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Th17: An Effector CD4 T Cell Lineage with Regulatory T Cell Ties

Review

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Summary

The naive CD4 T cell is a multipotential precursor with defined antigen recognition specificity but substantial plasticity for development down distinct effector or regulatory lineages, contingent upon signals from cells of the innate immune system. The range of identified effector CD4 T cell lineages has recently expanded with description of an IL-17-producing subset, called Th17, which develops via cytokine signals distinct from, and antagonized by, products of the Th1 and Th2 lineages. Remarkably, Th17 development depends on the pleiotropic cytokine TGF-β, which is also linked to regulatory T cell development and function, providing a unique mechanism for matching CD4 T cell effector and regulatory lineage specification. Here, we review Th17 lineage development, emphasizing similarities and differences with established effector and regulatory T cell developmental programs that have important implications for immune regulation, immune pathogenesis, and host defense.

The directed development of CD4 effector T cells by cytokines elicited from pathogen-activated cells of the innate immune system is a hallmark of adaptive immunity. Until recently, the known universe of adaptive CD4 T cell responses has been encompassed by the Th1-Th2 paradigm (Mosmann and Coffman, 1989; Murphy and Reiner, 2002). Development of T helper 1 (Th1) cells, which evolved to enhance clearance of certain intracellular pathogens, is coupled to the sequential actions of interferon-γ (IFN-γ) and interleukin-12 (IL-12) (Hsieh et al., 1993; Scharton and Scott, 1993). The development of Th2 cells, which evolved to enhance the clearance of parasites, is coupled to IL-4 (Min et al., 2004; Shinkai et al., 2002). The divergence of Th1 and Th2 differentiation is largely due to crossregulatory effects of these polarizing cytokines, providing a mechanism whereby first-line innate immune defenses guide appropriate effector T cell responses that, in turn, orchestrate the host response to efficiently clear pathogens and establish long-lived memory for enhanced recall responses. The benefits of adaptive CD4 T cell responses, however, come at a cost. Inappropriate or poorly controlled effector T cells can cause host pathology and are particularly deleterious when directed against self or ubiquitous environmental or commensal floral antigens, which, unlike most pathogens, cannot be effectively cleared. In this setting, persistent effector T cell responses drive chronic inflammatory disorders such as autoimmunity and allergy, or atopy. Effector T cell responses are therefore normally under stringent regulatory control.

Although a key mechanism whereby dysregulated effector responses are avoided is through intrathymic deletion of self-reactive clones, postthymic mechanisms are also critical because many antigens are developmentally or physically sequestered from developing thymocytes and because the recombinatorial capacity of antigen-recognition receptors in lymphocytes is so robust. Hence, evolutionary pressure to match the development of adaptive effector T cell responses with corresponding regulatory T cell programs has probably been critical to the successful emergence of adaptive immunity. Several subsets of regulatory T cells, or Tregs, have been described, albeit with incompletely defined lineage relationships and functions at present (Figure 1). At least one class of Tregs, so-called natural Tregs (nTregs), is the product of a developmental lineage distinct from Th1 and Th2 lineages and therefore represents the first well-defined expansion of the CD4 T cell functional repertoire.

In this review, we highlight yet another lineage of CD4 T cells, the newly described Th17 lineage. The Th17 lineage represents the only additional effector CD4 T cell arm to be described since the original discovery of Th1 and Th2 two decades ago. Th17 cells are characterized by the production of a distinct profile of effector cytokines, including IL-17 (or IL-17A), IL-17F, and IL-6, and have probably evolved to enhance host clearance of a range of pathogens distinct from those targeted by Th1 and Th2. Th17 cells develop via a pathway separate from Th1 and Th2, but with several notable parallels to the Th1 lineage that have led to some confusion over the role of the Th1 cells in autoimmunity. The development of Th17 effectors also shares with some Tregs a requirement for TGF-β, establishing an important link between Th17 and Treg development. As a basis for understanding recent advances in Th17 development and function, we first highlight features of Th1, Th2, and Treg development and function for comparison.

Th1 and Th2 Development: Mechanisms for Lineage Specification

Since the advent of T cell receptor (TCR) transgenic mouse models, it has been established that naive T cells of identical antigenic specificity can develop into distinct functional effectors, e.g., Th1 and Th2, contingent upon early signals received in concert with antigen (Hsieh et al., 1992; Seder et al., 1992). There has been extensive investigation of the factors and signaling pathways that distinguish differentiation of Th1 and Th2 cells (see Murphy and Reiner, 2002 for review), and although there is now general consensus on many features that control

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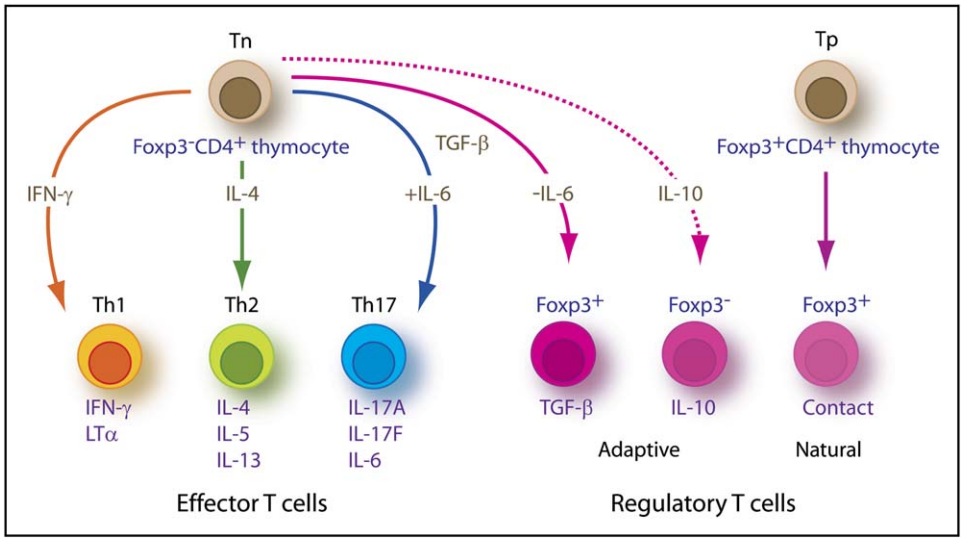

Figure 1. Diversification of CD4 T Cell Lineages

Although functional CD4 T cell development has been dominated by the Th1-Th2 paradigm for nearly two decades, the number of defined lineages has now increased. The cytokines associated with arrows indicate dominant cytokines involved in specification of each of the indicated lineages. The cytokines listed below each cell type indicate key effector or regulatory cytokines produced by differentiated cells of that lineage or, in the case of nTreg, a contact-dependent mechanism of suppression. Tn: naive, postthymic CD4 T cell precursors; Tp: thymic precursors. Dotted lines represent less well-defined lineage relationships.

these developmental programs, certain details remain contentious (Berenson et al., 2004). An important facet of the Th1-Th2 developmental paradigm, which is shared with many developmental strategies, is the presence of reiterative feedback mechanisms that propagate early lineage decisions once initiated. Th1 differentiation is initiated by coordinate signaling through the TCR and STAT1-associated cytokine receptors. Both type I and type II interferons can activate STAT1 via their respective receptors (Hibbert et al., 2003; Lucas et al., 2003; Pflanz et al., 2002), as can the IL-12 family member IL-27 (Hunter, 2005). Receptors for each of these cytokines are expressed on naive T cell precursors and are activated by products of pathogen-stimulated cells of the innate immune system. NK cells are a major source of IFN-γ, whereas plasmacytoid dendritic cells (DCs) are the primary source of IFN-α. STAT1 signaling downstream of IFNs in antigen-activated naive precursors upregulates the transcription factor T-bet, which is thought to be a "master regulator" of Th1 differentiation (Mullen et al., 2001; Szabo et al., 2000). The homeobox transcription factor Hlx is selectively expressed by Th1 cells by virtue of its being a target of T-bet (Mullen et al., 2002) and may complement T-bet as a Th1-maintenance factor (Zheng et al., 2004). T-bet potentiates expression of the *Ifng* gene and upregulates the inducible chain of the IL-12 receptor (IL-12Rβ2) while suppressing Th2-associated factors. Induction of a competent IL-12 receptor on developing Th1 cells licenses IL-12 signaling through STAT4, which further potentiates IFN-γ production and induces expression of IL-18Rα, thereby conferring responsiveness to IL-18 by mature Th1 cells. The IL-12-driven component of Th1 development results in mature effector cells that can produce IFN-γ through either TCR-dependent or -independent (IL-12 plus IL-18) pathways (Robinson et al., 1997; Yang et al., 1999). Thus, the later stage of Th1 differentiation induced by IL-12 enables mature Th1 cells to produce IFN-γ in an antigen-independent mode, not unlike cells of the innate immune system such as NK cells.

Although a T-bet-dependent pathway to Th1 development has been well described, alternative pathways appear to exist. For example, the innate phase of the IFN-γ response during *Listeria monocytogenes* (LM) infection is unaffected in the absence of T-bet (Way and Wilson, 2004). The adaptive immune response to LM showed only a modest reduction in the numbers of Th1 cells or IFN-γ secretion by CD4 T cells, suggesting that alternative pathways can independently contribute to Th1 development. Whether these include known pathways, such as IL-12 and IL-18, or unknown pathways is unclear. Nevertheless, there may be greater plasticity in pathways to effector cytokine gene expression than previously appreciated.

