Published in final edited form as:

Immunol Rev. 2008 December ; 226: 219–233. doi:10.1111/j.1600-065X.2008.00711.x.

Diversity in the contribution of IL-10 to T-cell-mediated immune regulation

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Summary

Recent progress in our understanding of mechanisms by which the immunosuppressive cytokine interleukin-10 (IL-10) participates in an ever-increasing diversity of T-cell lineages to maintain immune homeostasis has broadened the framework for defining regulatory and effector T cells and has blurred the lines between them. In this review, we highlight established and emerging roles for IL-10 produced by distinct CD4⁺ T-cell lineages that underlie its non-redundant role in curbing immune responses to the intestinal microbiota at steady state and its role to limit T-cell-driven inflammation in responses to pathogens.

Keywords

T cells; Th1/Th2/Th17 cells; cytokines; lineage commitment

Introduction

Interleukin-10 (IL-10) was originally termed cytokine synthesis inhibitory factor (CSIF), owing to its discovery as a product of T-helper type 2 (Th2) cells that suppressed the differentiation and effector functions of Th1 cells (1). It was subsequently discovered that IL-10 suppresses the production of pro-inflammatory cytokines by dendritic cells (DCs) and macrophages, including IL-12, thereby inhibiting the ability of antigen-presenting cells (APCs) to induce differentiation of Th1 cells (2). In contrast to many other hematopoietic cytokines, IL-10 has closely related homologues encoded in the genomes of several viruses, which are able to bind the IL-10 receptor and inhibit immune activity (3-6). This represents a possible adaptation for immune system evasion by viruses and emphasizes the important role of IL-10-like molecules in suppression of immune responses. Binding of IL-10 to its receptor, comprised of the IL-10 receptor 1 (IL-10R1) and IL-10R2 chains, initiates a STAT3-dependent signaling cascade that ultimately results in suppression of transcription of several target genes already described (7), but beyond this role, the IL-10-induced gene program is poorly understood.

Since its discovery, the diversity of hematopoietic cells that produce IL-10 has grown, and it is now known to be produced by both innate and adaptive immune cells, including, in addition to Th2 cells, monocytes, macrophages, DCs, B cells, CD8⁺ T cells, regulatory T cells (Tregs), Th1 cells, and, most recently, Th17 cells (8). Irrespective of the cellular source, the principal role of IL-10 appears to be containment and suppression of inflammatory responses so as to downmodulate effector adaptive immune responses and minimize tissue damage in response to microbial challenges. Accordingly, IL-10 induces

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downregulation of major histocompatibility complex (MHC) antigens, the intercellular adhesion molecule-1 (ICAM-1), as well as the costimulatory molecules CD80 and CD86 on APCs (9), and it has been shown to promote differentiation of DCs expressing low levels of MHC class II (MHC II), CD80, and CD86 (10). Thus, IL-10 is able to limit the ability of APCs to promote the differentiation and/or proliferation of CD4⁺ T cells, thereby regulating both initiation and perpetuation of adaptive T-cell responses. In addition, IL-10 downregulates or completely inhibits expression of several pro-inflammatory cytokines and other soluble mediators, thereby further compromising the capacity of effector T cells to sustain inflammatory responses to antigenic challenges.

Encounters between foreign organisms and the immune system are generally rare in comparison to the constant exposure to ingested food antigens and the commensal intestinal microbiota, each of which can trigger inflammatory responses when unrestrained. Thus, much of the on-going immune regulation at steady state is focused on intestinal immune homeostasis that involves a dynamic process of preventing immune cell activation and inflammation in response to harmless antigens while retaining the capacity to mount effective responses to those associated with potential pathogens. Whereas the cell types and anti-inflammatory mediators involved in this process are diverse, evidence from animal studies implicate IL-10, specifically CD4⁺ T-cell-derived IL-10, as a requisite, non-redundant mediator of intestinal immune homeostasis (11,12). Accordingly, much of our early understanding of mechanisms by which IL-10 participates in immune regulation has been contributed by studies of intestinal inflammation regulated by CD4⁺ T cells. Thus, in several models of inflammatory bowel disease (IBD), specific subsets of CD4⁺ T cells with immune regulatory properties have been shown to act via mechanisms that are either completely or partially dependent on IL-10 (13,14). A similar role for IL-10-producing T cells in restraining anti-pathogenic responses was also evident early on (15) and has gained momentum recently, as discussed below.

Historically, technical constraints have limited our ability to identify and study the cells that produce IL-10 *in vivo*, often leading to conflicting or contradictory results. The recent development of reporter models that permit identification and tracking IL-10-expressing cells have begun to facilitate a more refined understanding of the cell subsets that produce IL-10 and their developmental origins, both in the steady state as well as in response to infection (16-18), and promise new insights into the regulation of IL-10 expression (19). In this review, we focus on the role of IL-10-producing lymphocytes, particularly CD4⁺ T cells, in immune regulation, with special emphasis on recent advances in our understanding of the development of IL-10-producing regulatory and effector CD4⁺ T-cell subsets.

IL-10 expression by CD4⁺ T-cell subsets: a growing family of Treg cells

The involvement of IL-10 in T-cell-mediated immune regulation, until recently, has been primarily linked to its production by two general subsets of Tregs: 'natural' and 'induced' (or 'adaptive') Tregs, which develop intrathymically and extrathymically, respectively. Natural Tregs (nTregs) are characterized by their expression of the transcription factor forkhead box protein 3 (Foxp3) during thymic development. Foxp3 initiates and maintains a developmental program that specifies this Treg lineage during positive selection on high affinity T-cell receptor ligands. Unlike nTregs, which are fully functional upon thymic export, the regulatory function of induced Tregs (iTregs) is acquired in the periphery from antigen-naïve CD4⁺ T-cell precursors. iTregs can be subdivided on the basis of their expression of Foxp3. Foxp3⁺ iTregs differentiate from naïve precursors in the periphery in response to antigens presented on non-inflammatory APCs and transforming growth factor- β (TGF- β) and develop functional and phenotypic features that are largely indistinguishable from nTregs, reflecting the dominance of Foxp3⁺ in dictating the gene expression program

of both these cell types. Both types of Foxp3⁺ Tregs, natural and induced, can produce IL-10, although, as will be discussed below, many facets of their regulatory function are independent of IL-10. Foxp3⁻ iTregs also develop extrathymically from naive precursors, perhaps the best characterized of which are the IL-10-producing T regulatory type 1 (Tr1) cells, which are characterized by high level production of IL-10 that is critical for their regulatory function (20).

