage populations that lack IL-17RA, providing a mechanism for coordinating inflammation by subsets of the IL-17 family (41).

In contrast to IL-17A and IL-17F, IL-25 (IL-17E) induces the expression of CC chemokines, such as CCL5 (RANTES) and CCL11 (Eotaxin 1), both of which are important for the recruitment of eosinophils, as well as the expression of Th2 cytokines, IL-5, IL-13, and perhaps IL-4 (42, 67). Recent studies have identified a novel innate immune cell population that is a target for IL-25 actions (discussed below), hence the involvement in Th2-type allergic responses. Therefore, distinct IL-17 family members are potent orchestrators of innate immunity mediated by polymorphonuclear leukocytes; IL-17A and IL-17F recruit neutrophilic responses, whereas IL-25 recruits eosinophilic and basophilic responses.

THE Th17 LINEAGE: A NEW ARM OF ADAPTIVE IMMUNITY

Crosstalk Between the IL-17 and IL-12 Cytokine Families: New Lessons from Models of Infection and Autoimmunity

In a reprise of sorts of the seminal report that linked Th1 polarization to APC activation by the intracellular pathogen, L. monocytogenes (15), Kamradt and colleagues (68) examined the effects of the causative agent of Lyme
disease, the spirochete Borrelia burgdorferi, on the in vitro development of effector CD4 T cells. Compared with controls differentiated under classic Th1-polarizing conditions by addition of IL-12, the gene expression profile of T cells differentiated in the presence of B. burgdorferi sonicates or B. burgdorferi–derived lipopeptides was distinct and included increased levels of IL-17A mRNA. Importantly, single-cell analyses identified a fraction of effector T cells positive for IL-17A, GM-CSF, and TNF-α and negative for IFN-γ or IL-4, revealing a novel cytokine phenotype distinct from Th1 or Th2. This report not only established the first link between bacterial infection and a new effector T cell phenotype later to become Th17, but it also presaged description of a factor later identified as critical to Th17 development: IL-6.

Concurrent with these findings, Kastelein and colleagues (69, 70) reported the discovery of the first new member of what was to become the five-member IL-12 family (see also Reference 131 in this volume). IL-12, the family prototype, is composed of IL-12p40 and IL-12p35 subunits that form a disulfide-linked heterodimer required for function. On the basis of greater susceptibilities to infection of mice deficient for the IL-12p40 (Il12a−/−), compared with those deficient for IL-12p35 (Il12b−/−), investigators speculated that IL-12 might form functional complexes with other partners (70). In a database search for sequences homologous to IL-12p40, a novel protein was identified (IL-23p19) that could pair with IL-12p40 to form a distinct, heterodimeric cytokine that was named IL-23 (Figure 3). Soon after, Aggarwal et al. (71) reported that IL-23, but not IL-12, stimulated memory, but not naive, CD4 T cells to produce IL-17A and IL-17F, a pivotal finding that linked the IL-17 and IL-12 families and that was consistent with a unique effector CD4 T cell population similar to that previously reported by Kamradt and colleagues (68).

Definitive evidence identifying an IL-23–dependent pathway for IL-17–producing T cells came from studies of immune pathogenesis in murine models of autoimmunity. Experimental autoimmune encephalomyelitis (EAE) and collagen-induced arthritis (CIA) had been associated with aberrant Th1 responses, in part on the basis of studies demonstrating that disease development was blocked in mice deficient in the p40 subunit of IL-12 (Il12p40 or Il12b) or in mice treated with IL-12p40-specific neutralizing antibodies (72–74). However, there were also data from a number of studies that were inconsistent with a simple Th1 or IL-12–IFN-γ cytokine axis link in EAE and CIA: Mice deficient in IFN-γ were susceptible to both diseases, as were mice deficient in proximal components of IFN-γ signaling pathway (Ifngr−/− and Stat1−/−) (75–80).

Cua and coworkers (81, 82) provided a resolution of this paradox when they revisited the immunopathologic basis for EAE and CIA using mice deficient in either IL-12 or IL-23, or both. They found that disease development was ablated in mice deficient in IL-23 or both IL-23 and IL-12, but not in mice deficient in IL-12 only. Mice deficient in the IL-23p19 subunit (Il23a−/−, lacking IL-23 only) or the IL-12p40 subunit (Il12b−/−, lacking both IL-23 and IL-12) were resistant to EAE and CIA, whereas IL-12p35-deficient mice (Il12a−/−, lacking IL-12 only) remained susceptible. These findings were consistent with another study that found that mice lacking the IL-12 receptor complex (Il12rb2−/−) also succumbed to EAE (83). Thus, IL-23, not IL-12, is critically linked to autoimmunity in these models.

Consistent with the previous identification of induction of IL-17A by IL-23 (71) was the finding that mice deficient in IL-23 lost development of a population of an IL-17–expressing subset of CD4 T cells, while retaining IFN-γ-producing, Th1-type cells. A reciprocal pattern of IFN-γ- and IL-17A–positive cells was found in IL-12p35-deficient mice that developed exacerbated disease compared with wild-type controls (82). A positive correlation was established between IL-23, IL-17–producing effector T cells, and experimental autoimmune encephalomyelitis (EAE): a rodent model of multiple sclerosis induced by immunization with antigenic components of myelin, leading to a T cell–dependent central nervous system inflammation resulting in focal demyelination

Collagen-induced arthritis (CIA): a T cell– and antibody-mediated rodent model of autoimmune arthritis in which chronic joint inflammation is induced by immunization with collagen proteins; models many aspects of rheumatoid arthritis
Cytokine pathways involved in Th17 development. Shown are the key cytokines and receptor pathways by which Th17 lineage commitment is induced, or in the case of IL-27, suppressed. Structural homology between the IL-6, IL-23, and IL-27 receptors is evident, including the shared component of the IL-6 and IL-27 receptors, gp130. The TGF-β, IL-6, and IL-27 receptors are expressed by naive T cells, as is the IL-12Rβ1 chain that is common to the IL-12 and IL-23 receptors. The inducible chain of the IL-23 receptor, IL-23R, is expressed downstream of Th17 lineage commitment. The TGF-β receptor phosphorylates the receptor-regulated Smads (RSmads) 2 and 3 upon binding of activated ligand that has been released from latency-associated peptide components, permitting recruitment of the co-Smad, Smad4 and nuclear translocation (see References 129 and 130 for reviews). The mechanisms by which active TGF-β is generated for T cell differentiation are unknown. Activation of the principal STATs thought to be important in Th17 differentiation is indicated in black; other STATs recruited by these receptors are indicated in gray, but their function in Th17 biology is unclear.