Th2 differentiation is initiated by TCR signaling in concert with IL-4 receptor signaling via STAT6. Signals that emanate from the TCR and IL-4 receptors act cooperatively to upregulate low expression of GATA-3, a master regulator of Th2 differentiation (Ouyang et al., 1998, 2000; Zheng and Flavell, 1997). GATA-3 autoactivates its own expression and drives epigenetic changes in 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. In addition, IL-4 signaling prevents the colocalization of the TCR with IFN-γ receptors at the immunologic synapse of naive T cells activated by APCs, suggesting another way in which IL-4 may inhibit Th1 development (Maldonado et al., 2004). Thus, early IL-4 signaling rapidly initiates positive and negative feedback loops that operate at a number of levels to reinforce early commitment to Th2 development while blocking Th1 development.

Importantly, cytokines produced by mature effector cells themselves can reinforce their own developmental program through positive and negative feedback, acting both on naive T cells and innate immune cells. Thus, IFN-γ produced by mature Th1 cells or innate immune cells induces STAT1 signaling and T-bet expression in antigen-activated, naive CD4 T cells, leading to upregulation of the IL-12 receptor on developing Th1 cells and suppression of GATA-3. Similarly, IL-4 produced by mature Th2 cells initiates Th2 development through its upregulation of GATA-3 via STAT6 and suppresses Th1 development by blocking IL-12Rβ2 expression. The action of GATA-3 to promote its own transcription through a cell-intrinsic positive feedback loop represents a potent mechanism for rapidly stabilizing Th2 development. Remarkably, a further layer of counterregulation may reside at the level of physical interactions between chromatin domains that contain Th1- or Th2-specific cytokine genes. Direct contacts exist between juxtaposed regulatory regions in the Th2 cytokine gene cluster on chromosome 11 and the *Ifng* promoter on chromosome 10 in the mouse, providing the first demonstration of interchromosomal interactions that could reciprocally regulate genes involved in divergent lineages (Spilianakis et al., 2005). As a result of such robust counterregulatory pathways, Th1 and Th2 development diverge rapidly after antigen priming to produce mature effectors with stable, mutually exclusive expression of IFN-γ or IL-4, respectively.

Regulatory T Cells: Strategies for Controlling the Effectors

Besides effector subsets, CD4 T cells can differentiate into distinct regulatory subsets characterized by their ability to suppress adaptive T cell responses and prevent autoimmunity (Sakaguchi, 2000). While at least one class of regulatory T cells, nTregs, develops intrathymically, mounting evidence indicates that other Tregs develop from naive CD4 T cell precursors in the periphery, so-called induced, or adaptive, Tregs (aTregs). nTregs appear to act preferentially in T cell zones of secondary lymphoid tissues to preempt effector T cell development from naive precursors, thereby terminating effector CD4 T cell development before it begins. Obviously, if completely dominant, this mechanism would prevent adaptive responses to pathogens and risk destruction of self by preventing the anti-pathogen response in favor of the anti-self response. aTregs are distinguished by their differentiation from naive CD4 T cells in peripheral lymphoid tissues, where they may develop in parallel to effector T cells and track them to sites of inflammation to quell effector T cell-driven inflammation as pathogen-associated antigens are cleared. At least two types of aTregs have been described. One, called Tr1, develops under control of IL-10-conditioned DCs and is marked by high amounts of IL-10 production; as discussed below, it does not express the transcription factor Foxp3 (Groux et al., 1997; Wakkach et al., 2003). Another is induced from naive precursors under the influence of TGF-β; these cells are $Foxp3^+$ and display suppressive activities that are indistinguishable from nTregs, although they develop extrathymically (Chen et al., 2003; Fantini et al., 2004). Additional types of CD4 Tregs have been described but are less well characterized. There has been generous debate as to whether these subsets represent truly distinct lineages or overlapping and flexible activation states, although recent evidence that has emerged in parallel with new findings on the differentiation of Th17 cells (considered below) may provide some answers.

Following the original functional and phenotypic characterization of Tregs by Sakaguchi and coworkers (reviewed in Sakaguchi, 2004), which linked suppressive activity to a small subset of circulating T cells that stably express the high-affinity component of the IL-2 receptor CD25, insights into the developmental lineage of $CD4^+CD25^+$ nTregs have come from several studies showing that the forkhead-winged helix transcription factor Foxp3 (or Scurfin) is uniquely expressed by nTregs and is required for their development (Akbari et al., 2003; Coffer and Burgering, 2004; Fontenot et al., 2003; Gavin and Rudensky, 2003; Hori et al., 2003; Khattri et al., 2003). Foxp3 was reported initially as specific to Treg in mice (Fontenot et al., 2003; Hori et al., 2003; Khattri et al., 2003) and soon thereafter in humans (Walker et al., 2003b). Foxp3 appears to be a master regulator of nTreg development in mice and humans (Fontenot et al., 2003; Hori et al., 2003; Khattri et al., 2003) insofar as all nTregs express Foxp3 and enforced expression of Foxp3 in conventional, $CD4^+CD25^-$ T cells induces Treg function and phenotypic features, including the expression of CD25, the glucocorticoid-induced tumor necrosis factor receptor-related (GITR) protein, and CTLA4 (Fontenot et al., 2003; Hori et al., 2003; Khattri et al., 2003; Walker et al., 2003b). As in Scurfy mice, which have a frameshift mutation in the *Foxp3* gene, mice with a targeted deficiency of *Foxp3* develop a lymphoproliferative autoinflammatory disease caused by an absence of nTreg (Fontenot et al., 2003; Khattri et al., 2003). A similar syndrome was reported in humans with mutations in *Foxp3* (Walker et al., 2003b).

Subsequent identification of a subset of $Foxp3^+$ thymocytes led to the identification of an intrathymic developmental program for $Foxp3^+$ Tregs. Although the precise mechanisms by which nTregs develop in the thymus are unknown, results from TCR transgenic systems generally favor an "altered selection" model in which development of nTregs requires higher-affinity TCR interactions during positive selection that are not so high affinity as to induce negative selection (Apostolou et al., 2002; Bensinger et al., 2001; Jordan et al., 2001; von Boehmer et al., 2003). Although nTreg development was originally thought to require IL-2 and IL-2 signaling (Malek et al., 2002), recent studies indicate that induction of Foxp3 expression and suppressive function do not require IL-2 or CD25; rather, both are required for maintenance of nTreg homeostasis and function following thymic export (D'Cruz and Klein, 2005; Fontenot et al., 2005). Thus, autoimmune disease that is associated with the loss of Tregs in mice deficient for IL-2, IL-2Rα, or IL-2Rβ (Wolf et al., 2001; Almeida et al., 2002; Malek et al., 2002) is due to poor maintenance of peripheral nTregs, not a defect in intrathymic Treg development. Notably, an important effect of deficient IL-2 signaling in peripheral Tregs was decreased expression of TGF-β1 (Fontenot et al., 2005), which appears to be necessary for maintenance of Foxp3 expression by this population (Marie et al., 2005). Accordingly, it would

appear that at least one critical function for IL-2 signaling is the TGF-β1-dependent maintenance of Foxp3 expression by nTregs in the extrathymic environment.

Although programming for Foxp3 expression was initially associated with thymic selection, recent studies establish the potential for extrathymic development of Foxp3$^+$ Tregs from naive, CD4$^+$CD25$^-$ T cell precursors. Activation of naive murine CD4 T cells in the presence of TGF-β in vitro induced Foxp3, producing cells with suppressive action in vivo (Chen et al., 2003). Similarly, TGF-β reportedly induced Foxp3 expression in conventional human CD4 T cells (Fantini et al., 2004). This study also suggested an autoregulatory loop because TGF-β-induced Foxp3 appeared to inhibit expression of Smad7, an inhibitor of TGF-β signaling, thus augmenting TGF-β signaling. The use of Foxp3 reporter mice, in which development of Foxp3$^+$ Tregs from Foxp3$^-$ precursors was shown, clearly establish that this is not due to outgrowth of contaminating Foxp3$^+$ cells within the CD4$^+$CD25$^-$ fraction (Wan and Flavell, 2005). Additional studies have extended these findings in vivo, particularly in settings of chronic antigen exposure or antigen-specific tolerance induction. Cells functionally and phenotypically indistinguishable from nTregs developed from RAG-deficient, Foxp3$^-$ TCR transgenic precursors after continuous, low-dose administration of specific peptide (Apostolou and von Boehmer, 2004), and induction of Dby-specific transplantation tolerance resulting from in vivo T cell coreceptor blockade was associated with the TGF-β-dependent generation of Foxp3-expressing T cells (Cobbold et al., 2004). In studies of oral tolerance induction, the development of antigen-specific Foxp3$^+$ Tregs in the absence of nTregs was demonstrated (Curatto de Lafaille et al., 2004). Finally, Foxp3$^+$ cells with Treg activity also develop from RAG-deficient, monospecific naive T cell precursors following homeostatic proliferation after transfer into lymphopenic hosts (Curatto de Lafaille et al., 2004). Thus, although the association between Foxp3 expression and Treg functional activity remains valid, it appears that the induction of T cell expression of Foxp3 may not be limited to the thymus. In many cases, this appears to be dependent on the actions of TGF-β, although this has not been rigorously tested in all settings.