In keeping with its original links to an effector T-cell lineage, Th2, IL-10 has more recently been linked to each of the other effector lineages, Th1 and Th17. Similar to its production by *bona fide* Tregs, IL-10 production by cells that arise from each of the three effector CD4⁺ T-cell subsets (Th1, Th2, and Th17) appears important for downmodulation of at least certain immune responses, increasing the range of IL-10's importance as a regulator of inflammatory adaptive immunity.

Overlapping expression of IL-10 and Foxp3

It has long been accepted that IL-10 is one of the cytokines utilized by natural Foxp3⁺ Tregs to suppress immune responses. Sorted nTreg populations (CD25⁺ and/or Foxp3⁺) express IL-10 transcripts in the absence of *ex vivo* reactivation, and IL-10 is thus considered a part of the natural Foxp3⁺ Treg signature (21,22). The nTregs utilized in most analyses are commonly isolated from secondary lymphoid tissues, where a minority (~10% - 20%) of all Foxp3⁺ cells expresses IL-10 (18). The highest frequencies of IL-10-expressing Foxp3⁺ Tregs are found in the lymph nodes draining the intestines but, even in these sites, represent a minority of all Foxp3⁺ Tregs. The basis for restricted expression of IL-10 by Foxp3⁺ Tregs in secondary lymphoid tissues is not yet known, but based on these findings, it is not surprising that *in vitro* experiments and certain adoptive transfer studies utilizing splenic and/or lymph node-derived Foxp3⁺ Tregs revealed little or no role for IL-10 in nTreg cell function. These data are therefore consistent with the findings that mice deficient for genes that are more ubiquitously expressed within the natural Treg lineage such as *Foxp3*, *Ctla4*, and *Tgfb1*, develop fatal, systemic autoimmunity (23-26), while *Il10*^{-/-} mice do not. Instead, the inflammatory pathology associated with *Il10* deficiency is largely restricted to the intestines (27) and is eliminated in gnotobiotic mice lacking an intestinal microbiota (28). Thus, at least in the steady state, IL-10 is dispensable for systemic immune regulation but plays a non-redundant role in the maintenance of intestinal immune homeostasis to the intestinal flora.

nTregs in IL-10-mediated immune regulation

Studies that have examined the role of Foxp3⁺ Tregs in the control of intestinal inflammation have painted a complex picture regarding the role of IL-10. In the CD45RB^{hi} T-cell transfer model of colitis, disease prevention by CD45RB^{lo} Tregs was dependent on IL-10 (13,29). Conversely, when Foxp3⁺ Tregs were enriched on the basis of expression of CD25 instead of CD45RB, IL-10 was not required for disease prevention (13), and somewhat surprisingly, neither was TGF-β (30). Interestingly, the novel cytokine IL-35, recently identified as a constitutive product of Foxp3⁺ Tregs, was able to reverse ongoing colitis in this model (31) and could possibly compensate for the absence of either IL-10 or TGF-β. IL-35 is the most recently identified member of the IL-12 family of cytokines and is a heterodimer composed of an IL-12p35 chain (or IL-12a, which is shared with IL-12) and an Epstein-Barr virus-induced gene 3 (EBI-3) chain (shared with IL-27). However, the requirement for IL-35 in mediating immune homeostasis is still unclear, as mice lacking the genes encoding either subunit of IL-35 (*Il12a*^{-/-} or *Ebi3*^{-/-} mice) display no overt immunological abnormalities. It has been suggested that because deletion of either component of IL-35 also compromises IL-12- or IL-27-dependent pro-inflammatory cascades, the lack of inflammatory disease in IL-35-deficient mice might be attributed to

their concomitant loss of these critical pro-inflammatory cytokines (31). Nevertheless, IL-10-deficient mice, in which all regulatory and pro-inflammatory pathways associated with the subunits of IL-35 are intact, succumb to commensal flora-dependent colitis, indicating that in otherwise immune sufficient mice, IL-35 cannot compensate for IL-10.

An alternative explanation for the IL-10-independent contribution of Foxp3⁺ Tregs in intestinal immune homeostasis derives from the fact that in the CD45RB transfer model used for many of these studies, disease development requires homeostatic expansion of the transferred CD4⁺ T cells that is accompanied by their differentiation into effector cells. Because this occurs predominantly in the secondary lymphoid tissues, where Foxp3⁺ cells can act to inhibit effector T-cell differentiation from naive precursors, development of flora-reactive effector T cells that would otherwise traffic to the intestines and drive inflammation there is blocked at the inductive stage. Accordingly, co-transfers of Foxp3⁺ Treg-enriched CD25⁺ cells deficient for the integrin β_7 , which is important for entry of lymphocytes into the intestines, could still prevent induction of disease by CD45RB^{hi} T cells, because they retain trafficking to secondary lymphoid sites (32). Therefore, although Treg activity can normally be detected in both the mesenteric lymph nodes and colon (13,32), in this model, protection does not require the presence of Tregs in the intestines. However, the ability of CD25⁺ Tregs to reverse ongoing inflammation, which was driven by effector cells that had already reached the intestines, was dependent on IL-10 produced by the Treg cells (33).

Prior to the onset of intestinal inflammation, the number and frequency of Foxp3-expressing cells present in the intestines of IL-10-deficient mice closely resemble those of wildtype mice (our unpublished observations). Thus, expression of Foxp3 in the absence of IL-10 expression is inadequate for the maintenance of intestinal homeostasis in otherwise immunocompetent mice. Using IL-10 reporter mice, we have found that at steady state, the large majority of IL-10-expressing CD4⁺ T cells are resident in the intestines (18). Furthermore, we and others have shown that the colonic *lamina propria* is enriched for Foxp3⁺ cells, a large fraction of which express IL-10 both in the steady state and during cure of CD45RB^{hi}-induced colitis (17,18,33). Thus, the protective role of Foxp3⁺ cells in the intestinal tissues is largely dependent on their ability to express IL-10. This was reinforced by a recent study demonstrating that inactivation of the *Il10* locus in Foxp3-expressing cells results in spontaneous colonic inflammation (34). Collectively, these studies suggest a two-tiered model of immune regulation by Foxp3⁺ Tregs, one component of which is aimed at blocking induction of effector T-cell development in T-cell zones of secondary lymphoid tissues and is IL-10-independent, and a second component of which is aimed at downmodulating effector T-cell-driven inflammation in tissues where target antigens enter, such as the intestines, through IL-10-dependent mechanisms. This partitioning of the sites where IL-10-dependent regulation is most critical may prove important for continued efforts to use delivery of exogenous IL-10 to treat ongoing inflammatory bowel diseases (35,36).