Figure 3

Annu. Rev. Immunol. 2007.25:821-852. Downloaded from arjournals.annualreviews.org by CNRS-multi-site on 12/06/07. For personal use only.
antigen proteolipid protein peptide, and enriched by culture with IL-23, induced severe EAE in recipient mice following passive transfers, whereas Th1 cells enriched by culture with IL-12 did not (88). This study further defined functional and phenotypic differences of so-called ThIL-17 cells and Th1 cells, demonstrating that gene expression profiles of these two subsets were quite distinct. Thus, whereas IL-12-polarized cells, i.e., prototypic Th1 cells, preferentially expressed genes associated with cytotoxicity (IFN-γ, FasL, and granzymes), IL-23-polarized cells expressed genes associated with chronic inflammation (IL-17/IL-17A, IL-17F, IL-6, TNF-α, and proinflammatory chemokines). These results confirmed a new role for IL-17-producing effectors cells (later termed Th17) in immunopathogenesis and strongly suggested that Th1 and Th17 cells represent distinct effector subsets that develop under differential conditions of IL-12 or IL-23 conditioning.

Administration of neutralizing antibody specific for IL-23 proved an effective therapy for both inhibiting and treating EAE (89). Treatment with anti-IFN-γ resulted in significant worsening of clinical disease, associated with elevated levels of serum IL-17A and consistent with an antagonistic role for IFN-γ in Th17-mediated CNS inflammation. Treatment with anti-IL-17A had a significant, but modest, effect on amelioration of CNS inflammation and clinical disease score compared with treatment with anti-IL-23, in line with previous studies showing only partial re-mediation by blockade of IL-17. Whether this is due to compensatory effects of IL-17F, which is also produced by Th17 cells, IL-17-independent effects of IL-23, or both remains to be determined.

**Th17: An Effector Lineage Distinct from Th1 and Th2**

On the basis of these findings, investigators proposed two alternative models for differentiation of Th17 cells (64, 90). In one model, the early differentiation of Th1 and Th17 from naive CD4 T cell precursors was shared, such that IFN-γ- and IL-17-expressing subsets diverged contingent upon selective availability of IL-12 and IL-23 acting on a common Th1-precursor or pre-Th1 intermediate that coexpressed both IL-12 and IL-23 receptors. In the other model, the development of IFN-γ and IL-17 producers was nonoverlapping and represented distinct lineages. Because T-bet and STAT4 are required for disease development in EAE and CIA, whereas IL-12, IFN-γ, and STAT1 are not, a corollary of the latter model predicts that T-bet and STAT4 contribute to disease development through Th17-independent mechanisms, or perhaps have critical roles in both lineages.

In a pair of reports, one from our group and the other from Dong and colleagues (91, 92), direct support for a Th1-independent pathway of Th17 differentiation was established (Figure 4). We found that IL-23 failed to induce IL-17 production from Th1-polarized cells, indicating that Th1 cells are not IL-23-responsive. Furthermore, both type II and type I IFNs, which activate STAT1-induced expression of T-bet and Th1 commitment, strongly inhibited Th17 development in vitro. Together, these findings indicated not only that the Th1 pathway is nonpermissive to Th17 development, but also that its defining cytokine, IFN-γ, actively suppresses Th17 development. Indeed, neutralization of IFN-γ and IL-4—whether by blocking antibodies or genetic deficiency—was required to induce appreciable IL-17-producing effectors under the conditions examined. Key signaling components of Th1 and Th2 differentiation—STAT1, T-bet, STAT4, and STAT6—were dispensable for Th17 development. These findings were extended in vivo by Park et al. (92), who showed that Th17 development was unimpaired in immunized mice deficient for
Type 17 pathogens
(Bacteria, fungi)

IFN-γ or T-bet. Collectively, these studies established that IL-17-producing effectors develop via a lineage that is distinct from, and antagonized by, the Th1 and Th2 lineages.

These findings offer explanations for a number of the paradoxical effects observed in the development of EAE and CIA in mice with Th1-lineage defects. Given the pathogenic potential of Th17 cells and the suppressive effects of IFN-γ on Th17 development, a mechanism now exists to explain why mice with targeted deletions of IFN-γ, IFN-γR, IL-12, or STAT1 display exacerbated disease development. On the contrary, the disease resistance of mice with genetic or siRNA-induced T-bet deficiency does not appear to be due to a defect in Th17 development (92, 93), implicating T cell–independent mechanisms by which T-bet contributes to pathology. Thus, T-bet may have complex roles in the Th17 inflammatory cascade outside of the T cell compartment. In a similar vein, why STAT4-deficient mice are also resistant to EAE (94),...
despite an absence of requirement for STAT4 in commitment to Th17 development, is unclear. Because, at least in vitro, STAT4 is dispensable for Th17 development, it remains to be determined whether the disease resistance of STAT4-deficient mice is due to a role for this factor in Th17 function or survival, amplification of Th17 cytokine production via IL-23, or a Th17-independent mechanism.