Interestingly, although TGF-β can induce Foxp3 expression by naive, peripheral CD4 T cells, it does not appear to be required for intrathymic development of nTregs. Using transgenic mice that express a dominant-negative TGF-β type II receptor (dnTGF-βRII) under control of the CD4 promoter, CD4$^+$CD25$^+$ Tregs develop and function normally, suggesting that TGF-β signaling is not required for their intrathymic development (Fahlen et al., 2005). A caveat here is the timing of ablation of TGF-β signaling by dnTGF-βRII during thymic development. However, normal numbers of Foxp3$^+$ nTregs are found in thymuses of TGF-β1-deficient mice despite reduced numbers in the periphery (Marie et al., 2005), again arguing against a requisite role for TGF-β in nTreg development. Thus, current data indicate that although TGF-β is dispensable for intrathymic expression of Foxp3 and nTreg development, TGF-β is required for the maintenance of Foxp3 expression and Treg fitness in the extrathymic environment, and TGF-β can induce the development of Foxp3$^+$ Tregs from naive CD4 precursors. Accordingly, administration of TGF-β expands the in vivo pool of antigen-specific CD25$^+$ Foxp3-expressing Treg cells (Peng et al., 2004), and transgenic overexpression of TGF-β1 increases the frequency of Treg cells in mice (Schramm et al., 2004).

In addition to its role in induction of adaptive Treg development, TGF-β is involved in at least some aspects of Treg function, although this is an area of considerable controversy. The contact-dependent suppressor activity of CD4$^+$CD25$^+$ T cells could be abrogated by incubation with TGF-β1 antibody in both mouse and human Tregs (Nakamura et al., 2001, 2004). This finding could be explained by the observation that activation of CD4$^+$CD25$^+$ T cells resulted in the expression of high amounts of membrane bound TGF-β, mostly in latent form. Since then, it has been reported that the activation of CD4$^+$CD25$^+$ T cells leads to the upregulation of TGF-βRII and induces the secretion of TGF-β. Furthermore, it has been shown that the administration of the TGF-β1-blocking molecule recombinant latency-associated peptide of TGF-β1 (rLAP) inhibits the suppressive activity of human and mouse CD4$^+$CD25$^+$ T cells (Nakamura et al., 2004). In a murine model of diabetes mediated by CD8 T cells, TGF-β1-expressing Foxp3$^+$ Treg cells selectively accumulated in pancreatic lymph nodes and islets (Green et al., 2003) and suppressed pathogenic CD8 T cells via a TGF-β-dependent mechanism. Thus, adoptive transfers of CD4$^+$CD25$^+$ Treg cells did not suppress naive or activated islet-reactive CD8 T cells bearing dnTGF-βRII. Similarly, the inhibition of CD45RB^{hi} CD4 T cells by Treg in an adoptive model of colitis required TGF-β signaling (Fahlen et al., 2005). Interestingly, however, neither intact TGF-β signaling nor TGF-β production was required by Treg for their ability to suppress the CD45RB^{hi} population, suggesting that, at least in this model, Treg may act instead to induce or process TGF-β production by other cells.

Given the dominance of Tregs in suppressing naive and effector T cell functions in many experimental settings, how is the effector T cell response recruited to enhance pathogen clearance, or, how are the Tregs themselves suppressed to allow protective effector responses? Pasare and Medzhitov found that Treg suppression was reversed in the setting of TLR-induced activation of DCs, thereby linking pathogen-associated molecular patterns, or PAMPs, to abrogation of Treg dominance (Pasare and Medzhitov, 2003). Furthermore, reversal of Treg suppression required TLR-induced, MyD88-dependent production of IL-6 and an unidentified cofactor by DCs. This finding provided a vital link between pathogen-induced activation of innate immune cells and the release of Treg dominance to permit initiation of an effector response by adaptive immune cells. In an extension of these studies, we found that IL-1 synergized with IL-6 to subvert Treg suppression through a mechanism dependent on potentiation of Treg responsiveness to IL-2 (Kubo et al., 2004). In these and other studies (Yamazaki et al., 2003), TLR activation of DCs also reversed the anergic state of Tregs, permitting their robust proliferation while retaining their suppressive function once proinflammatory cytokine production subsided. Additional studies (Klein et al., 2003; Walker et al., 2003a) showed that Tregs can proliferate in vivo similarly to naive T cells after immunization without

losing suppressive function either in vivo or in vitro. Thus, Treg suppression is reversed in the context of pathogen-elicited activation of DCs through TLR-stimulated production of IL-6 and IL-1, permitting linked reversal of Treg dominance to pathogen-induced activation of innate immune cells. Furthermore, the active recruitment of Treg proliferation in the same setting provides a mechanism whereby the effector T cell response is accompanied by an expanded pool of Tregs, which are poised to reassert dominant suppression once the inciting pathogen is cleared and ongoing development of effector T cells ceases.

Th17: Effector T Cells with Immunopathogenic Potential

The breakthrough leading to discovery of the Th17 lineage came from murine models of autoimmunity. Experimental autoimmune encephalitis (EAE) and collagen-induced arthritis (CIA), two prototypical autoimmune mouse models, have historically been associated with unchecked Th1 responses, based largely on studies in which disease development was ablated by treatment with neutralizing antibodies specific for IL-12p40 or gene-targeted mice deficient in the p40 subunit of IL-12 (Constantinescu et al., 1998; Leonard et al., 1995; Segal et al., 1998). However, the link with IL-12 in these diseases came into question with the discovery by Oppmann et al. that a new IL-12 family member, IL-23, shares with IL-12 the p40 subunit (Oppmann et al., 2000). The IL-12 heterodimer is composed of IL-12p40 and IL-12p35, whereas the IL-23 heterodimer is composed of the IL-12p40 chain paired with IL-23p19. Given that key experimental data linking EAE and CIA to Th1 autoimmunity were based on protection associated with manipulations that targeted the IL-12p40 subunit, it became unclear whether protective effects were truly due to inhibition of IL-12 or might involve IL-23. Indeed, data from a number of studies were inconsistent with a simple Th1 or IL-12–IFN-γ cytokine axis link, as mice deficient in IFN-γ were susceptible to EAE and CIA, as were mice deficient in IFN-γR signaling (Bettelli et al., 2004; Ferber et al., 1996; Kageyama et al., 1998; Matthys et al., 1998, 1999; Willenborg et al., 1996).

In an elegant series of studies, Cua and coworkers resolved this paradox when they revisited the immunopathologic basis for EAE and CIA using mice deficient in IL-12, IL-23, or both (Cua et al., 2003; Murphy et al., 2003). Strikingly, it was found that disease development was ablated in mice deficient in IL-23, but not IL-12. Thus, mice deficient in the IL-23p19 subunit (lacking IL-23 only) or the IL-12p40 subunit (lacking both IL-23 and IL-12) were resistant to EAE and CIA, whereas IL-12p35-deficient mice (lacking IL-12 only) remained susceptible. These findings were consistent with another study that found that mice lacking the IL-12 receptor complex also succumbed to EAE (Zhang et al., 2003). Thus, it appears that IL-23, not IL-12, is critically linked to autoimmunity in these models.

Clues to the pathogenic role for IL-23 came from analyses of the cytokine phenotypes of effector CD4 T cells primed with type II collagen in the CIA studies by Murphy et al. (Murphy et al., 2003). In previous studies, it had been found that IL-23 elicited production of the proinflammatory cytokine IL-17 from CD4 T cells of the effector and memory phenotype (Aggarwal et al., 2003). IL-17, or IL-17A, is the founding member of a six-member family of cytokines (IL-17A–F; reviewed by Kolls and Lindén, 2004) that was initially described as a product of human CD4 T cells and has been linked to a number of T cell-driven inflammatory conditions. Notably, diminished frequencies of IL-17$^+$, but not IFN-γ^+, T cells were recovered from the draining lymph nodes of collagen-immunized, IL-23p19-deficient mice that were protected from arthritis development; a reciprocal pattern of IFN-γ^+ and IL-17$^+$ cells was found in IL-12p35-deficient mice that developed exacerbated disease compared to wild-type controls. Thus, a positive correlation was established between the availability of IL-23 and IL-17-producing effector T cells and disease development, and a negative correlation was established between IL-12 and IFN-γ-producing Th1 cells and disease development. In accord with these findings, impaired joint inflammation was reported in IL-17-deficient mice after type II collagen immunization (Nakae et al., 2003), and neutralization of IL-17 also decreased disease severity (Koenders et al., 2005a; Lubberts et al., 2001, 2004). Furthermore, overexpression of IL-17 in the joints exacerbated disease (Koenders et al., 2005b; Lubberts et al., 2001). Collectively, these data strongly implicated a role for the IL-23-induced development of IL-17-producing effector T cells in autoimmune inflammation; the production of classical Th1 cells alone did not induce disease.

More direct proof of a link between IL-17-producing T cell effectors and immune pathogenesis came with data showing that proteolipid protein peptide (PLP) primed CD4 T cells enriched for production of IL-17 by culture in IL-23 induced severe EAE in recipient mice after passive transfers, whereas Th1 cells enriched by culture in IL-12 did not (Langrish et al., 2005). This study further defined functional and phenotypic differences between so-called $Th_{IL\text{-}17}$, or Th17, cells and Th1 cells, demonstrating that gene-expression profiles of these two subsets were quite distinct. Thus, although 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-17F, IL-6, TNF-α, and proinflammatory chemokines). These results therefore confirmed a new role for Th17 cells in immunopathogenesis and strongly suggested that Th1 and Th17 cells represent distinct effector subsets that develop under differential IL-12 or IL-23 conditioning.