Mechanisms of induction of IL-10 in developing Foxp3⁻ and Foxp3⁺ Tregs

Foxp3⁺ T cells present in mucosal tissues are generally believed to be a mixture of Foxp3⁺ cells of thymic origin (nTregs) and peripherally induced Foxp3⁺ iTregs (37). In the steady state, adoptively transferred naive Foxp3⁻ T cells can upregulate Foxp3 expression in peripheral tissues, which is enhanced in intestinal tissues (38). In fact, adoptive transfer of Foxp3⁻ thymocytes resulted in the differentiation of three populations of Treg-based on coordinate or differential expression of Foxp3 and IL-10 - Foxp3⁻IL-10⁺, Foxp3⁺IL-10⁺, and Foxp3⁺IL-10⁻ - with the frequencies and tissue distribution of each population resembling that of intact mice. Interestingly, the development of all three subsets was dependent on TGF- β (18). Thus, similar to the induction of Foxp3 by naive CD4⁺ T-cell precursors *in vitro*, peripheral induction of both Foxp3 and IL-10 *in vivo* required TGF- β . Although it is still not known whether Foxp3⁺IL-10⁺ Tregs present in peripheral tissues develop from

Foxp3⁻ or Foxp3⁺ thymic precursors, or more likely a mixture of both, IL-10 was not expressed by either subset of thymocytes and adoptive transfer of Foxp3⁺ thymic precursors resulted in induction of IL-10 in a fraction of cells, with increased induction in intestinal tissues (18). Thus, whereas Foxp3 can be induced intrathymically or extrathymically, IL-10 induction in both Foxp3⁺ nTregs and iTregs occurs in the periphery.

A recent report demonstrated that Foxp3⁺ precursors isolated from the human thymus that co-expressed the receptor for inducible costimulator ligand (ICOS-L) preferentially upregulated IL-10 expression in response to stimulation with APCs that express ICOS-L (39). It was suggested that expression of ICOS by T cells identifies precursors of the Foxp3⁺IL-10⁺ cells found in the periphery. However, in our studies in mouse, we have found no role for ICOS expression or signaling in the induction of IL-10 in CD4⁺ T cells *in vivo* at the steady state (Maynard *et al.*, manuscript in preparation). Instead, consistent with earlier findings associating expression of ICOS with high-level expression of IL-10 (40), we have found that costimulation via ICOS enhances the IL-10 output of already differentiated IL-10-competent cells. Thus, although repetitive costimulation via ICOS probably does promote elevated expression of IL-10, this pathway is apparently not essential for commitment to any IL-10-producing lineage in mice. Further studies will be required to determine whether this represents differences between mouse and human.

In addition to its role in maintaining Foxp3 expression in nTregs following thymic export (41), TGF- β also promotes the extrathymic induction of Foxp3 in naive CD4 precursors (42-44). Importantly, it was recently shown that the vitamin A metabolite, *all-trans* retinoic acid (*at*-RA), which is produced by mucosal DCs, particularly the CD103⁺ subset, is a co-factor that enhances the TGF- β -dependent induction of Foxp3 in naive CD4⁺ T cells (38,45-47). This observation suggests that *at*-RA, which also promotes gut tropism in lymphocytes (48), is a key player in the induction and maintenance of Foxp3⁺ Treg cell-mediated mucosal homeostasis. However and somewhat paradoxically, we have found that *at*-RA produced by mucosal DCs inhibits the TGF- β -mediated induction of IL-10 in CD4⁺ T cells (Maynard *et al.*, manuscript submitted). This included both the CD103⁻ and CD103⁺ subsets, although CD103⁻ DCs allowed a modest induction of IL-10 slightly above background. Therefore, it appears that Foxp3 and IL-10 are differentially induced in naive precursors, perhaps via interactions with distinct populations or differentially activated APCs. Collectively, our data suggest that the APC populations that support development of IL-10-producing Tregs do not metabolize vitamin A to generate *at*-RA but either produce TGF- β themselves or, if residing in an environment rich in TGF- β such as the intestines, may activate latent TGF- β to initiate induction of IL-10.

Along these lines, it was recently shown that mice with a DC-specific deficiency of the TGF- β -activating integrin $\alpha_v\beta_8$ spontaneously develop colonic inflammation as they age (49). This was attributed to the diminished capacity of $\alpha_v\beta_8$ -deficient DCs to induce Foxp3⁺ Tregs extrathymically, as there was a substantial reduction in the frequency of Foxp3⁺ cells isolated from the colon of mice lacking $\alpha_v\beta_8$ on DCs relative to their wildtype counterparts. An alternative possibility is that, consistent with our findings that TGF- β promotes both Foxp3 and IL-10 in the periphery, colitis in the *Itgb8* conditional knockout mice was more directly a result of reduced induction of IL-10 in colonic CD4⁺ T cells. Thus, $\alpha_v\beta_8$ activation of TGF- β might be one mechanism that contributes to the development of IL-10-producing Tregs in the steady state, although this idea will require further study.

Tr1 cells

The first CD4⁺ T-cell population to be defined as 'Tr1' was isolated from human severe combined immunodeficient (SCID) patients transplanted with human leukocyte antigen (HLA)-mismatched hematopoietic cells (50). Despite the mismatch, patients failed to

develop graft versus host disease (GVHD), even in the absence of immunosuppressive drugs, and were found to have significantly elevated levels of IL-10 in the blood. It was soon discovered that most of this IL-10 was produced by a subset of donor-derived T cells. Subsequent studies have identified Tr1 cells, CD4⁺ T cells with regulatory ability predicated on IL-10 expression, in various settings *in vivo*. For instance, self MHC-reactive Tr1 clones that inhibit naive and antigen-specific CD4⁺ T-cell proliferation in an IL-10/TGF- β -dependent manner have been isolated from healthy individuals (51). It was demonstrated that in non-allergic humans, the majority of allergen-specific CD4⁺ T cells in the peripheral blood are IL-10-producing Tr1-type cells. In contrast, in allergic patients, these cells are significantly outnumbered by IL-4-producing allergen-responsive Th2 cells (52). Finally, Tr1 cells specific for Desmoglein 3 (Dsg 3), the autoantigen of *Pemphigus vulgaris* (PV), were isolated from 80% of healthy carriers compared with just 17% of PV patients (53). Earlier studies using rodent models also suggested the existence of Tr1-like cells *in vivo*, particularly in the murine intestines (54).

Like natural Tregs, Tr1 cells proliferate poorly *in vitro*, hindered, at least in part, by the autocrine effects of IL-10. Thus, antibody blockade of IL-10 partially restores Tr1 proliferation (14,50). *In vivo*, Tr1 cells also proliferate in response to IL-15, which is considered a T-cell receptor (TCR)-independent growth factor for Tr1 cells (55). Tr1s display an enhanced capacity to migrate to inflamed tissues and have been shown to inhibit inflammatory responses in the colon and central nervous system (14,56). Consistent with their functional dependence on IL-10 production, antibody blockade of the IL-10 receptor following adoptive transfer of Tr1 cells abrogated their protective effect against CD4⁺ T-cell-mediated intestinal inflammation. While the trafficking of Tr1 cells to inflammatory sites appears to be antigen-independent, their regulatory activity at inflamed sites appears to be antigen-dependent. Nevertheless, activated Tr1s can suppress local inflammation driven by other T cells with distinct antigenic specificities (14) and are thus believed to function via 'bystander' suppression - in accordance with their secretion of IL-10 as a suppressive mechanism.