**Th17 Differentiation: A New Role for Old Cytokines**

Despite the clear role for IL-23 in Th17-mediated immunopathology and host protection, three independent studies found that IL-23 is not required for Th17 commitment (95–97). In each study, development of IL-17A/IL-17F-producing effectors was undiminished under conditions of IL-23 deficiency, and Th17 development was not enhanced by addition of exogenous IL-23. Furthermore, differential upregulation of IL-12Rβ2 and IL-23R was associated with signals that induced Th1 and Th17 differentiation, respectively (91). Thus, as in Th1 development, Th17 development is initiated independently of a requirement for signaling by an IL-12 family member; in analogous fashion to the induced expression of the variable component of the IL-12 receptor, IL-12Rβ2, the variable component of the IL-23 receptor, IL-23R, is upregulated downstream of signals that initiate Th17 differentiation, thereby conferring IL-23 responsiveness. IL-23 signaling is therefore not required for Th17 commitment and early IL-17 production, but instead appears to be important for amplifying and/or stabilizing the Th17 phenotype, a basis for the finding that IL-23 augments IL-17 production from memory, but not naive, CD4 T cells (71).

In efforts to define IL-23-independent factors that initiated Th17 cell development, investigators found that TGF-β and IL-6 act cooperatively and nonredundantly to achieve Th17 commitment (95–97) (Figures 3 and 4). Stockinger and coworkers (95) showed that naive T cells activated in the presence of CD4+CD25+ regulatory T cells (Tregs) exhibited suppressed levels of IFN-γ and IL-2 production but expressed high levels of IL-17A. Antibody blockade of TGF-β in cultures containing LPS-activated DCs, Tregs, and naive CD4 T cells activated with anti-CD3 identified TGF-β as a critical factor for Th17 development. Importantly, in addition to TGF-β, differentiation of IL-17-producing effector cells required a soluble DC factor elicited by TLR- and MyD88-dependent signaling, which proved to be IL-6. TNF-α and IL-1β could amplify the frequency of IL-17-producing effectors, but they were nonessential cofactors. Analysis of transcription factor expression by these Th17 cells showed that compared with Th1 and Th2 cells, Th17 effectors lacked expression of T-bet, Hlx, and GATA3, supporting and extending previous findings identifying the Th17 cell as a product of an effector lineage distinct from Th1 and Th2 (91, 92).

Our group, and Kuchroo and coworkers, independently identified TGF-β as a critical factor for Th17 commitment and showed further that IL-6 acted to deviate TGF-β-driven development of Foxp3-expressing Tregs toward Th17 (96, 97). Thus, the addition of IL-6 suppressed the TGF-β-induced generation of Foxp3+ Tregs, while reciprocally promoting the generation of Th17 cells (reviewed in 56). TGF-β addition under conditions of endogenous IL-6 production resulted in upregulation of the IL-23R component of the IL-23 receptor, in contrast to the effects of IFN-γ, which upregulated the IL-12Rβ2 component of the IL-12 receptor, but not IL-23R. More recent studies indicate that high levels of IL-6 can induce IL-23R independently of TGF-β (P. Mangan & C. Weaver, unpublished observations). Accordingly, the antagonistic effects of TGF-β/IL-6 versus IFN-γ signaling early in the activation of naive T cells deviate lineage development toward Th17 or Th1, with concomitant upregulation of the inducible components of the
IL-23 or IL-12 receptor components, respectively. To extend these findings in vivo, we explored TGF-β1-deficient mice (96). Mice homozygous for TGF-β1 deficiency were essentially devoid of Th17 cells, which are normally enriched in the lamina propria of the intestine and in mesenteric lymph nodes (59; P. Mangan & C. Weaver, unpublished observations). Mice hemizygous for TGF-β1 deficiency showed an intermediate phenotype compared with controls, and circulating levels of IL-17 correlated with these phenotypes. Bettelli et al. (97) showed that transgenic expression of TGF-β under control of the IL-2 promoter enhanced Th17 development and exacerbated EAE, whereas IL-6 deficiency inhibited Th17 development. Complementary studies by Stockinger and coworkers (97a) found that deficiency of TGF-β signaling specific to T cells blocked EAE development, consistent with the in vitro observations.

Collectively, these studies support a model in which TGF-β plus IL-6, IFN-γ, or IL-4 initiate commitment to the Th17, Th1, or Th2 lineages, respectively (Figures 1 and 4). The requirement for TGF-β in Th17 differentiation is shared with adaptive Tregs, which default to Foxp3 induction in the absence of IL-6. Thus, IL-6, elicited by pathogen-induced activation of innate immune cells via TLRs, is a critical switch factor that diverts antigen-activated naive T cells toward a proinflammatory rather than anti-inflammatory adaptive response (98, 99; reviewed in 56). The dichotomous actions of TGF-β on Th17 versus Treg development extend and reinforce previous studies indicating that TGF-β actions are contextual (reviewed in 100) and provide a new framework in which to view TGF-β actions in adaptive immunity.

What Role for IL-23?

The foregoing studies identified critical roles for TGF-β and IL-6 as proximal factors essential for induction of Th17 development and established that IL-23 functioned subsequent to Th17 commitment, contingent upon upregulation of the inducible component of the IL-23 receptor, IL-23R. Given the critical requirement for IL-23 in Th17 function in vivo, IL-23 appears to be essential to expand and maintain committed Th17 effectors and/or extend their function. In this regard, it is noteworthy that IL-12 acts in Th1 lineage development not only to amplify IFN-γ production by Th1-committed precursors that have upregulated the inducible chain of the IL-12 receptor, but also to induce upregulation of the IL-18Rα chain, thereby inducing a TCR-independent pathway to IFN-γ production via the coordinated signaling of IL-12 and IL-18 (Figure 1a). A similar pathway is now apparent for the Th17 lineage.