Th17 Differentiation: A Distinct Developmental Pathway and New Role for TGF-β in Adaptive Immunity

Although many of the characteristics that identified Th17 as a unique effector T cell subset were defined in the foregoing studies, the pathway leading to Th17 differentiation has begun to emerge only more recently and has held some interesting surprises. Two distinct models of Th17 differentiation were proposed after establishment of the central role for Th17 cells in EAE and CIA (Bettelli and Kuchroo, 2005; McKenzie et al., 2006). In one model, it was proposed that the early differentiation of Th1 and Th17 from naive CD4 T cell precursors was shared, and thus Th1 and Th17 diverged contingent upon selective availability of IL-12 and IL-23 acting on a common

"Th1 precursor" or "pre-Th1" intermediate that coexpressed IL-12 and IL-23 receptors. In the second model, it was proposed that Th1 and Th17 differentiation were nonoverlapping and represented distinct lineages. In view of the fact that intact T-bet and STAT4 are strictly 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.

In a pair of reports (Harrington et al., 2005; Park et al., 2005), direct support for a Th1-independent pathway of Th17 differentiation was established (Figure 2). In our studies, 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 interferons, which activate STAT1-induced expression of T-bet and Th1 commitment, strongly inhibited Th17 development. Together, these findings indicated that not only was the Th1 pathway nonpermissive to Th17 development, but the Th1 product IFN-γ actively suppressed development of Th17 development. Parallel findings were made for the Th2 lineage: Th2-polarized cells were unresponsive to IL-23, and IL-4 potently inhibited 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. Accordingly, we found that key signaling components of Th1 and Th2 differentiation—STAT1, T-bet, STAT4, and STAT6—were each dispensable for Th17 development. Park et al. extended these studies in vivo, showing that immunization of mice deficient in IFN-γ or T-bet led to unimpaired Th17 development (Park et al., 2005). In sum, these data established that IL-17-producing effectors develop via a lineage that is distinct from, and antagonized by, the Th1 and Th2 lineages. These findings provide potential explanations for a number of the paradoxical effects observed in the development of EAE and CIA in mice with Th1-lineage defects. In view of the pathogenic potential of Th17 cells and the potent suppressive effects of IFN-γ on Th17 development, it is now not surprising that mice with targeted deficiencies of IFN-γ, IFN-γR, IL-12, and STAT1 display exacerbated disease development. With respect to the observed disease resistance of T-bet-deficient mice, it is now apparent that this is probably not due to a defect in Th17 development, implicating T cell-independent mechanisms by which T-bet contributes to pathology.

Although IL-23 appears to be required for Th17-mediated immunopathology, three new reports indicate that IL-23 is not required for Th17 commitment (Bettelli et al., 2006; Mangan et al., 2006; Veldhoen et al., 2006). In each of these studies, the development of IL-17-producing effectors in a primary response in vitro was relatively undiminished under conditions of IL-23 deficiency, and Th17 development was not enhanced by addition of exogenous IL-23. In our own studies, differential upregulation of IL-12Rβ2 and IL-23R was associated with signals that induced Th1 and Th17 differentiation, respectively (Harrington et al., 2005). Thus, in striking parallel to 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. This is in agreement with early reports that found that IL-23 was shown to augment IL-17 production from the memory pool of CD4 T cells, but not from naive cells (Aggarwal et al., 2003). In this regard, it is notable that IL-12 actions in Th1 development are linked to additional differentiation events, and it is not unlikely that this is paralleled for Th17. Specifically, IL-12 induces expression of the IL-18Rα chain, resulting in an alternative pathway for IFN-γ expression that is TCR independent. Assuming that a similar pathway exists downstream of IL-23 signaling in developing Th17 cells, this could provide an important mechanism by which Th17 effector function is augmented. In this regard, preliminary studies from our lab indicate that both IL-1, whose receptor shares signaling components with the IL-18R, and IL-18 may act in concert with IL-23 to activate a TCR-independent pathway of IL-17 expression (Y. Lee and C.T.W., unpublished data). Thus, in another parallel with the Th1 lineage, Th17 cells appear to have TCR-dependent and -independent mechanisms for expression of effector cytokines.

If IL-23 is not required for Th17 commitment, what is? Remarkably, three independent groups that addressed this question from different initial premises arrived at the same conclusion: TGF-β is necessary for initiation of Th17 differentiation (Bettelli et al., 2006; Mangan et al., 2006; Veldhoen et al., 2006). Keying off the observation that LPS-activated dendritic cells stimulate naive T cell proliferation by subverting the suppressive activity of Tregs (Kubo et al., 2004; Pasare and Medzhitov, 2003), Veldhoen et al. showed that naive T cells activated in the presence of $CD4^+CD25^+$ Tregs exhibited suppressed amounts of IFN-γ and IL-2 production but expressed high amounts of IL-17. Antibody blockade of TGF-β in cultures of LPS-activated DCs, Tregs, and naive CD4 T cells identified TGF-β as a critical factor for Th17 development. Importantly, in addition to TGF-β, differentiation of IL-17-producing effector cells required soluble dendritic cell factors elicited by TLR- and MyD88-dependent signaling. In addition to TLR4, TLR3 and TLR9 signals (elicited by LPS, polyI:C, and CpG, respectively) were effective at inducing DC factors that could act in concert with TGF-β to differentiate Th17 cells. Strikingly, analysis of DC-derived factors that acted in concert with TGF-β led to the identification of IL-6 as a critical cofactor. Thus, the same inflammatory cytokine previously identified as a signal for reversal of Treg suppression was now shown to participate in development of the Th17 lineage. Using an APC-free culture system, it was shown that Th17 development could be reconstituted by TGF-β and IL-6 alone, indicating that other DC-drive factors (e.g., costimulators) were not required. IL-1β and TNF-α were found to amplify the Th17 response induced by TGF-β and IL-6 but could not substitute for either of these cytokines. Finally, an analysis of transcription-factor expression by IL-17-producing T cells showed that Th17 effectors

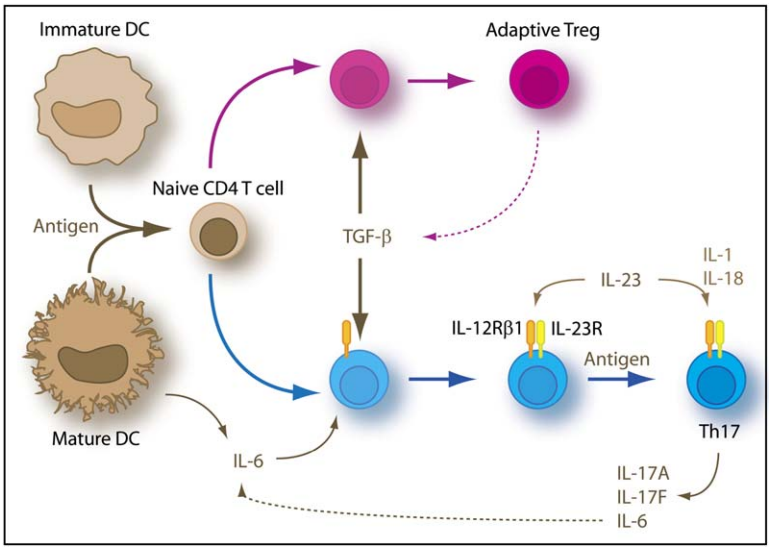

Figure 2. Model of Branching Th17 and Adaptive Treg Lineage Development

This model emphasizes distinct pathways leading to mature Th17 effector cells or Foxp3$^+$ adaptive Tregs (aTreg), induced by a common requirement for TGF-β but differential effects of IL-6 and IL-23. Naive CD4 T cells (Tn) activated by antigen presented on immature DCs that do not produce IL-6 production are induced by TGF-β to express Foxp3 and develop into aTregs (top panel). Tns activated by mature, TLR-activated DCs that produce IL-6 are induced by TGF-β to upregulate IL-23R and become competent for IL-17 production and IL-23 signaling. IL-23 signaling induces responsiveness to IL-18 and IL-1, which can act synergistically with IL-23 to induce Th17 cytokine production independently of TCR stimulation. Alternatively, TCR stimulation by antigen can induce Th17 cytokine production directly, without a requirement for IL-23, IL-1, or IL-18. Dotted lines indicate possible positive feedback loops by which cytokine products of Th17 (IL-6) or aTreg cells (TGF-β1) may reinforce lineage development.

lacked expression of T-bet and Hlx in comparison to Th1-polarized cells and GATA-3 compared to Th2-polarized cells, supporting and extending previous findings that identified the Th17 cell as a product of an effector lineage distinct from Th1 and Th2 (Harrington et al., 2005; Park et al., 2005).