The observation that T-cell-specific deficiency of IL-10 results in spontaneous colonic inflammation highlights the importance of IL-10-producing Tregs in the maintenance of gut homeostasis (11). This role coupled with the observation that targeting of IL-10 deficiency to the Foxp3⁺ subset of CD4⁺ T cells results in less severe disease (34), suggests that Foxp3⁺ Tr1 cells are at least partially responsible for intestinal immune regulation. Indeed, we have observed that Foxp3⁺IL-10⁺ cells are enriched in the colon, albeit at a reduced frequency relative to Foxp3⁺IL-10⁺ cells, and are highly enriched in the small intestine, where they outnumber Foxp3⁺IL-10⁺ cells (18).

Tr1 versus IL-10 Tregs: distinct subsets?

Concerning IL-10-producing Tregs that do not express Foxp3, there remains considerable controversy over developmental origins and functions, complicating nomenclature and immune regulatory properties. Murine Tr1 cells can be generated *in vitro* by repetitive antigenic stimulation of naive CD4 precursors in the presence of IL-10 (14) or IL-10-conditioned, 'tolerogenic' DCs (10) and can also develop *in vivo* from T cells primed in the presence of IL-10. Besides IL-10, Tr1 have been reported to produce other cytokines, including IL-5 and IFN- γ , although the function of these cytokines, if any, has not been critically examined. Tr1 cells also produce TGF- β , which is partially responsible for Tr1-mediated suppression *in vitro* (57). It has been proposed that the term Tr1 be used to refer to Tregs whose development and function are absolutely dependent on IL-10. This definition excludes other IL-10-producing Tregs that can develop under other experimental conditions, and such cells are alternatively referred to as 'IL-10 Tregs.' Included under this moniker are IL-10-producing T cells induced in the presence of vitamin D3 (Vit D3) and dexamethasone

(Dex) (58), which display some differences in cytokine profiles from that of the prototypical Tr1 cells. However, Tr1-like cells have also been differentiated *in vitro* under other conditions that were also devoid of IL-10. For example, fully functional Tr1-like cells have been induced by CD2 costimulation (59) as well as by CD3 and CD46 activation (60). In fact, when Tregs were differentiated *in vitro* using artificial APCs that expressed elevated levels of CD58 and CD80, addition of exogenous IL-10 resulted in only a very modest increase in Tr1 differentiation (61). Clearly, at least *in vitro*, there appear to be multiple pathways to induction of IL-10-expressing T cells with regulatory activity akin to that originally described for Tr1 cells, although much work needs to be done to clarify *in vivo* correlates and their origins.

The implementation of dual reporter mice that permit identification of Tregs on the basis of coordinate or differential expression of IL-10 and Foxp3 have begun to permit studies to better define Tregs, such as Tr1 and IL-10 Tregs, distinguished from natural or induced Tregs on the basis of their lack of Foxp3 expression (62,63). Interestingly, we have observed that secondary lymphoid tissues of dual reporter mice harbor a small fraction of Foxp3⁺IL-10⁺ 'Tr1-like' cells that express high levels of IL-10, limited IFN- γ and no IL-4 (18). Similar to Tr1 cells generated *in vitro*, isolated Foxp3⁺IL-10⁺ cells suppressed responder cell proliferation *ex vivo* via the additive effects of IL-10 and TGF- β . Interestingly, Foxp3⁺IL-10⁺ T cells are highly enriched in the small intestines, particularly among the intra-epithelial lymphocytes (17,18), confirming earlier findings suggesting the presence of Tr1-like cells in the intestines (54).

Somewhat surprisingly and in apparent conflict with *in vitro* studies, we found that the development of Foxp3⁺IL-10⁺ CD4⁺ T cells *in vivo* could occur in the absence of IL-10 (18). Thus, cells with phenotypic features characteristic of Tr1 cells developed in dual reporter mice deficient for IL-10. However, a role for IL-10 in the maintenance of this subset cannot be ruled out without further experimentation. Moreover, because these experiments were performed in mice completely deficient in IL-10, production of IL-10 protein could not be examined in these cells. Thus, while IL-10 might be dispensable for the induction of *Il10* gene transcription in CD4⁺ T cells *in vivo*, it is still unknown whether it is essential for actual or optimal expression of IL-10 protein. Further, we observed a remarkably polarized distribution of Tr1-like cells (Foxp3⁺IL-10⁺) versus Foxp3⁺IL-10⁺CD4⁺ T cells in the small and large intestines of dual reporter mice, respectively, implying regional differences in the development of IL-10 competent Treg subsets, the basis for which is unknown and requires further investigation.

A role for nTregs in the development of Tr1 cells?

A possible role for nTregs in the development of Tr1 cells *in vitro* has been investigated. Co-culture of CD25⁺ (Foxp3-enriched) Tregs with CD25⁻CD4⁺ T cells resulted in the differentiation of cells with regulatory properties that suppressed in an IL-10- and/or TGF- β -dependent manner (64,65). However, Levings *et al.* (66) have shown that Tr1 differentiation *in vitro* in the presence of immature DCs requires IL-10 but not nTregs. Furthermore, adoptive transfer of Foxp3⁺ CD4⁺ thymocytes into naive hosts resulted in the induction of IL-10 expression in a subset of cells, particularly in cells recovered from intestinal tissues (18). Importantly, treatment of recipient mice with a TGF- β blocking antibody resulted in significantly reduced frequencies of IL-10-expressing cells. In addition, DCs conditioned by induced Foxp3⁺ Tregs induced development of Tr1-like cells via an IL-27-dependent mechanism that was augmented by TGF- β (67). Hence, it appears that although Foxp3⁺ Tregs, via secretion of TGF- β and in certain situations IL-10, can induce Tr1 development, it appears that the latter subset can arise independently of the former, suggesting that Tr1 are a distinct subset of Tregs whose development does not include an obligatory requirement for Foxp3⁺ Tregs.