IL-18 is a member of the IL-1 superfamily of proinflammatory cytokines that is produced by cells of the innate immune system (e.g., DCs) and signals through the IL-18 receptor (IL-18R), a heterodimer consisting of the ligand-binding subunit (IL-18Rα) and a signaling subunit (IL-18Rβ; also known as IL-1R87 or IL-1RacPL) (101, 102). Signaling through the IL-18R has parallels with the IL-1 and TLRs via activation of MyD88 and IRAK4. IL-18Rα upregulation on Th1 cells is IL-12- and STAT4-dependent (27, 103–105). In view of initial reports of STAT4 activation by IL-23 (70), we recently explored the possibility that Th17 cells, like Th1 cells, might use IL-23-induced IL-18 signaling to amplify IL-17 production. We found that Th17 cells polarized by TGF-β and IL-6 in the absence of IL-23 produce IL-17 when stimulated by IL-23 plus IL-18 or IL-23 plus IL-1β, but were poorly responsive to IL-23 alone (Y. Lee & C.T. Weaver, unpublished observation). Thus, Th17 cells appear to parallel Th1 cells with respect to development of a TCR-independent mechanism for cytokine production (Figure 4). Furthermore, Th17 commitment induced by TGF-β and IL-6 may prime developing Th17 cells for IL-23 responsiveness, but IL-23 may be essential for further differentiation of Th17 cells.
that is required for their optimal production of IL-17A and IL-17F at inflammatory sites. Accordingly, we found that IL-23 deficiency did not prevent the development of comparable numbers of IL-17-competent CD4 T cells in vivo in response to challenge with the intestinal pathogen, Citrobacter rodentium, although host protection was ablated (56, 96). Thus, IL-23 has nonredundant functions in host protection and autoimmunity that are independent of Th17 induction.

Remarkably, Th17 cells may depend on a novel ligand to activate IL-18R for IL-17A/IL-17F production in vivo. In a study that examined the IL-18 pathway in EAE, Gutcher et al. (106) found that IL-18-deficient mice were fully susceptible to disease, whereas IL-18Rα-deficient mice were resistant and characterized by a deficient Th17 response. Disease development in Il18<sup>−/−</sup> mice was attenuated by blocking antibodies to IL-18Rα, implicating an alternative IL-18Rα ligand that drives Th17-dependent immunopathology in this model. Although these data are in conflict with other studies that indicate protection in IL-18-deficient mice (107), they nevertheless suggest that an alternative IL-18R ligand exists and may be important for Th17 development or maintenance. Thus, the IL-18 signaling cascade appears to be important in Th17-mediated immunopathology, through binding of an as yet unidentified ligand distinct from IL-18. Interestingly, IL-18Rα-expression on APCs, not T cells, appeared to be necessary for disease development, implicating a more complex cytokine network for the IL-18R pathway in Th17-mediated disease pathogenesis than previously appreciated. Another study reported that mice deficient for IL-1 receptor type I are resistant to IL-1-induced IL-17 production and are resistant to EAE (108). Thus, both IL-1- and IL-18-associated pathways appear important for Th17 function, although additional studies are needed to clarify their respective roles.

Although the principal focus on IL-23 function has been the Th17 pathway and thus adaptive immune induction and function, a report by Powrie’s group (109) identifies an important role for IL-23 in directly activating the innate immune system. Using a model in which systemic administration of the DC-activating antibody, anti-CD40, bypassed the normal T cell requirement for induction of murine inflammatory bowel disease (IBD), these investigators found that many of the pathologic features of IBD could be demonstrated in RAG-deficient mice and were therefore T cell– and B cell–independent. Anti-CD40-induced immunopathology was effectively inhibited by administration of anti-IL-12p40 antibody, as well as with neutralizing antibodies to TNF-α and IFN-γ. Treatment with anti-IL-12- or anti-IL-23-specific antibodies revealed distinct roles for IL-12 and IL-23 in this setting. Neutralization of IL-12 resulted in the reversal of systemic wasting and inhibition of elevated serum levels of proinflammatory cytokines (TNF-α, IL-6, and MCP-1, albeit modestly). Conversely, neutralization of IL-23 resulted in dramatic reductions in intestinal expression of these same cytokines, whereas treatment with anti-IL-12 had either no effect or, in the case of IL-6, resulted in exaggerated levels of expression. Furthermore, treatment with anti-IL-23, but not anti-IL-12, attenuated intestinal inflammation, supporting a specific role for IL-23 in local mucosal inflammation. These results were supported by gene ablation studies using RAG-deficient mice crossed into IL-12−, IL-23−, and IL-12/IL-23-deficient mice. Remarkably, anti-CD40 administration induced intestinal expression of IL-17A mRNA independently of T cells, and this was ablated in IL-12p40- and IL-23p19−, but not IL-12p35-deficient mice. Thus, IL-23 can act on non-T cell populations to induce expression of IL-17A. Although the cellular sources responsible for IL-17 production were not identified, these data nevertheless provide striking evidence that the IL-23–IL-17 cytokine axis may play a central role in intestinal immune pathology via T cell–independent mechanisms.
Recent studies have extended links with the IL-23-IL-17 axis to other models of intestinal inflammation (110), identifying a positive correlation with this pathway and IBD development akin to that identified for EAE and CIA. Mice deficient in IL-10 develop a CD4 T cell–dependent, spontaneous colitis within several weeks of birth, depending on strain background (111). Rennick and colleagues (111) crossed IL-10-deficient mice with either IL-23-deficient ($p^{19-/-}$) or IL-12-deficient ($Il^{12p_{35}-/-}$) mice and assessed development of colitis. Mice doubly deficient for IL-10 and IL-12 developed comparable colitis, whereas mice doubly deficient for IL-10 and IL-23 failed to develop disease. On the basis of these findings, IL-23 was administered to RAG-deficient mice reconstituted with naive or memory T cells from IL-10-deficient mice. IL-23 accelerated the onset of colitis in both groups and was associated with elevations of IL-17A and IL-6. Examination of T cell populations from mice doubly deficient for IL-10 and IL-23 showed the persistence of a small number of IL-17A+ T cells, suggesting that generation of Th17 cells in this setting can occur in the absence of IL-23, consistent with our own studies indicating that p19-deficient mice developed IL-17A-competent T cells independently of IL-23 (96). Administration of anti-IL-6 and anti-IL-17A, either alone or in combination, attenuated disease development in mice administered IL-23, indicating that at least part of IL-23’s effects could be mediated by these cytokines. Elson at al. (C. Elson, Y. Cong, C. Weaver, T. Schoeb, T. McClanahan, R. Fick, and R. Kastelein, manuscript submitted) found that passive transfers of Th17 cells reactive to the intestinal bacterial flora potentially induced IBD compared with transfers of equivalent numbers of Th1 cells. Treatment of Th17 recipients with anti-IL-23 both blocked disease development and treated established disease, indicating, again, that even mature Th17 cells require IL-23 to exercise their pathogenic effects.