We independently identified TGF-β as a critical factor for Th17 commitment (Mangan et al., 2006). Following the identification of IFN-γ and IL-4 as potent inhibitors of Th17 development, we reasoned that TGF-β might promote Th17 development through its effects to inhibit Th1 and Th2 development and the cytokines driving Th1 and Th2 specification. The addition of exogenous TGF-β1 to primary cultures of naive CD4 T cells induced a modest but appreciable population of Th17 effectors, which was markedly enhanced in the presence of neutralizing antibodies to IL-4 and IFN-γ. Using IFN-γ-deficient APCs and T cells, or IFN-γR-deficient T cells, exogenous TGF-β induced even greater Th17 development, independently of IL-23. Importantly, TGF-β upregulated 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. Accordingly, the antagonistic effects of TGF-β versus IFN-γ signaling early in the activation of naive T cells deviates lineage development toward Th17 or Th1, with concomitant upregulation of the inducible components of the IL-23 or IL-12 receptor, respectively.

Because TGF-β had been previously linked to the development of Foxp3$^+$ Tregs, we examined the phenotypes of T cell populations generated by exogenous TGF-β addition; we found that an appreciable fraction of Foxp3$^+$ T cells were induced, albeit minor in comparison to the numbers of IL-17-positive cells. Notably, expression of IL-17 or Foxp3 was restricted to separate subsets; thus, TGF-β-driven Th17 and Treg development from naive precursors were mutually exclusive. Importantly, we found that under conditions where IL-6 was supplemented, development of Foxp3$^+$ cells was eliminated. Conversely, blockade of IL-6 permitted enhanced development of the Foxp3$^+$ subpopulation, suggesting that IL-6 blocked Treg development while enhancing Th17 development.

To examine the requirement for TGF-β in Th17 development in vivo, we explored TGF-β1-deficient mice. 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 (unpublished data; Stark et al., 2005). Mice hemizygous for TGF-β1 deficiency showed an intermediate phenotype compared to controls, and circulating amounts of IL-17 correlated with these phenotypes. Thus, although not directly establishing an in vivo link between TGF-β and Th17 development, these studies support a critical role for TGF-β1 in the development of Th17 cells.

In a final, independent set of studies, Kuchroo and coworkers confirmed that Th17 development was TGF-β dependent and also showed that the addition of IL-6 controlled the relative frequency of Th17 versus Tregs that developed in the presence of TGF-β (Bettelli et al., 2006). Using Foxp3-EGFP knockin reporter mice to track Treg development, it was found that naive, Foxp3$^-$ CD4 T cells were induced to express Foxp3 upon activation in the presence of TGF-β. In a survey that examined the effects of proinflammatory cytokines on the development of Foxp3$^+$ T cells, only IL-6 potently suppressed the frequency of cells expressing Foxp3; cocultures of TGF-β with IL-7, IL-10, IL-11, IL-12, IL-13, IL-15, IL-18, and TNF-α did not appreciably suppress the frequency of Foxp3$^+$ T cells that developed from naive precursors. When the phenotype of the cells cultured with TGF-β plus IL-6 was examined, a majority were IL-17$^+$. Thus, in agreement with our own studies, IL-6 appeared to divert the development of Foxp3$^+$ regulatory cells towards the Th17 lineage, an effect that was independent of IL-23. The addition of IL-6 therefore suppressed the TGF-β-induced generation Foxp3$^+$ Tregs, while reciprocally promoting the generation of Th17 cells. These in vitro studies were supplemented by the finding that resistance of EAE development in IL-6-deficient mice was correlated with a deficiency of IL-17$^+$ cells in EAE infiltrates.

The foregoing studies identified a critical role for TGF-β, and IL-6, in Th17 development and suggested that IL-23 likely functioned subsequent to Th17 commitment, perhaps to expand committed Th17 effectors or maintain and extend their function. In view of the in vitro findings that IL-17 effectors could develop independently of IL-23, we reexamined the requirements for IL-23 in vivo using an infectious model based on sublethal challenge with the intestinal bacterial pathogen *Citrobacter rodentium*, for which we had found an intact IL-23–IL-17 axis essential for host protection (Mangan et al., 2006). In wild-type mice, challenge with *C. rodentium* stimulated a rapid, robust CD4 T cell effector response in the distal colon and draining lymph nodes that was dominated by Th17 cells. Notably, upon inoculation of IL-23p19-deficient mice with this pathogen, comparable, robust Th17 responses were identified despite the absence of IL-23. However, unlike the wild-type controls, which cleared the infection and resolved the colonic inflammation, IL-23-deficient animals had a markedly impaired inflammatory response in the colonic tissues, failed to clear the pathogen, and rapidly succumbed to infection, indicating that induction of IL-17-producing effectors alone was inadequate for host protection. Thus, while IL-23 is dispensable for the differentiation of IL-17-competent T cells in vitro and in vivo, it is indispensable for a fully protective Th17 response. This could be due to limitations of a positive feedback loop that upregulates proinflammatory cytokines induced by Th17 cells (Figure 2), especially IL-6, IL-1, and TNF-α.

Concluding Remarks

The remarkable balancing act of adaptive immunity—how to facilitate the targeted destruction of pathogens without excessive collateral damage to self—is nowhere better exemplified than in the shared use of TGF-β in controlling the newly described Th17 effector lineage and adaptive Treg development. It is perhaps fitting that TGF-β should be central to this yin/yang interplay, given its complex and often apparently inconsistent biology (Wahl, 1994). We now have a glimpse of an elegant and self-correcting or homeostatic mechanism, wherein the same factor driving one response drives a compensatory response that controls or terminates the initial response. Thus, in a setting of pathogen-driven inflammation, naive T cells that recognize foreign antigen may receive signals from regulatory T cells themselves (TGF-β) to initiate Th17 development, while the pathogen-induced signal that acts in concert with TGF-β to switch the response to a protective Th17 response (IL-6) also subverts Treg dominance and drives expansion of Tregs that could be poised to terminate further T effector differentiation as the pathogen responsible for initiating the inflammatory response is cleared. Alternatively, the production of TGF-β, contributed perhaps by the turnover of effete, apoptotic host cells under normal, nonpathogenic states (Chen et al., 2001), may initiate the development of Tregs that could maintain dominant suppressive function in the absence of pathogenic challenges but contribute to Th17 effector development when necessary. The unique role of TGF-β is made all the more intriguing by the fact that it is also required for the maintenance of Foxp3$^+$ Tregs in the periphery, induced through the actions of IL-2 (Fontenot et al., 2005; Marie et al., 2005).

Although the complexities of TFG-β biology remain a formidable challenge, the newly identified links between TGF-β and Th17 development promise the solution of many conundrums that have precluded a complete understanding of autoimmune pathogenesis. They also raise many intriguing questions. First, elucidation of this new effector lineage and the description of cytokine factors driving its development beg the question of which transcription factor (or factors) controls Th17 lineage specification. For Th1, Th2, and Treg lineages, key transcription factors have been identified that specify the genotypic and phenotypic characteristics of these lineages. Thus, T-bet specifies Th1, GATA-3 specifies Th2, and Foxp3 specifies Treg development. Is there an analogous factor that links TGF-β and IL-6 signaling to Th17 development? Related to this, how do TGF-β and IL-6 signaling cooperate to specify Th17 commitment, whereas the absence of IL-6 induces Foxp3, thereby specifying Treg development? Given the abundance of existing data on signaling pathways that TGF-β and IL-6 recruit and the target-gene responses they elicit, answers to these questions are likely to be answered soon. Our current understanding of competitive inhibition of the TGF-β and IFN-γ signaling cascades offers good predictions as to how IFN-γ acts on the naive T cell to inhibit Th17 development (Ulloa et al., 1999) and vice versa (Lin et al., 2005). It is likely that similar considerations of intersections of the STAT3-mediated IL-6 signaling pathway with the Smad2-, Smad3-, and Smad4-mediated TGF-β signaling pathway will contribute additional new insights.

Although discovery of the Th17 lineage originated in revisionist studies of autoimmune mechanisms, its true origins no doubt lie in the evolutionary pressures to protect vertebrates from certain classes of microbial pathogens (McKenzie et al., 2006). In retrospect, it is apparent that the Th1-Th2 paradigm was insufficient to encompass the entire spectrum of pathogenic challenges. As the classes of pathogens for which Th1 and Th2 are protective became better defined—primarily those adapted for intracellular survival in phagocytes for at least a portion of their life cycle, or parasites such as helminths, respectively—it became increasingly apparent that Th1 and Th2 cells offer protection against only a portion of the pathogen landscape, implying the existence of additional effector lineages. Almost certainly, therefore, the Th17 lineage evolved to control certain classes of pathogens not covered by Th1 and Th2. Given the growing association of IL-23 and/or IL-17 to host protection in a number of bacterial infection models (e.g., *Klebsiella pneumoniae, Borrelia burgdorferi*, *Bordetella pertussis*, and *Citrobacter rodentium*), it is likely that Th17 cells evolved to cope with a range of extracellular bacterial pathogens (Fedele et al., 2005; Happel et al., 2005; Infante-Duarte et al., 2000; Mangan et al., 2006) as well as to contribute to homeostatic maintenance of mucosal tissues such as the gut, which are colonized by abundant commensal bacterial species. Th17 cells may also play an important role in the clearance of fungi, although more studies will be needed to define the range of pathogens linked to this lineage. In this regard, our own recent studies have found that mice deficient in

IL-6 fail to develop a protective Th17 response upon challenge with *C. rodentium*, establishing a critical role for this cytokine in Th17 development induced by infection (D. O'Quinn, P.R.M., and C.T.W., unpublished data). The links between IL-6, Th17 development, and host protection offer new possibilities for improved vaccine development that were previously unappreciated.