Tr1-promoting features of APCs

Despite our limited understanding of the mechanisms controlling differentiation of Tr1-like cells *in vivo*, the *in vitro* protocols for generation of these cells that have been reported involve chronic TCR stimulation and/or the use of immunosuppressive reagents that downmodulate APC function. These protocols suggest the importance of tolerogenic APCs, and/or the mechanisms that render DCs as such, in the *in vivo* development of Tr1 cells. Treatment of DCs with IL-10 (68,69) or TGF- β (70) enhanced their ability to induce Treg cell differentiation *in vitro*. The enrichment of Tr1 cells in the small intestines suggests the presence of APCs that preferentially mediate induction of IL-10 in CD4⁺ T cells in the intestines and/or associated lymphoid tissues. Interestingly, chronic stimulation of T cells with CD8 α ⁺ DCs from mesenteric lymph nodes induced Tr1-like cell development (71). In addition, the *lamina propria* of the small intestine was shown to harbor a population of CD11c⁺CD11b⁻CD103^{hi} DCs with superior ability to induce IL-10 production by CD4⁺ T cells relative to their CD11c⁺CD11b⁺CD103^{lo} counterparts (72).

In addition to the tissue site, the relative expression of certain molecules by APCs has been linked to their ability to induce IL-10 expression in CD4⁺ T cells. Splenic CD11c^{lo}CD45RB^{hi} DCs, which expressed low levels of MHC II and CD86 even after lipopolysaccharide (LPS) treatment, have been shown to induce antigen-specific Tr1 cells both *in vitro* and *in vivo* through their secretion of IL-10 (10). In contrast, elevated levels of CD40, CD86, and programmed-death ligand 1 (PD-L1) have been detected on APC subsets that efficiently induced Tr1 cells (73,74). LPS treatment was shown to induce antigen-specific IL-10-producing CD4⁺ T cells that suppressed CD8⁺ T-cell responses *in vivo* (75). Finally, maturation-dependent expression of ICOS-L was shown to enhance the ability of plasmacytoid DCs (pDCs) to induce Tr1-like cell development (76). Thus, expression of IL-10 in CD4⁺ T cells can be promoted by several different APC populations, reinforcing the multiplicity of inductive pathways that can culminate in expression of IL-10 by T cells. Conversely, similar to its inhibition of IL-10 expression in Th2 cells, OX40 ligand (OX40L) suppressed the development of Tr1 cells induced by Vit D3 and Dex, ICOSL, or immature DCs (77).

Elusiveness of a specific Tr1 marker

Detailed characterization of Tr1 cells that may arise physiologically has been hampered by the absence of specific markers that permit their identification. Because high IL-10 secretion is the only unifying feature ascribed to this population, with the attendant difficulties of isolating pure cell populations defined by a secreted product, it has been difficult to isolate pure populations of IL-10-expressing cells in numbers sufficient for detailed analyses. Thus, each of the Tr1-like cells described as arising *in vivo* have been identified by high-level expression of IL-10 only following *ex vivo* re-stimulation. Although expression of ICOS has recently shown promise as a marker in human studies (39), our own studies using reporter mice suggest that this may be more complicated in mice and/or is species specific. Tr1 cells have also been shown to express the gene encoding repressor of GATA-3 (ROG) (78). However, while ROG could be useful in distinguishing Foxp3⁺ Tregs and Foxp3⁻ Tregs, it is also expressed by other activated CD4⁺ T cells, limiting its usefulness as a potential marker of Tr1 cells.

Tr1 cells can also express Th1-associated (CXCR3 and CCR5) and Th2-associated (CCR3, CCR4, and CCR8) chemokine receptors (79), limiting usefulness of these markers, and raising issues concerning the true origins of Tr1 cells *in vivo*. Do the overlapping phenotypic features between Tr1 cells and effector T cells represent convergent features of distinct developmental programs, do they imply that the Tr1 cells are derived from effector T cells, or both? As discussed below, it is increasingly apparent that IL-10 producers with regulatory

features are generated in concert with certain protective or autoimmune Th1, Th2, and Th17 responses. In this regard, our own microarray analyses of Foxp3⁺IL-10⁺ cells isolated from secondary lymphoid tissues of Foxp3/IL-10 dual reporter mice indicate considerable overlap with gene expression profiles of Th1, Th2, and Th17 cells (unpublished observations). Nevertheless, as discussed above, there remain substantial, albeit complex, experimental data in support of Tr1-like subsets that develop independently of classical effector T cell lineages, and this remains a central, open issue, resolution of which will have important implications for strategies to modulate immune regulation for therapeutic ends.

IL-10-producing, 'effector-regulatory' CD4⁺ T cells

Despite its initial discovery as a product of Th2 cells, subsequent studies have revealed that IL-10 can be induced in each of the three effector T-cell subsets defined to date, highlighting the diverse requirements and effects of IL-10 as a cytokine critical for restraint of adaptive immunity (Fig. 1). In an increasing number of studies, IL-10-producing cells are being identified as products of Th1, Th2, or Th17 responses to chronic antigenic stimulation, whether induced by different classes of pathogens, autoantigens, or the commensal microbiota. Thus, whereas deficiency of IL-10 under pathogen-free conditions results in uncontrolled effector responses limited to the intestinal flora, in infectious settings, IL-10 appears essential to limit host tissue injury that can result from unbridled T-cell effector-driven inflammation, irrespective of the type of adaptive response. How this coupling of effector and regulatory facets of effector T-cell development is achieved to prevent perpetuation of an effector response once the inciting pathogen has been controlled, and how the buffering of effector responses by IL-10-producers is balanced to enable effective recall responses to pathogens remain to be determined. Here, we highlight studies that are beginning to frame these issues, and are providing insights into the complexity of their implications for regulation of expression of the *Il10* gene locus.

IL-10 and Th2 cells

In contrast to the original reports, it is increasingly apparent that IL-10, despite being a product of Th2 cells, can actually suppress classical Th2 responses (80). Following stimulation of naïve ovalbumin (OVA)-specific CD4⁺ T cells with DCs isolated from the bronchial lymph nodes of OVA-challenged mice, induced Th2 cells that initially expressed IL-4 and IL-10 transitioned to exclusive production of IL-10 with successive rounds of activation. Consistent with their origin from true Th2 precursors, these cells were later reported to express the Th2-specific transcription factor, GATA-3 (81). In another study, adoptive transfer of OVA-specific, CD4⁺CD25⁺ nTregs prevented OVA-induced airway hyperreactivity in a Th2 model of asthma, but protection could be inhibited by IL-10-blockade. Interestingly, the IL-10 was not derived from the nTreg cells (or macrophages, DCs, or B cells), as IL-10-deficient CD4⁺CD25⁺ cells were still protective (82). These data support a model in which the nTregs contribute to the suppression of Th2-driven airway inflammation via amplification of the IL-10 production Th2 effectors.