ROR$\gamma$t: A MASTER REGULATOR OF Th17 IDENTIFIED

For Th1, Th2, and a subset of Tregs, key transcription factors have been identified that can specify most of the genotypic and phenotypic characteristics of these lineages and have been termed master regulators. Thus, T-bet specifies Th1, GATA3 specifies Th2, and Foxp3 specifies Treg development. It had been speculated that Th17 development may be similarly linked to its own master. This now appears to be the case. A new report by Littman and coworkers (112) has identified the orphan nuclear receptor, retinoic orphan receptor (ROR) $\gamma$t, as a transcription factor that appears necessary, and can be sufficient, for Th17 development, providing compelling evidence that Th17 cells develop contingent upon a central transcriptional pathway that specifies their differentiation. ROR$\gamma$t is normally expressed in developing thymocytes (CD4+CD8+) as well as in lymphoid tissue inducer (LTi) cells and LTi-like cells important for development of cryptopatches and isolated lymphoid follicles in the lamina propria of intestinal tissues (113). To examine the role of ROR$\gamma$t in T cell development and lymphoid organogenesis, Littman and colleagues generated mice with the coding sequence for green fluorescent protein (GFP) knocked into the initiation site of ROR$\gamma$t translation, thereby reporting cells activating this locus while knocking out expression of ROR$\gamma$t from the targeted allele (114, 115). In a reanalysis of intestinal cells derived from these mice, a subpopulation of lymphocytes distinct from LTi cells and expressing lower GFP levels was identified. Independent studies that had examined genes differentially expressed by Th1 and Th17 cells identified elevated ROR$\gamma$t mRNA in Th17, but not Th1, cells, suggesting a link between the ROR$\gamma$t+ T cells in the intestinal lamina propria and the Th17 lineage. When lamina propria lymphocyte cells from ROR$\gamma$t(GFP)+ mice were isolated and stimulated, a large fraction of the GFP+ TCR $\alpha$$\beta$ T cells expressed IL-17 (approximately
60%), whereas the GFP− fraction did not. Furthermore, in mice homozygous for the reporter-targeted allele (and thus RORγt-deficient), the fraction of T cells expressing IL-17 was markedly diminished, though not completely eliminated. Collectively, these data established a strong correlation between expression of RORγt and IL-17A in intestinal effector sites. To investigate the link between RORγt and Th17 development further, these investigators derived Th17 effectors in vitro under Th17-polarizing conditions. Mice deficient for RORγt (which lack both RORγ and RORγt, products of alternative transcriptional start sites) showed impaired Th17 development in vitro compared with wild-type controls. Conversely, the induction of Th17 development was correlated with both RORγt expression and IL-17 and IL-17F expression. Importantly, the defect in RORγt-deficient mice was confined to IL-17-producing T cells; differentiation of IFN-γ-producing Th1 cells under Th1-polarizing conditions was normal or even enhanced compared with wild-type controls. Enforced expression of RORγt in naive CD4 T cells resulted in IL-17 production by approximately one-half of cells that expressed RORγt; this was in contrast to cells transduced with T-bet or empty vector retroviral vectors, neither of which showed significant IL-17 expression. Additional studies correlated the expression of RORγt with Th17 development and immunopathology in a model of EAE and demonstrated that IL-17F expression. Importantly, the defect in RORγt-deficient mice was confined to IL-17-producing T cells; differentiation of IFN-γ-producing Th1 cells under Th1-polarizing conditions was normal or even enhanced compared with wild-type controls. Enforced expression of RORγt in naive CD4 T cells resulted in IL-17 production by approximately one-half of cells that expressed RORγt; this was in contrast to cells transduced with T-bet or empty vector retroviral vectors, neither of which showed significant IL-17 expression. Additional studies correlated the expression of RORγt with Th17 development and immunopathology in a model of EAE and demonstrated that IL-17A-deficient mice showed a correlative loss of Th17 and RORγt+ intestinal lymphocytes. Thus, RORγt expression is IL-6- and TGF-β-dependent and is both necessary and sufficient to induce Th17 development by many CD4 T cells.

Collectively, results from this study establish that RORγt has a role in Th17 differentiation analogous to that for T-bet and GATA3 in the differentiation of Th1 and Th2 cells, respectively (112) (Figures 1 and 4). Unlike T-bet and GATA3, however, RORγt is a nuclear receptor whose putative ligand remains unidentified, and it is unknown whether RORγt activity is regulated by a ligand or is ligand-dependent. Assuming that the ligand(s) for RORγt must be coordinately expressed to effect RORγt function and, hence, Th17 specification, this could explain the imperfect correlation found in this study between RORγt expression and IL-17 production, although there are other explanations. It is also unknown whether RORγt directly induces IL-17 transcription or acts indirectly through the induction or suppression of other factors, although a ROR response element (RORE) has been identified in an evolutionarily conserved region of the IL-17A promoter, consistent with a direct role for RORγt in IL-17 transcription. In any case, linkage of Th17 differentiation to RORγt represents a major advance that should accelerate understanding of the signaling circuitry that specifies Th17 programming, including how Smad and STAT signals emanating from TGF-β and IL-6 receptors might cooperate to specify Th17 commitment.