If TGF-β and IL-6 can induce IL-17-producing effectors independently of IL-23, what is the precise role of IL-23 in amplifying or maintaining Th17 responses in vivo, and where does it act? The studies of Cua and coworkers clearly identify a requirement for IL-23 in Th17-dependent autoimmune pathogenesis, and there are now equally compelling data indicating a similar requirement in at least some bacterial infections. Hence, Th17 lineage commitment is independent of IL-23, although host protection and autoimmunity linked to Th17 development are not. This indicates that IL-23 signaling in developing Th17 cells is necessary in vivo for critical downstream actions on Th17 precursors; whether this is related to Th17 survival, cell number amplification, or enhanced functional properties is yet to be defined. Direct effects on non-T cells are also possible. In this regard, it will be important to define at what anatomic sites IL-23 actions on Th17 precursors occur. While it has been assumed that IL-23 actions are exerted at sites of T effector cell induction—i.e., T cell zones of secondary lymphoid tissues—in fact, we have very little information regarding the temporal and spatial effects of IL-12 family-member actions in situ. The principal site of IL-23 production in normal mice was limited to the distal ileum of the intestines (Becker et al., 2003), consistent with an important role for IL-23 in homeostasis to the intestinal flora but also raising the possibility that IL-23 acts primarily at effector sites, rather than inductive sites, whether to recruit Th17 cells, enhance their function, or prevent their death. Given the finding that IL-1 and IL-18 can synergize with IL-23 to induce potent IL-17 production from Th17-polarized effectors independently of TCR stimulation (Y. Lee and C.T.W., unpublished data), a potent positive feedback loop with IL-23 at its epicenter exists at sites of inflammation.

Finally, what are the implications for the other arm of the adaptive response, the B cell? The connection between Th17 and host protection against extracellular bacteria has important implications for immunoglobulin isotype switching. Because opsonizing antibodies represent a fundamental component of the adaptive response for enhancing phagocytic clearance of extracellular bacteria, it stands to reason that Th17 cells enhance immunoglobulin class switching that favors targeting to activating Fc receptors on phagocytic cells. In this regard, it is notable that TGF-β has long been recognized as a cytokine favoring class switching of IgA and IgG2b, Ig subclasses important in mucosal barrier function and antibacterial protection. This is contrasted with the propensity for IgG2a and IgG3 class switching by the Th1 cytokine IFN-γ and IgG1 and IgE class switching by the Th2 cytokine IL-4 (Stavnezer, 1996). IL-6 is also a potent B cell growth and differentiation factor. Thus, TGF-β and IL-6, the cytokines critical for Th17 development, have symmetrical links with B cell maturation and class-switching functions aimed at blocking mucosal colonization by commensal bacteria and clearing invasive extracellular bacteria. Accordingly, B cell responses in IL-23- and IL-17A-deficient mice are defective (Ghilardi et al., 2004; Nakae et al., 2002), although detailed pathogen challenge studies have not yet been reported. Clearly, a great deal is yet to be learned, but, with the discovery of this unique pathway and its intimate relationship to T regulatory cell and B cell pathways, the foundation has been laid for a new era in understanding adaptive immune regulation, with its attendant opportunities for improved therapies for host defense and autoimmunity.

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References

Aggarwal, S., Ghilardi, N., Xie, M.H., de Sauvage, F.J., and Gurney, A.L. (2003). Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. J. Biol. Chem. *278*, 1910–1914.

Akbari, O., Stock, P., DeKruyff, R.H., and Umetsu, D.T. (2003). Role of regulatory T cells in allergy and asthma. Curr. Opin. Immunol. *15*, 627–633.

Almeida, A.R., Legrand, N., Papiernik, M., and Freitas, A.A. (2002). Homeostasis of peripheral CD4+ T cells: IL-2R alpha and IL-2 shape a population of regulatory cells that controls CD4+ T cell numbers. J. Immunol. *169*, 4850–4860.

Apostolou, I., and von Boehmer, H. (2004). In vivo instruction of suppressor commitment in naive T cells. J. Exp. Med. *199*, 1401–1408.

Apostolou, I., Sarukhan, A., Klein, L., and von Boehmer, H. (2002). Origin of regulatory T cells with known specificity for antigen. Nat. Immunol. *3*, 756–763.

Becker, C., Wirtz, S., Blessing, M., Pirhonen, J., Strand, D., Bechthold, O., Frick, J., Galle, P.R., Autenrieth, I., and Neurath, M.F. (2003). Constitutive p40 promoter activation and IL-23 production in the terminal ileum mediated by dendritic cells. J. Clin. Invest. *112*, 693–706.

Bensinger, S.J., Bandeira, A., Jordan, M.S., Caton, A.J., and Laufer, T.M. (2001). Major histocompatibility complex class II-positive cortical epithelium mediates the selection of CD4(+)25(+) immunoregulatory T cells. J. Exp. Med. *194*, 427–438.

Berenson, L.S., Ota, N., and Murphy, K.M. (2004). Issues in T-helper 1 development–resolved and unresolved. Immunol. Rev. *202*, 157–174.

Bettelli, E., and Kuchroo, V.K. (2005). IL-12- and IL-23-induced T helper cell subsets: birds of the same feather flock together. J. Exp. Med. *201*, 169–171.

Bettelli, E., Sullivan, B., Szabo, S.J., Sobel, R.A., Glimcher, L.H., and Kuchroo, V.K. (2004). Loss of T-bet, but not STAT1, prevents the development of experimental autoimmune encephalomyelitis. J. Exp. Med. *200*, 79–87.

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T.B., Oukka, M., Weiner, H.L., and Kuchroo, V.K. (2006). Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature *441*, 235–238.

Chen, W., Frank, M.E., Jin, W., and Wahl, S.M. (2001). TGF-beta released by apoptotic T cells contributes to an immunosuppressive milieu. Immunity *14*, 715–725.

Chen, W., Jin, W., Hardegen, N., Lei, K.J., Li, L., Marinos, N., McGrady, G., and Wahl, S.M. (2003). Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by

TGF-beta induction of transcription factor Foxp3. J. Exp. Med. *198*, 1875–1886.

Cobbold, S.P., Castejon, R., Adams, E., Zelenika, D., Graca, L., Humm, S., and Waldmann, H. (2004). Induction of foxP3+ regulatory T cells in the periphery of T cell receptor transgenic mice tolerized to transplants. J. Immunol. *172*, 6003–6010.

Coffer, P.J., and Burgering, B.M. (2004). Forkhead-box transcription factors and their role in the immune system. Nat. Rev. Immunol. *4*, 889–899.

Constantinescu, C.S., Wysocka, M., Hilliard, B., Ventura, E.S., Lavi, E., Trinchieri, G., and Rostami, A. (1998). Antibodies against IL-12 prevent superantigen-induced and spontaneous relapses of experimental autoimmune encephalomyelitis. J. Immunol. *161*, 5097–5104.

Cua, D.J., Sherlock, J., Chen, Y., Murphy, C.A., Joyce, B., Seymour, B., Lucian, L., To, W., Kwan, S., Churakova, T., et al. (2003). Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature *421*, 744–748.

Curatto de Lafaille, M.A., Lino, A.C., Kutchukhidze, N., and Lafaille, J.J. (2004). CD25- T cells generate CD25+Foxp3+ regulatory T cells by peripheral expansion. J. Immunol. *173*, 7258–7268.

D'Cruz, L.M., and Klein, L. (2005). Development and function of agonist-induced CD25+Foxp3+ regulatory T cells in the absence of interleukin 2 signaling. Nat. Immunol. *6*, 1152–1159.

Fahlen, L., Read, S., Gorelik, L., Hurst, S.D., Coffman, R.L., Flavell, R.A., and Powrie, F. (2005). T cells that cannot respond to TGF-beta escape control by CD4(+)CD25(+) regulatory T cells. J. Exp. Med. *201*, 737–746.

Fantini, M.C., Becker, C., Monteleone, G., Pallone, F., Galle, P.R., and Neurath, M.F. (2004). Cutting edge: TGF-beta induces a regulatory phenotype in CD4+CD25- T cells through Foxp3 induction and down-regulation of Smad7. J. Immunol. *172*, 5149–5153.

Fedele, G., Stefanelli, P., Spensieri, F., Fazio, C., Mastrantonio, P., and Ausiello, C.M. (2005). Bordetella pertussis-infected human monocyte-derived dendritic cells undergo maturation and induce Th1 polarization and interleukin-23 expression. Infect. Immun. *73*, 1590–1597.

Ferber, I.A., Brocke, S., Taylor-Edwards, C., Ridgway, W., Dinisco, C., Steinman, L., Dalton, D., and Fathman, C.G. (1996). Mice with a disrupted IFN-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). J. Immunol. *156*, 5–7.

Fontenot, J.D., Gavin, M.A., and Rudensky, A.Y. (2003). Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat. Immunol. *4*, 330–336.

Fontenot, J.D., Rasmussen, J.P., Gavin, M.A., and Rudensky, A.Y. (2005). A function for interleukin 2 in Foxp3-expressing regulatory T cells. Nat. Immunol. *6*, 1142–1151.

Gavin, M., and Rudensky, A. (2003). Control of immune homeostasis by naturally arising regulatory CD4+ T cells. Curr. Opin. Immunol. *15*, 690–696.