In vitro, Th2-polarized cultures display considerable intraclonal heterogeneity that increases contingent upon the number of cytokines analyzed simultaneously in single cells (83). For example, a simple pairwise analysis of IL-4 and IL-10 in Th2 cells results in three subpopulations based on differential or coordinate expression of IL-4 and IL-10: IL-4⁺IL-10⁻, IL-4⁺IL-10⁺, and IL-4⁻IL-10⁺. Exactly what controls this skewing of differentiating cells to each of these subsets is unclear. Thus, it is yet to be determined whether these subsets arise through predominant expression of an effector cytokine (e.g. IL-4) early in the response that gives rise through gradual silencing of effector cytokine expression and transition through a 'double-positive' intermediate to regulatory cytokine (i.e. IL-10) production later in the response, or rather through parallel and stochastic

probabilities of expression of individual cytokine genes results in these subsets, or both. At least in some studies, there is support for the former mechanism, as Th2 cells can transition from IL-4⁺IL-10⁺ cells to IL-4⁻IL-10⁺ cells with repetitive stimulation in the presence of IL-10-producing CD8 α ⁻ pulmonary DCs (84). Although the mechanism of IL-4 induction was not examined, DC-derived IL-10 may have contributed to the induction of IL-10 (and eventual silencing of IL-4), analogous to its effects in Tr1 differentiation. These IL-10-producing 'regulatory' Th2 cells expressed GATA-3, suggesting their origin from Th2 precursors.

A key unknown is the mechanism whereby a Th2 cell might transition to IL-10 expression, seemingly secondary to IL-4 expression. GATA-3 has been shown to bind the IL-10 locus and promote remodeling of the *Il10* locus (85). Although this can occur independently of the Th2 differentiation program, it is possible that in the context of Th2 development, increasing expression of GATA-3 that accompanies Th2 commitment progressively promotes chromatin accessibility at the *Il10* locus, allowing for late but sustained expression of IL-10 that is persistent as IL-4 expression is extinguished. However, the silencing of IL-4 in favor of IL-10 production would likely be a hindrance in the initiation of a rapid recall response. An alternative scenario was suggested by Akdis *et al.* (52), who showed that allergen-specific effector (Th2) and suppressor (Tr1) cells co-existed in the peripheral blood of both healthy and allergic humans in certain baseline quantities. The relative frequencies of Th2 and Tr1 cells contributed to the propensity towards an allergic or healthy state, respectively. Despite varying distributions within each population, the antigenic specificities of both Th2 and Tr1 cells were similar, suggesting that the same antigen can induce both lineages either via divergent pathways, although a linear pathway involving an IL-4 and IL-10 'double-positive' Th2 intermediate population, a fraction of which transitions over time to a strict IL-10-producer, could not be excluded. In any case, the original conception that IL-10 might represent a component of the 'effector' Th2 response due to its association in Th2 clones is giving way to a model in which IL-10 counteracts Th2 effector cytokine-driven inflammation, through mechanisms yet to be fully elucidated.

IL-10 expression by Th1 cells

Although a key feature that implicated IL-10 as a Th2-specific cross-regulator of Th1 responses was the fact that it was not produced by Th1 clones, several disease models have revealed that IL-10 is expressed by T cells that share numerous features with conventional Th1 cells. While in retrospect this observation likely reflects the fact that IL-10's potent suppression of IL-12 by APCs would suppress the cytokine by which Th1 clones were defined (i.e. IFN- γ), thereby precluding co-expression of IL-10 and IFN- γ in long-term clones, the expression of IL-10 has now been observed in at least a subset of Th1 cells induced in response to several pathogens *in vivo*, including *Brucella abortus* (86), *Borrelia burgdorferi* (87), *Mycobacterium tuberculosis* (88), *Mycobacterium avium* (89), *Listeria monocytogenes* (81), as well as *Toxoplasma gondii* and *Trypanosoma cruzi* (90). Despite the common expression of Th1-specific factors such as T-bet and IFN- γ , the cells defined in these various systems have differed in their Treg marker profile. Thus, *L. monocytogenes*-induced Th1 cells expressed both Foxp3 and IL-10 (81), whereas *T. gondii*- and *T. cruzi*-induced Th1 cells expressed IL-10 but not Foxp3 (90). Importantly, the protective ability of both Foxp3⁺ and Foxp3⁻ subsets was strictly IL-10-dependent.

Factors controlling the expression of IL-10 by Th1 cells

Stimulation of primary human T cells in the presence of IL-12 *ex vivo* was shown to induce IL-10 (91,92). In contrast, *T. gondii* induction of IL-10-expressing Th1 cells was reported to occur independently of IL-12, as well as IL-18, IL-23, or STAT4 (89,90). However, IL-27, another member of the IL-12 cytokine family, has also been shown to induce both Th1

development and IL-10 induction in CD4⁺ T cells (67,93,94). The requirement for IL-12 in stimulating production of IL-10 by established Th1 clones is also controversial (91,95,96), and the kinetics of IL-10 expression by Th1 effectors appears to vary based on the experimental system. An intriguing example of this was demonstrated in a study showing that most of the IL-10 production by Th1 effectors induced by *T. gondii* occurred rapidly and transiently post-activation. Thus, the Th1 response was characterized by simultaneous expression of IFN- γ and IL-10 in the acute phase, but the latter was largely downregulated in the chronic phase (90,97). This is in contrast to other experimental systems where chronic antigenic stimulation leads to stabilization of IL-10 expression, usually with downregulation of effector activity (14,81,84).

Interestingly, in at least one study wherein IL-10 expression was silenced while IFN- γ expression persisted, IL-10 protein could be induced by re-stimulation *ex vivo*, albeit with delayed kinetics (90). Thus, despite a block in translation of IL-10 protein, the differentiated cells maintain low levels of *Il10* transcripts that might be subject to one or more post-transcriptional or pre-translational regulatory mechanisms. Indeed, cells can actively transcribe the *Il10* gene even though *Il10* mRNA is undetectable, implicating potent post-transcriptional regulatory mechanisms that control IL-10 production (98). Accordingly, it is feasible that Th1 cells may be committed to expression of *Il10* for the life of the cell, yet post-transcriptional mechanisms that confer suppressed or delayed kinetics of protein expression relative to the acute response could permit rapid, robust, and unimpeded IFN- γ secretion that is characteristic of Th1 recall responses.

The mechanism whereby IL-10 expression by Th1 cells is suppressed remains to be elucidated. Early expression of IFN- γ by Th1 cells drives a positive feedback loop that involves induction of the Th1 transcription factor, T-bet, thereby reinforcing expression of IFN- γ (99). Interestingly, mice with a deletion of gene encoding T-bet (*Tbx21*) display elevated levels of IL-10 relative to wildtype controls (100), suggesting that T-bet might be involved in the negative regulation of *Il10* gene transcription and/or post-transcriptional regulation such that, as Th1 differentiation proceeds and T-bet levels increase, *Il10* transcription is increasingly repressed. The transcriptional repressor B-lymphocyte induced maturation protein-1 (Blimp-1) represses both *Tbx21* and *Ifng* gene expression, thereby inhibiting Th1 differentiation, while *in vitro*-generated Blimp-1-deficient Th2 cells express significantly less IL-10 than their wildtype counterparts (101). Thus, it is possible that Blimp-1, via a mechanism that involves repression of T-bet expression, directly or indirectly promotes induction of IL-10.