IL-27: A BRAKE ON TH17 DEVELOPMENT?

Much as early characterization of IL-23 identified it as an inducer of Th1 immunity, a third IL-12 family member, IL-27, has been linked to Th1 responses, owing in part to its activation of the STAT1 signaling pathway and induction of T-bet (22, 23, 117). However, studies in infectious models have also identified a role for IL-27 in limiting adaptive immunity, at least in part through its actions on IL-2 production (118, 119). More recently, two studies have redefined the actions of IL-27 by demonstrating its important role in curbing Th17 responses by limiting development of Th17 effectors (120, 121).

Like IL-12 and IL-23, IL-27 is a heterodimeric cytokine and is composed of Epstein-Barr virus-induced gene 3 (EBI3) and p28, homologs of IL-12p40 and IL-12p35, respectively (see 69, 122 for review). IL-27 appears to be produced by similar cells as
IL-12 and IL-23 (e.g., activated DCs and macrophages), although few studies have directly addressed the cellular sources of IL-27. The receptor for IL-27 is composed of two subunits, IL-27Rα (also called WSX-1 or TCCR), expression of which is limited to immune cells, and gp130, which is shared with several members of the IL-6 receptor family and is expressed more widely on both immune and nonimmune cells (123). Unlike the IL-12 and IL-23 receptors, which require upregulation of their specific subunits (IL-12Rβ2 and IL-23R) downstream of early activation events, both components of the IL-27 receptor are expressed by naive T cells; it is therefore poised for early actions in regulating naive T cell activation and effector T cell development.

Clues to IL-27’s function in limiting effector T cell responses came initially from studies of parasitic infections in IL-27Rα-deficient mice (118). Although previous studies indicated that IL-27 could stimulate Th1 development from naive CD4 T cells in vitro, Hunter and colleagues (118) found that IL-27Rα-deficient mice nevertheless generated robust Th1 responses when challenged with *Toxoplasma gondii* and that they succumbed to an acute, lethal, CD4-dependent inflammatory disease (121). To explore the basis for this, *T. gondii*-infected mice were treated to promote longer-term survival and induction of more chronic disease. In this setting, IL-27Rα-deficient mice showed pronounced inflammatory changes in the CNS that were not evident in wild-type mice and that were eliminated by anti-CD4 treatment. When the CD4 T cells in CNS infiltrates were phenotyped, they were found to express IL-17, IL-6, and TNF-α, identifying them as Th17 cells and suggesting that IL-27 signaling might be required to suppress unchecked Th17 immunity.

Examination of the development of Th17 cells induced by TGF-β and IL-6 in vitro indicated that IL-27 suppressed development of Th17 effectors (121). In view of the fact that IL-6 is required for the development of Th17 cells, and that IL-6 and IL-27 share the receptor component gp130, it was speculated that IL-27 may act to inhibit Th17 development through competition with IL-6 for receptor binding. Notably, however, even in the setting of IL-6 neutralization, which substantially blocked Th17 development, the residual IL-17 production was further reduced by addition of IL-27, indicating that although competition for gp130 signaling cannot be absolutely excluded, IL-27 appears to have additional effects beyond simple competition for gp130 signaling. The inhibitory effect of IL-27 was abrogated in naive CD4 T cells from STAT1-deficient mice, establishing that suppression of Th17 development by IL-27 is STAT1-dependent, consistent with previous studies demonstrating inhibition by type I and type II IFNs, which also signal via STAT1 (91). In addition, and consistent with previous reports (91, 92), T-bet-deficient naive T cells developed into Th17 effectors without compromise, and inhibition by IL-27 was undiminished in T-bet-deficient precursors, indicating that activation of T-bet through a STAT1-dependent mechanism is not required for the suppressive effects of IL-27 on Th17 development.

Although the precise signaling mechanisms through which IL-6 and TGF-β cooperate to induce Th17 differentiation have not been delineated, a central role for IL-6-induced STAT3 activation is evident. Like many type 1 cytokines, IL-6 signaling via STAT3 activates SOCS3 (suppressor of cytokine signaling 3) expression, which provides a negative feedback mechanism for blocking cytokine expression, including IL-17 (124). IL-23 also induces IL-17 via STAT3, and loss of SOCS3 increases binding of STAT3 to the IL-17 promoter and enhances IL-17 production in response to IL-6 and TGF-β (124). IL-27 signaling can also induce SOCS3 via STAT1, raising the possibility that IL-27 might block Th17 development (as may type I and II IFNs) via sequential activation of STAT1 and SOCS3, resulting in STAT3 antagonism. However, although IL-17...
production induced by TGF-β and IL-6 was modestly increased in Soc3−/− mice, the suppressive effects of IL-27 were not greatly diminished (121). Therefore, the effects of IL-27 to inhibit IL-17 production are not due to enhanced SOCS3-mediated dampening of IL-6/gp130. Collectively, these data indicate that IL-27 acts directly on naive T cells to suppress the development of Th17 effectors through a STAT1-dependent, T-bet-independent mechanism that is not exclusively mediated by competition for IL-6/gp130 signaling or suppression of SOCS3 inhibition of IL-6 signaling.

In an independent report, Ghilardi and coworkers (120) also identified Th17-suppressive effects of IL-27 using an EAE model and further explored mechanisms by which IL-27 acts. In agreement with the findings of Hunter and colleagues (121), EAE induction was exacerbated in IL-27Rα-deficient mice and was associated with increased frequencies of Th17 cells in the CNS. In vitro, IL-27 suppression was associated with STAT1-dependent blockade of upregulation of IL-23R on naive T cells by IL-6. Therefore, IL-27 and IL-6 act antagonistically to effect Th17 development, in part through effects on upregulation of the inducible component of the IL-23 receptor. Importantly, IL-27 blocked Th17 differentiation more efficiently than did IFN-γ in vitro, and suppression via IL-27 or IFN-γ was not redundant, but additive. Furthermore, despite the effect of IL-6 on abrogating Treg-mediated suppression (98, 99), inhibition of IL-6-mediated hyperproliferation by IL-27 was independent of IL-6’s actions on Tregs. Therefore, enhanced proliferation was abrogated only when IL-27Rx was absent from effector cells, not regulatory cells, and IL-27-mediated inhibition of T cell hyperproliferation to IL-6 was blocked even when Treg cells were removed. Thus, antagonism between IL-27 and IL-6 occurred via direct actions on naive T cells, not through actions on Tregs.