Ghilardi, N., Kljavin, N., Chen, Q., Lucas, S., Gurney, A.L., and De Sauvage, F.J. (2004). Compromised humoral and delayed-type hypersensitivity responses in IL-23-deficient mice. J. Immunol. *172*, 2827–2833.

Green, E.A., Gorelik, L., McGregor, C.M., Tran, E.H., and Flavell, R.A. (2003). CD4+CD25+ T regulatory cells control anti-islet CD8+ T cells through TGF-beta-TGF-beta receptor interactions in type 1 diabetes. Proc. Natl. Acad. Sci. USA *100*, 10878–10883.

Groux, H., O'Garra, A., Bigler, M., Rouleau, M., Antonenko, S., de Vries, J.E., and Roncarolo, M.G. (1997). A CD4+ T-cell subset inhibits antigen-specific responses and prevents colitis. Nature *389*, 737–742.

Happel, K.I., Dubin, P.J., Zheng, M., Ghilardi, N., Lockhart, C., Quinton, L.J., Odden, A.R., Shellito, J.E., Bagby, G.J., Nelson, S., and Kolls, J.K. (2005). Divergent roles of IL-23 and IL-12 in host defense against Klebsiella pneumoniae. J. Exp. Med. *202*, 761–769.

Harrington, L.E., Hatton, R.D., Mangan, P.R., Turner, H., Murphy, T.L., Murphy, K.M., and Weaver, C.T. (2005). Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat. Immunol. *6*, 1123–1132.

Hibbert, L., Pflanz, S., De Waal Malefyt, R., and Kastelein, R.A. (2003). IL-27 and IFN-alpha signal via Stat1 and Stat3 and induce T-Bet and IL-12Rbeta2 in naive T cells. J. Interferon Cytokine Res. *23*, 513–522.

Hori, S., Nomura, T., and Sakaguchi, S. (2003). Control of regulatory T cell development by the transcription factor Foxp3. Science *299*, 1057–1061.

Hsieh, C.S., Heimberger, A.B., Gold, J.S., O'Garra, A., and Murphy, K.M. (1992). Differential regulation of T helper phenotype development by interleukins 4 and 10 in an alpha beta T-cell-receptor transgenic system. Proc. Natl. Acad. Sci. USA *89*, 6065–6069.

Hsieh, C.S., Macatonia, S.E., Tripp, C.S., Wolf, S.F., O'Garra, A., and Murphy, K.M. (1993). Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. Science *260*, 547–549.

Hunter, C.A. (2005). New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. Nat. Rev. Immunol. *5*, 521–531.

Infante-Duarte, C., Horton, H.F., Byrne, M.C., and Kamradt, T. (2000). Microbial lipopeptides induce the production of IL-17 in Th cells. J. Immunol. *165*, 6107–6115.

Jordan, M.S., Boesteanu, A., Reed, A.J., Petrone, A.L., Holenbeck, A.E., Lerman, M.A., Naji, A., and Caton, A.J. (2001). Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. Nat. Immunol. *2*, 301–306.

Kageyama, Y., Koide, Y., Yoshida, A., Uchijima, M., Arai, T., Miyamoto, S., Ozeki, T., Hiyoshi, M., Kushida, K., and Inoue, T. (1998). Reduced susceptibility to collagen-induced arthritis in mice deficient in IFN-gamma receptor. J. Immunol. *161*, 1542–1548.

Khattri, R., Cox, T., Yasayko, S.A., and Ramsdell, F. (2003). An essential role for Scurfin in CD4+CD25+ T regulatory cells. Nat. Immunol. *4*, 337–342.

Klein, L., Khazaie, K., and von Boehmer, H. (2003). In vivo dynamics of antigen-specific regulatory T cells not predicted from behavior in vitro. Proc. Natl. Acad. Sci. USA *100*, 8886–8891.

Koenders, M.I., Lubberts, E., Oppers-Walgreen, B., van den Bersselaar, L., Helsen, M.M., Di Padova, F.E., Boots, A.M., Gram, H., Joosten, L.A., and van den Berg, W.B. (2005a). Blocking of interleukin-17 during reactivation of experimental arthritis prevents joint inflammation and bone erosion by decreasing RANKL and interleukin-1. Am. J. Pathol. *167*, 141–149.

Koenders, M.I., Lubberts, E., Oppers-Walgreen, B., van den Bersselaar, L., Helsen, M.M., Kolls, J.K., Joosten, L.A., and van den Berg, W.B. (2005b). Induction of cartilage damage by overexpression of T cell interleukin-17A in experimental arthritis in mice deficient in interleukin-1. Arthritis Rheum. *52*, 975–983.

Kolls, J.K., and Lindén, A. (2004). Interleukin-17 family members and inflammation. Immunity *21*, 467–476.

Kubo, T., Hatton, R.D., Oliver, J., Liu, X., Elson, C.O., and Weaver, C.T. (2004). Regulatory T cell suppression and anergy are differentially regulated by proinflammatory cytokines produced by TLR-activated dendritic cells. J. Immunol. *173*, 7249–7258.

Langrish, C.L., Chen, Y., Blumenschein, W.M., Mattson, J., Basham, B., Sedgwick, J.D., McClanahan, T., Kastelein, R.A., and Cua, D.J. (2005). IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J. Exp. Med. *201*, 233–240.

Leonard, J.P., Waldburger, K.E., and Goldman, S.J. (1995). Prevention of experimental autoimmune encephalomyelitis by antibodies against interleukin 12. J. Exp. Med. *181*, 381–386.

Lin, J.T., Martin, S.L., Xia, L., and Gorham, J.D. (2005). TGF-beta 1 uses distinct mechanisms to inhibit IFN-gamma expression in CD4+ T cells at priming and at recall: differential involvement of Stat4 and T-bet. J. Immunol. *174*, 5950–5958.

Lubberts, E., Joosten, L.A., Oppers, B., van den Bersselaar, L., Coenen-de Roo, C.J., Kolls, J.K., Schwarzenberger, P., van de Loo, F.A., and van den Berg, W.B. (2001). IL-1-independent role of IL-17 in synovial inflammation and joint destruction during collagen-induced arthritis. J. Immunol. *167*, 1004–1013.

Lubberts, E., Koenders, M.I., Oppers-Walgreen, B., van den Bersselaar, L., Coenen-de Roo, C.J., Joosten, L.A., and van den Berg, W.B.

(2004). Treatment with a neutralizing anti-murine interleukin-17 antibody after the onset of collagen-induced arthritis reduces joint inflammation, cartilage destruction, and bone erosion. Arthritis Rheum. *50*, 650–659.

Lucas, S., Ghilardi, N., Li, J., and de Sauvage, F.J. (2003). IL-27 regulates IL-12 responsiveness of naive CD4+ T cells through Stat1-dependent and -independent mechanisms. Proc. Natl. Acad. Sci. USA *100*, 15047–15052.

Maldonado, R.A., Irvine, D.J., Schreiber, R., and Glimcher, L.H. (2004). A role for the immunological synapse in lineage commitment of CD4 lymphocytes. Nature *431*, 527–532.

Malek, T.R., Yu, A., Vincek, V., Scibelli, P., and Kong, L. (2002). CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rbeta-deficient mice. Implications for the nonredundant function of IL-2. Immunity *17*, 167–178.

Mangan, P.R., Harrington, L.E., O'Quinn, D.B., Helms, W.S., Bullard, D.C., Elson, C.O., Hatton, R.D., Wahl, S.M., Schoeb, T.R., and Weaver, C.T. (2006). Transforming growth factor-beta induces development of the T(H)17 lineage. Nature *441*, 231–234.

Marie, J.C., Letterio, J.J., Gavin, M., and Rudensky, A.Y. (2005). TGF-beta1 maintains suppressor function and Foxp3 expression in CD4+CD25+ regulatory T cells. J. Exp. Med. *201*, 1061–1067.

Matthys, P., Vermeire, K., Mitera, T., Heremans, H., Huang, S., and Billiau, A. (1998). Anti-IL-12 antibody prevents the development and progression of collagen-induced arthritis in IFN-gamma receptor-deficient mice. Eur. J. Immunol. *28*, 2143–2151.

Matthys, P., Vermeire, K., Mitera, T., Heremans, H., Huang, S., Schols, D., De Wolf-Peeters, C., and Billiau, A. (1999). Enhanced autoimmune arthritis in IFN-gamma receptor-deficient mice is conditioned by mycobacteria in Freund's adjuvant and by increased expansion of Mac-1+ myeloid cells. J. Immunol. *163*, 3503–3510.

McKenzie, B.S., Kastelein, R.A., and Cua, D.J. (2006). Understanding the IL-23-IL-17 immune pathway. Trends Immunol. *27*, 17–23.

Min, B., Prout, M., Hu-Li, J., Zhu, J., Jankovic, D., Morgan, E.S., Urban, J.F., Jr., Dvorak, A.M., Finkelman, F.D., LeGros, G., and Paul, W.E. (2004). Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. J. Exp. Med. *200*, 507–517.

Mosmann, T.R., and Coffman, R.L. (1989). Th1 and Th2 cells: Different patterns of lymphokine secretion leads to different functional properties. Annu. Rev. Immunol. *7*, 145–173.