Importantly, mice with T-cell-specific deletion of *Prdm1* (the gene encoding Blimp-1) develop spontaneous colitis, and IL-10 production by CD4⁺ T cells was impaired in these mice (102). This finding suggests that in addition to its effects on Th1 and Th2, Blimp-1 may be involved in the induction of IL-10 Treg cell development in the steady state. Because Blimp-1 is a transcriptional repressor that mediates multiple functions through its interaction with other transcription factors, it is not unlikely that factors downstream of Blimp-1 cooperate to regulate *Il10* gene transcription. One such target is the transcriptional repressor BCL-6, which is repressed by Blimp-1 (101). Because T cells from *Bcl6*^{-/-} mice produce increased levels of IL-10 (103), indicating a role for BCL-6 in repression of IL-10, it is possible that Blimp-1 indirectly enhances T-cell production of IL-10 via repression of BCL-6.

IL-27 was shown to be a powerful inducer of both T-bet and IL-10 in T cells (67,93,94). Early reports demonstrated that mice deficient in the gene encoding the α chain of the IL-27 receptor (*Il27ra*^{-/-}) showed defective Th1 responses to *Listeria* as well as *Leishmania*, prompting the conclusion that IL-27, like IL-12, promotes Th1 development (104,105).

However, it is now clear that IL-27 can also suppress Th1-specific factors in certain contexts, while favoring high-level expression of IL-10 (94). Interestingly, the induction of IL-10 by IL-27 required STAT-3, but importantly, not T-bet or STAT-4. This is in direct contrast to the development of IFN- γ /IL-10 ‘double producers’ in differentiating and differentiated Th1 cells that were induced by ectopic expression of Notch and required activation of STAT-4 by IL-12 or IL-27 (106).

Whereas T-bet deficiency is associated with elevated IL-10 production by CD4⁺ T cells, in an *in vitro* system, T-bet and IL-10 were coordinately upregulated by IL-27. Surprisingly, the IL-27-mediated induction of T-bet (as well as IL-10) was enhanced by the addition of TGF- β (67), which is a potent inhibitor of Th1 development and IFN- γ expression (107) and is important for the induction of IL-10-producing Tregs *in vivo* (18). However, the IL-27 signaling pathway is unlikely to be a dominant mechanism for IL-10 Treg development in the steady state, because *Il27ra*^{-/-} mice display no discernable differences in CD4⁺CD25⁺ nTreg development and function, and importantly, no intestinal pathology (104,105).

IL-10 expression by Th17 cells

Recent findings indicate that IL-10 is also expressed by Th17 cells induced in the presence of TGF- β and IL-6 (94,108). As with Th1 and Th2, IL-10 limits the pathogenic potential of Th17 cells in an autocrine manner (108). Further work will be required to dissect the mechanisms controlling the induction and regulation of IL-10 production by Th17 cells. IL-10 induction associated with Th17 development *in vitro* is dependent on TGF- β and STAT-3-dependent IL-6 signaling; IL-10 production increases with increasing concentrations of either cytokine (94,108). Remarkably, while IL-27 inhibits induction of Th17 differentiation (93,109,110), it also enhances IL-10 production by Th1 and Th2 but not Th17 cells (94).

Additional studies are also required to clarify the role of IL-23, if any, in limiting the suppressive effects of Th17 cell-derived IL-10, thereby allowing the pro-inflammatory functions of IL-17 to dominate. It has been suggested that TGF- β and IL-6 are required for commitment to the Th17 lineage, including the induction of the ‘autoregulatory’ IL-10 cascade, whereas IL-23 is required for the maintenance and/or pathogenicity of Th17 cells. Consistent with this theory, polarization of naive T cells or re-stimulation of *in vivo*-derived Th17 cells in the presence TGF- β and IL-6 resulted in increased expression of IL-17 as well as IL-10, and these cells failed to induce experimental autoimmune encephalomyelitis (EAE), except when recipients were also treated with an IL-10-blocking antibody. Conversely, Th17 cells cultured with IL-23 did not produce IL-10 and induced severe disease. Paradoxically, however, Th17 cells re-activated *ex vivo* in the presence of TGF- β , IL-6, and IL-23 produced more IL-10 than cells re-activated in the presence of TGF- β and IL-6 alone (108).

Another issue yet to be completely addressed is the overlapping expression of IL-17 and IL-10 in Th17 cells and the relevance of this to control of Th17-mediated pathology. Interestingly, when naive CD4⁺ T cells were activated *in vitro* in the presence of TGF- β and IL-6, approximately 50% of IL-17⁺ cells co-expressed IL-10 (94). However, we have found that in the intestines, the IL-10- and IL-17-expressing CD4⁺ T-cell populations are largely distinct, both at steady state and during active intestinal inflammation (18, Authors’ unpublished data). Thus, although it is possible that these subsets developed from a common Th17 precursor population from which distinct progeny developed under control of different post-differentiation signals, this will need to be directly addressed.

IL-10-producing CD8⁺ T cells

The first immunoregulatory T cells described, originally referred to as suppressor T cells, were predominantly CD8⁺ T cells, which proved difficult to characterize due to the absence of unique lineage markers. The subsequent discovery of CD4⁺CD25⁺ cells with potent immunosuppressive properties led to the re-emergence of the concept of T-cell-mediated immunoregulation, the preferred use of the term 'regulatory T cells', and widespread focus on CD4⁺ T cells as major players in immunoregulation. However, subsets of CD8⁺ T cells with regulatory activity have been described and continue to be studied as important immunoregulatory cells, although unlike CD4⁺ Tregs, no 'naturally occurring' CD8⁺ Tregs have been forthcoming, since expression of Foxp3 is largely restricted to αβ CD4⁺ T cells at steady state (21). More recent studies have found, however, that CD8 precursors can be induced to express Foxp3, with acquisition of induced Treg activity akin to that of Foxp3⁺ CD4⁺ Tregs (111).