These studies are consistent with a mechanism whereby Th17-mediated inflammation may negatively feed back to limit propagation of Th17 production through actions of IL-27 production, and they raise important questions about the conditions under which IL-27 is produced and by what cells. In the case of the CNS, where IL-27 may be regulated at the level of p28 expression by astrocytes (121), this may provide a critical mechanism for protection of the brain, which is highly sensitive to Th17-mediated inflammation. Clearly, additional studies are needed to define pathways and factors that induce IL-27, as well as signaling mechanisms by which IL-27 (and other STAT1-activating cytokines such as the IFNs) act to antagonize IL-17 expression. Considering the link between RORγt expression and Th17 induction (112), one may speculate that cytokines activating the STAT1 pathway antagonize induction of RORγt expression stimulated by cosignaling through TGF-β and IL-6. In any case, elucidation of the link between IL-27 and Th17 suppression may open new pathways by which Th17 development can be blunted proximally rather than after the proinflammatory developmental pathway and cascade have been established.

IL-25 AND TYPE 2 IMMUNITY

Participation of IL-17 family cytokines in the Th17 arm of immunity is mirrored by family participation in the Th2 arm. Whereas IL-17A and IL-17F are Th17 products, IL-25 (IL-17E) can be produced by Th2 cells and now appears to have an important role in amplifying and/or initiating Th2 responses. IL-25 was originally discovered via database search for genes homologous to IL-17 and other IL-17 family members, and its expression identified in Th2-polarized cells (42). IL-25 expression was not detected in naive T cells, Th1-polarized cells, B cells, bone marrow–derived DCs, macrophages, mast cells, or endothelial cells, and the in vivo expression of IL-25 mapped to mucosal tissues almost exclusively. Remarkably, administration of IL-25 to mice recapitulated many of
the features of type 2 immunity via induced expression of prototypical Th2 cytokines (IL-4, IL-5, and IL-13): increased serum IgE, IgG1, and IgA; intestinal and pulmonary epithelial hyperplasia, goblet cell hypertrophy, and increased mucus production; and eosinophilia and eosinophilic tissue infiltrates. With the exception of upregulation of IL-4 and its effects on immunoglobulin subclasses, all other effects of systemic IL-25 administration could occur independently of lymphocytes (42, 67). Thus, IL-25 could induce IL-5 and IL-13 and their effects on eosinophil recruitment and mucosal epithelial responses, without requirement for the adaptive immune system.

In an effort to identify the cellular source of these cytokines, Fort et al. (42) found that IL-25-responsive cells identified in the spleen had an unusual phenotype. These cells lacked classical B and T cell markers, as well as granulocytes and macrophage markers, and expressed low levels of CD11c and F4/80 and high levels of MHC class II (MHC-IIb, CD11c, F4/80, CD3+, B220−, CD4−, CD8α−, CD11b−, and GR-1−). In a parallel report, Hurst et al. (67) identified a similar cell that was responsible for IL-5 production in response to IL-25. This population was lost in mice doubly deficient for RAG-2 and the common γ chain, which lack, in addition to lymphocytes, cells of NK, γδ T cell lineages, and perhaps other cells of hematopoietic origin. Thus, the IL-25-responsive cell type is likely of hematopoietic origin and dependent on common γ chain cytokines for its development (or perhaps responsiveness to IL-25). An uncharacterized innate immune cell, present in many different tissues, may therefore be responsible for IL-25-induced expression of IL-5 and IL-13, and possibly IL-4. Collectively, these data support a model in which IL-25 production by Th2 cells markedly amplifies type 2 responses through both adaptive and innate immune cells.

Subsequent reports identified a critical role for IL-25 in host defense to parasitic helminthes (125,126). Fallon et al. (125) examined the host response to Nippostrongyulus brasiliensis in wild-type or IL-25-deficient mice and found IL-25 critical for an early burst of type 2 cytokines that facilitates rapid worm expulsion but not essential for eventual pathogen clearance or Th2 response. Thus, although IL-25 deficiency blunted the early induction of IL-5 and IL-13, it did not prevent a late, IL-25-independent response. Administration of IL-25 to IL-25-deficient mice enhanced N. brasiliensis clearance via a type 2 cytokine-dependent mechanism and could reconstitute parasite clearance in RAG-deficient mice. Therefore, IL-25 induced a type 2 cytokine response sufficient to eliminate parasitic infection, via a T cell– and B cell–independent mechanism. As in the study of Fort et al. (42), the target for IL-25’s actions was identified as a CD19−, CD45R−, CD8− (non-B, non-T), c-kit+, FcεRI− cell found in mesenteric lymph nodes of infected wild-type, but not IL-25-deficient, mice. Expansion of this cell population was induced by IL-25 administered to wild-type and RAG-deficient mice, as well as mice pan-deficient for Th2 cytokines (Il4−/−, Il5−/−, Il9−/−, Il13−/−). A unique population of c-kit+ cells was induced in an IL-25-dependent and lymphocyte- and type 2 cytokine–independent manner (125). Interestingly, IL-25 induced the production of IL-5 and IL-13 protein in this cell population, but only IL-4 mRNA; IL-4 protein production could not be demonstrated. Thus, in agreement with previous studies, IL-25 appears to act on a novel cell population whose production of type 2 cytokines is independent of Th2 cells.