Mullen, A.C., High, F.A., Hutchins, A.S., Lee, H.W., Villarino, A.V., Livingston, D.M., Kung, A.L., Cereb, N., Yao, T.P., Yang, S.Y., and Reiner, S.L. (2001). Role of T-bet in commitment of TH1 cells before IL-12-dependent selection. Science *292*, 1907–1910.

Mullen, A.C., Hutchins, A.S., High, F.A., Lee, H.W., Sykes, K.J., Chodosh, L.A., and Reiner, S.L. (2002). Hlx is induced by and genetically interacts with T-bet to promote heritable T(H)1 gene induction. Nat. Immunol. *3*, 652–658.

Murphy, C.A., Langrish, C.L., Chen, Y., Blumenschein, W., McClanahan, T., Kastelein, R.A., Sedgwick, J.D., and Cua, D.J. (2003). Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. J. Exp. Med. *198*, 1951–1957.

Murphy, K.M., and Reiner, S.L. (2002). The lineage decisions of helper T cells. Nat. Rev. Immunol. *2*, 933–944.

Nakae, S., Komiyama, Y., Nambu, A., Sudo, K., Iwase, M., Homma, I., Sekikawa, K., Asano, M., and Iwakura, Y. (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.

Nakae, S., Nambu, A., Sudo, K., and Iwakura, Y. (2003). Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. J. Immunol. *171*, 6173–6177.

Nakamura, K., Kitani, A., and Strober, W. (2001). Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. J. Exp. Med. *194*, 629–644.

Nakamura, K., Kitani, A., Fuss, I., Pedersen, A., Harada, N., Nawata, H., and Strober, W. (2004). TGF-beta 1 plays an important role in the mechanism of CD4+CD25+ regulatory T cell activity in both humans and mice. J. Immunol. *172*, 834–842.

Oppmann, B., Lesley, R., Blom, B., Timans, J.C., Xu, Y., Hunte, B., Vega, F., Yu, N., Wang, J., Singh, K., 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.

Ouyang, W., Ranganath, S.H., Weindel, K., Bhattacharya, D., Murphy, T.L., Sha, W.C., and Murphy, K.M. (1998). Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. Immunity *9*, 745–755.

Ouyang, W., Lohning, M., Gao, Z., Assenmacher, M., Ranganath, S., Radbruch, A., and Murphy, K.M. (2000). Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. Immunity *12*, 27–37.

Park, H., Li, Z., Yang, X.O., Chang, S.H., Nurieva, R., Wang, Y.H., Wang, Y., Hood, L., Zhu, Z., Tian, Q., and Dong, C. (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat. Immunol. *6*, 1133–1141.

Pasare, C., and Medzhitov, R. (2003). Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. Science *299*, 1033–1036.

Peng, Y., Laouar, Y., Li, M.O., Green, E.A., and Flavell, R.A. (2004). TGF-beta regulates in vivo expansion of Foxp3-expressing CD4+CD25+ regulatory T cells responsible for protection against diabetes. Proc. Natl. Acad. Sci. USA *101*, 4572–4577.

Pflanz, S., Timans, J.C., Cheung, J., Rosales, R., Kanzler, H., Gilbert, J., Hibbert, L., Churakova, T., Travis, M., Vaisberg, E., et al. (2002). IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4(+) T cells. Immunity *16*, 779–790.

Robinson, D., Shibuya, K., Mui, A., Zonin, F., Murphy, E., Sana, T., Hartley, S.B., Menon, S., Kastelein, R., Bazan, F., and O'Garra, A. (1997). IGIF does not drive Th1 development but synergizes with IL-12 for interferon-gamma production and activates IRAK and NFkappaB. Immunity *7*, 571–581.

Sakaguchi, S. (2000). Regulatory T cells: Key controllers of immunologic self-tolerance. Cell *101*, 455–458.

Sakaguchi, S. (2004). Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu. Rev. Immunol. *22*, 531–562.

Scharton, T.M., and Scott, P. (1993). Natural killer cells are a source of interferon gamma that drives differentiation of CD4+ T cell subsets and induces early resistance to Leishmania major in mice. J. Exp. Med. *178*, 567–577.

Schramm, C., Huber, S., Protschka, M., Czochra, P., Burg, J., Schmitt, E., Lohse, A.W., Galle, P.R., and Blessing, M. (2004). TGFbeta regulates the CD4+CD25+ T-cell pool and the expression of Foxp3 in vivo. Int. Immunol. *16*, 1241–1249.

Seder, R.A., Paul, W.E., Davis, M.M., and Fazekas de St Groth, B. (1992). The presence of interleukin 4 during in vitro priming determines the lymphokine-producing potential of CD4+ T cells from T cell receptor transgenic mice. J. Exp. Med. *176*, 1091–1098.

Segal, B.M., Dwyer, B.K., and Shevach, E.M. (1998). An interleukin (IL)-10/IL-12 immunoregulatory circuit controls susceptibility to autoimmune disease. J. Exp. Med. *187*, 537–546.

Shinkai, K., Mohrs, M., and Locksley, R.M. (2002). Helper T cells regulate type-2 innate immunity in vivo. Nature *420*, 825–829.

Spilianakis, C.G., Lalioti, M.D., Town, T., Lee, G.R., and Flavell, R.A. (2005). Interchromosomal associations between alternatively expressed loci. Nature *435*, 637–645.

Stark, M.A., Huo, Y., Burcin, T.L., Morris, M.A., Olson, T.S., and Ley, K. (2005). Phagocytosis of apoptotic neutrophils regulates granulopoiesis via IL-23 and IL-17. Immunity *22*, 285–294.

Stavnezer, J. (1996). Immunoglobulin class switching. Curr. Opin. Immunol. *8*, 199–205.

Szabo, S.J., Kim, S.T., Costa, G.L., Zhang, X., Fathman, C.G., and Glimcher, L.H. (2000). A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell *100*, 655–669.

Ulloa, L., Doody, J., and Massague, J. (1999). Inhibition of transforming growth factor-beta/SMAD signalling by the interferon-gamma/STAT pathway. Nature *397*, 710–713.

Veldhoen, M., Hocking, R.J., Atkins, C.J., Locksley, R.M., and Stockinger, B. (2006). TGFbeta in the context of an inflammatory cytokine

milieu supports de novo differentiation of IL-17-producing T cells. Immunity *24*, 179–189.

von Boehmer, H., Aifantis, I., Gounari, F., Azogui, O., Haughn, L., Apostolou, I., Jaeckel, E., Grassi, F., and Klein, L. (2003). Thymic selection revisited: how essential is it? Immunol. Rev. *191*, 62–78.

Wahl, S.M. (1994). Transforming growth factor beta: the good, the bad, and the ugly. J. Exp. Med. *180*, 1587–1590.

Wakkach, A., Fournier, N., Brun, V., Breittmayer, J.-P., Cottrez, F., and Groux, H. (2003). Characterization of denritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo. Immunity *18*, 605–617.

Walker, L.S., Chodos, A., Eggena, M., Dooms, H., and Abbas, A.K. (2003a). Antigen-dependent proliferation of CD4+ CD25+ regulatory T cells in vivo. J. Exp. Med. *198*, 249–258.

Walker, M.R., Kasprowicz, D.J., Gersuk, V.H., Benard, A., Van Landeghen, M., Buckner, J.H., and Ziegler, S.F. (2003b). Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25- T cells. J. Clin. Invest. *112*, 1437–1443.

Wan, Y.Y., and Flavell, R.A. (2005). Identifying Foxp3-expressing suppressor T cells with a bicistronic reporter. Proc. Natl. Acad. Sci. USA *102*, 5126–5131.

Way, S.S., and Wilson, C.B. (2004). Cutting edge: immunity and IFN-gamma production during Listeria monocytogenes infection in the absence of T-bet. J. Immunol. *173*, 5918–5922.

Willenborg, D.O., Fordham, S., Bernard, C.C., Cowden, W.B., and Ramshaw, I.A. (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.

Wolf, M., Schimpl, A., and Hunig, T. (2001). Control of T cell hyperactivation in IL-2-deficient mice by CD4(+)CD25(-) and CD4(+)CD25(+) T cells: evidence for two distinct regulatory mechanisms. Eur. J. Immunol. *31*, 1637–1645.

Yamazaki, S., Iyoda, T., Tarbell, K., Olson, K., Velinzon, K., Inaba, K., and Steinman, R.M. (2003). Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. J. Exp. Med. *198*, 235–247.

Yang, J., Murphy, T.L., Ouyang, W., and Murphy, K.M. (1999). Induction of interferon-gamma production in Th1 CD4+ T cells: evidence for two distinct pathways for promoter activation. Eur. J. Immunol. *29*, 548–555.

Zhang, G.X., Gran, B., Yu, S., Li, J., Siglienti, I., Chen, X., Kamoun, M., and Rostami, A. (2003). Induction of experimental autoimmune encephalomyelitis in IL-12 receptor-beta 2-deficient mice: IL-12 responsiveness is not required in the pathogenesis of inflammatory demyelination in the central nervous system. J. Immunol. *170*, 2153–2160.

Zheng, W., and Flavell, R.A. (1997). The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell *89*, 587–596.

Zheng, W.P., Zhao, Q., Zhao, X., Li, B., Hubank, M., Schatz, D.G., and Flavell, R.A. (2004). Up-regulation of Hlx in immature Th cells induces IFN-gamma expression. J. Immunol. *172*, 114–122.