Peptide pulsed immature DCs induced antigen-specific CD8⁺ Tregs *in vivo*, and these cells suppressed effector and memory cell function in a cell-contact-dependent manner (64). Importantly, the expression of IFN-γ and IL-10 in this setting resembled the situation later reported in *T. gondii*-infected mice (90), in which IL-10 expression peaked early in the response but was absent at later time points, when IFN-γ levels were once again elevated. Xystrakis *et al.* (112) described a CD8⁺CD45RC^{lo} Treg cell subset in rats with a cytokine profile resembling that of Th2 cells: IL-4⁺IL-10⁺IL-13⁺. In addition, these cells were reported to express *Foxp3* and *Ctla4* mRNA, were anergic to TCR stimulation, and suppressed responder cell proliferation in a cell contact-dependent manner. Interestingly, it has been reported that expression of the IL-2-receptor β chain (CD122) identifies a naturally occurring subset of CD8⁺ regulatory T cells present in the murine spleen which can suppress responder cell proliferation in the absence of APCs (113). It was subsequently demonstrated that the suppression is mediated by IL-10 (114).

CD122 is also part of the IL-15 receptor complex, which is a TCR-independent growth factor for Tr1 cells (55). Thus, it is tempting to speculate that the CD8⁺CD122⁺ Tregs are CD8⁺ counterparts of Tr1 cells and require IL-15 for their maintenance and possibly function. Despite these data, in our IL-10 reporter model, we detected no reporter expression on CD8⁺ T cells in the spleen or any other lymphoid tissue, although our studies only examined unchallenged mice and reflect non-infectious, steady-state conditions (18). In a different IL-10 reporter model, repetitive TCR activation resulted in modest reporter expression on subsets of CD8⁺ T cells in the spleen and mesenteric lymph nodes (17). In contrast, approximately 50% of Peyer's patch CD8⁺ T cells expressed the IL-10-reporter molecule after activation. One explanation for this discrepancy is that despite never having expressed IL-10, this population of cells has a greater propensity towards expression of IL-10 upon TCR activation due to the expression of CD122. Alternatively, it is possible that cell activation and the corresponding secretion of IL-10 represent a terminal event in the life of this subset, such that the cells that have expressed IL-10 are eliminated and not readily detectable at steady state. Additional studies will be needed to address this possibility.

Unlike the secondary lymphoid tissues, IL-10-expressing CD8⁺ T cells are prominent among the intra-epithelial lymphocytes (IELs) of the small intestine of naive mice (18,115). This diverse population includes subsets of CD8αα⁺CD4⁻, CD8αβ⁺CD4⁻, CD8αβ⁺CD4⁺, and CD8αα⁺CD4⁺ IELs. Interestingly, the majority of the CD8αα⁺ cells, which accounted for greater than 70% of CD8⁺IL-10⁺ IELs, expressed γδ TCRs (18). Currently, it is unclear what factors induce development of these subsets and how critical each is to immune regulation in the small intestines. It has been reported that reconstitution of SCID mice with TCRαβ⁺CD4⁻CD8α⁺β⁻ but not γδ TCR⁺ or TCRαβ⁺CD4⁻CD8α⁺β⁺ IELs prevented colonic inflammation caused by subsequent transfer of CD4⁺CD45RB^{hi} T cells and that protection

was dependent on IL-10 production by the transferred IELs. In agreement with the likely ability of CD8⁺ IL-10-producing to regulate intestinal immune homeostasis, IL-10⁺ CD8⁺ T cells were detected in the colonic *lamina propria* of immunocompetent mice chronically treated with α CD3 (17).

Concluding remarks

As discussed herein, it is now apparent that expression of IL-10 is a feature shared by each of the CD4⁺ T-cell lineages described to date, including classical Tregs and effector T-cell subsets. Although there remains considerable ambiguity with regard to the diversity and origins of Foxp3⁺ Treg subsets, new insights into the plasticity of the IL-10 expression program across the entire range of T-cell lineages, while perhaps obscuring a complete understanding in the near-term, promises greater clarity down the road. Established and emerging data support a model in which IL-10 has a non-redundant role in curbing immune responses, both at steady state as a means to prevent autoinflammatory responses to the intestinal microbiota, and in the context of a range of anti-pathogenic responses as a means to limit T-cell driven inflammation as pathogen clearance is achieved. Because in each of these settings IL-10 expression limits the innate and adaptive immune responses, robust mechanisms must exist to regulate both the kinetics and magnitude of the IL-10 response to enable the immune system to achieve its primary objective of eliciting effector T-cell responses to coordinate resistance to potential pathogens, before downmodulating the response to avoid chronic inflammation.

Implicit in the remarkable promiscuity of *Il10* gene expression across each of the T-cell subsets is a substantial plasticity in the cell-intrinsic regulatory networks that control production of IL-10, at transcriptional and post-transcriptional levels. Our understanding of these mechanisms is currently in its infancy, but future studies aimed at a more detailed understanding of the *Il10* gene regulation should provide new opportunities for harnessing this cytokine for more effective immune modulatory therapies.

Acknowledgments

The authors thank members of the Weaver laboratory for their helpful comments and suggestions. We acknowledge the UAB Transgenic Mouse Facility, the UAB Digestive Diseases Research Developmental Center (DDRDC), the UAB Epitope Recognition and Immunoreagent Core Facility, and the UAB Comprehensive Cancer Center Gene Expression Shared Facility for infrastructure support for published and unpublished studies referenced herein. We also gratefully acknowledge grants from the NIH (to C.L.M. and C.T.W.) and a grant from the Crohns and Colitis Foundation of America (to C.T.W.) that have supported this work.

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Fig. 1. Expression of IL-10 by regulatory and effector CD4⁺ T cells *in vivo*

In response to specific antigenic stimuli, naive T cells (Tn) differentiate into Th1, Th2, or Th17 effector cells. Besides their signature transcription factors and cytokines, Th1: T-bet and IFN- γ , Th2: GATA-3 and IL-4, Th-17: ROR γ t and IL-17, all effector subsets activate expression of IL-10. In Th1, IL-10 is expressed by T-bet⁺IFN- γ ⁺ cells, which is able to suppress the Th1 development and function, but in some situations, expression of IL-10 is extinguished over time. Th2 cells transition through an IL4⁺IL-10⁺ stage to divergent subsets of IL-10⁻ and IL-4-producing cells, with the latter IL-10 producers suppressing the IL-4 producers to reinstate immune homeostasis. Th17 cells also express IL-10, which can suppress the pathogenic functions of this subset. However, the inhibition of IL-10 and thus restoration of effector function can be inhibited by cytokines such as IL-23. At this time, it is unclear whether subsets of Th1 and Th17 downregulate effector cytokines IFN- γ and IL-17, respectively, over time and persist as Foxp3⁺IL-10⁺ cells, thereby contributing to the

peripheral 'Tr1' pool, and if so the signaling networks controlling this divergence are yet to be elucidated. IL-10 is also upregulated by Foxp3⁻ and Foxp3⁺ subsets that are concentrated in intestinal tissues.