Owyang et al. (126) examined the role of IL-25 in defense against another helminthic parasite infection, Trichuris muris. In response to T. muris challenge, IL-25-deficient mice demonstrated significantly reduced induction of type 2 cytokines (IL-4 and IL-13) but elevated type 1 cytokines (IFN-γ). This response was accompanied by diminished induction of IgG1 and IgE, diminished goblet cell hyperplasia, and failure to eradicate worm burden. A mouse strain susceptible to T. muris infection (AKR) was successfully
treated by administration of IL-25, which resulted in reconstitution of a type 2 response typical of resistant strains (BALB/c and C57Bl/6). Consistent with these findings, resistant strains of mice demonstrated increased IL-25 and IL-17RB mRNA in the intestines of unchallenged animals. In an effort to identify the cellular source of IL-25, an IL-25-lacZ knock-in reporter system was developed, with which CD4+ and CD8+ T cells were identified in the cecal patch (analog of the human appendix) (126). No reporter expression was found in B cells (CD19+), basophils or mast cells (FcεRI+), eosinophils (CCR3+), NK cells (NK1.1+), DCs (CD11c+), or macrophage/monocytes (CD11b+). Thus, IL-25 appears to be constitutively expressed by subpopulations of CD4 and CD8 T cells found in the cecum of normal mice. Unfortunately, analyses of IL-25/lacZ-positive cells for coexpression of other Th2 cytokines (e.g., IL-4) were not reported, raising the possibility that the IL-25+ cells might not be synonymous with classical Th2 cells.

Owing to the enhanced type 1 response observed in the absence of IL-25, Owyang et al. (126) treated T. muris-infected mice with anti-IL-12 and anti-IFN-γ. Remarkably, neutralization of the Th1 pathway resulted in restitution of an effective Th2 response to parasitic helminthic infection despite IL-25 deficiency. Furthermore, the development of chronic infection in IL-25-deficient mice infected with T. muris resulted in a florid colitis that was associated with elevated intestinal production of IFN-γ and IL-17. Similarly, IL-25 deficiency resulted in high susceptibility to EAE that was associated with elevated CNS infiltration by Th17 cells. Collectively, these data suggest that IL-25 may play a dual role in host protection: enhanced induction of type 2 responses and suppression of type 1, or perhaps Th17, responses. Additional studies are needed to clarify the mechanism by which IL-25 deficiency leads to enhanced type 1 or type 17 responses, although this may not be dissimilar to previous reports in which deficient type 2 immunity based on cytokine blockade or signaling deficiency (e.g., GATA3) leads to a default enhancement of type 1 immunity.

**CONCLUDING REMARKS**

Bioinformatics-based discovery in the genomic/postgenomic era has propelled the identification and characterization of new members of the IL-12 and IL-17 cytokine families that have led in turn to discovery of a new arm of the adaptive immune response (Th17) and an important new player in another arm (Th2). IL-17 cytokines are now centrally positioned as products of two of the three arms of effector CD4 T cell lineages: IL-17A and IL-17F in Th17 and IL-25 in Th2. Particularly striking has been the identification of new members of the IL-12 family cytokines, IL-23 and IL-27, and IL-6, prototype for IL-12 cytokines, as innate immune cell products that control Th17 responses. Perhaps it is fitting that IL-6, patriarch of this extended family, has lately become appreciated as a key danger signal that switches the otherwise proregulatory cytokine, TGF-β, into a Th17-diverting mediator, making it a pivotal cytokine in self-nonself discrimination (56, 127).

In a broad sense, members of the IL-6 superfamily can now be viewed as integrators of more primitive type 1 and type 17 immunity, which probably evolved to handle simpler types of infectious agents, such as viruses, bacteria, protozoal parasites, and perhaps fungi. IL-12 is central to Th1 responses, whereas IL-6 and IL-23 are central to Th17. IL-27 may modulate the balance between Th1 and Th17, extinguishing Th17 development in favor of Th1. Whether this serves to facilitate transition from early Th17-dominated responses to late Th1-dominated responses, serves to dampen the risk of Th17-mediated autoimmunity, or both remains to be determined. In contrast, Th2 responses, and the cytokines that define them (IL-4, IL-5, and IL-13), probably evolved only later, as more complex multicellular organisms became hosts for more complex
pathogens—such as parasitic helminthes. IL-25 appears to have developed to orchestrate the adaptive and innate mechanisms for eradicating these pathogens. Regardless of origins, a common theme is that T cells have leveraged IL-17 family members to mobilize the rapid cellular responders of type 17 and type 2 immunity—the granulocytes—to enhance pathogen clearance.

The impact of the discovery of the IL-17 cytokines and the Th17 pathway for treatment of human disease is likely to be profound. The Th17 lineage is now implicated in a number of autoinflammatory disorders, including some previously associated with Th1 dysregulation: rheumatoid arthritis, multiple sclerosis, IBD, and psoriasis, as well as others likely to be defined. The Th17 arm has also been implicated in allograft rejection. The identification of IL-17 family participation in allergic diseases is equally promising. As our understanding of the pathways and mediators responsible for these diseases becomes better defined, so too will the therapies, with attendant lessening of untoward treatment risks. Existing therapies that target common proinflammatory mediators (e.g., TNF-α) have relatively high risk for infectious complications. Similarly, recent strategies based on administration of anti-p40 neutralizing antibodies have shown excellent clinical efficacy for IBD, but are also associated with a high risk of infection (128), owing to their inhibition of both type 1 and type 17 immunity. In this regard, the efficacy of IL-23 ablation for mouse models of autoimmunity has been compelling, and, notably, IL-23-deficient mice remain resistant to intracellular pathogens such as Mycobacterium, Listeria, Toxoplasma, and Leishmania, whereas IL-12- and p40-deficient mice are highly susceptible. Thus, there is good reason to hope that IL-23- and IL-17-targeting therapies will be highly effective in controlling certain autoinflammatory disorders, with decreased risk of infectious complications. New vaccine strategies for enhanced protection against type 17 and type 2 pathogens are also likely to result.

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