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IL-10 production by CD4⁺ effector T cells: a mechanism for self-regulation

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The development of Th1 lymphocytes is essential for cell-mediated immunity and resistance against intracellular pathogens. However, if left unregulated, the same response can cause serious damage to host tissues and lead to mortality. A number of different paracrine regulatory mechanisms involving distinct myeloid and lymphoid subpopulations have been implicated in controlling excessive secretion of inflammatory cytokines by Th1 cells. Much of this work has focused on interleukin (IL)-10, a cytokine with broad anti-inflammatory properties, one of which is to counteract the function of Th1 lymphocytes. While studying the role of IL-10 in regulating immunopathology during infection with the intracellular parasite *Toxoplasma gondii*, we discovered that the host-protective IL-10 derives in an autocrine manner from conventional interferon- γ (IFN- γ)-producing T-bet⁺ Foxp3^{neg} Th1 cells. In the following review, we will discuss these findings that support the general concept that production of IL-10 is an important self-regulatory function of CD4⁺ T lymphocytes.

INTRODUCTION

Effector function in CD4 T lymphocytes is mediated by three major subsets: Th1, Th2, and Th17.¹ These different CD4 populations have distinct roles in both host defense against pathogens and in immunopathology. Each subset was initially defined on the basis of their cytokine secretion signature and unique production of interferon- γ (IFN- γ), interleukin (IL-4), or IL-17, respectively. IL-10, a cytokine with broad immunoregulatory function, was originally described as a unique product of Th2 cells,² but was later shown to be expressed in a variety of lymphocytic as well as myeloid cell populations.³ It was also established that IL-10 affects T-cell function indirectly by inhibiting the function of antigen-presenting cells.^{3,4} Early studies with IL-10-deficient mice revealed a major tissue-protective role for the cytokine in preventing Th1/Th17-mediated immunopathology induced by endogenous gut flora.⁵ It was later recognized that IL-10 can perform a similar function in protecting mice against both Th1- and Th2-associated tissue damage induced by infection with pathogens.^{6–10}

An important model of the protective role of IL-10 in pathogen-induced immunopathology is murine infection with *Toxoplasma gondii*. This intracellular protozoan parasite triggers a highly polarized IL-12-dependent Th1 response required for control of pathogen replication.¹¹ That the same response is potentially immunopathogenic was revealed in experiments in

which infected IL-10-deficient mice were found to rapidly succumb to a “cytokine storm”-associated disease despite effective restriction of parasite growth.⁶ The observed immunopathology, which was marked by uncontrolled production of Th1 cytokines, acute inflammatory markers, and necrotic tissue damage, was shown to be dependent on CD4 T cells and thus was proposed to result from Th1 cell dysregulation.⁶ In addition to these observations made after intraperitoneal injection of *T. gondii*, the protective role of IL-10 was also shown in the natural peroral route of infection in both genetically susceptible C57BL/6 and resistant BALB/c mice.¹² Moreover, a similar requirement for IL-10 in prevention of immunopathology was subsequently described during infections with other pathogens, such as *Trypanosoma cruzi*,^{7,8} *Plasmodium chabaudi*,¹³ *Listeria monocytogenes*,¹⁴ murine cytomegalovirus,¹⁵ and respiratory influenza virus,¹⁶ which are associated with strong Th1 responses. In addition, there is an abundant literature showing a role for endogenous IL-10 in suppressing autoimmune pathology.^{3,17}

Although there is now considerable information regarding the regulatory effects of IL-10 on immune responses and pathology, there is much less known about the cellular sources of the cytokine and the specific signals that govern its induction. We have addressed this issue in the murine *T. gondii* infection model and identified Th1 cells as the unexpected source of the biologically relevant IL-10.

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CELLULAR ORIGINS OF HOST-PROTECTIVE IL-10

Traditionally, the IL-10 that regulates T-cell effector functions was thought to derive from paracrine sources (accessory cells or specialized CD4⁺ T lymphocytes). Indeed, in addition to Th2 lymphocytes themselves, macrophages, dendritic cells (DCs), B lymphocytes, mast cells, and eosinophils, as well as different regulatory CD4⁺ T subsets have been shown to be capable of producing the cytokine.³ From numerous studies, it is clear that a major function of the IL-10 derived from activated Th2 cells is to cross-regulate Th1 development and function. However, in the case of highly polarized Th1 infections IL-10 must originate from other non-Th2 sources. Although myeloid cells under certain circumstances can contribute to IL-10 production,^{3,18–20} a large number of studies have pointed to T cells as the major source of the cytokine in this situation. In the case of the *T. gondii* model, the latter conclusion is based on an important experiment in which infection of mice with a targeted deletion of IL-10 production in T cells recapitulated the phenotype observed with conventional IL-10-deficient animals.²¹ Experiments in which RAG/IL-10 double-deficient mice were reconstituted with CD4⁺ T lymphocytes from naive wild-type or IL-10-deficient donors and infected with *T. gondii* established that IL-10 from this T-cell population is sufficient to protect mice from lethal immunopathology.²²

In addition to Th2 cells, a number of different CD4 T-cell subsets are known to secrete IL-10 as a part of their regulatory function. These include natural as well as adaptive Foxp3⁺ T regulatory (Treg) cells,^{23–26} Th3²⁷ and Tr1^{28,29} cells. To further characterize the CD4⁺ T-cell population involved in host protection in *T. gondii* infection, we performed a single-cell analysis of IL-10 production in acutely infected mice. Surprisingly, we found that most of the IL-10⁺ CD4⁺ T cells were IFN- γ ⁺ T-bet⁺ Th1 cells and did not express Foxp3.²² The latter finding clearly dissociates IL-10-producing *T. gondii*-specific CD4⁺ T lymphocytes from both natural and adaptive Tregs. Their cytokine secretion pattern and mode of action also distinguish them from Th3 cells, which are selectively induced after oral antigen (Ag) administration and exert their regulatory functions through transforming growth factor- β rather than IL-10 secretion.

Because both Tr1 and IL-10⁺ Th1 cells are Foxp3^{neg} CD4 T lymphocytes that express regulatory IL-10, the question often arises as to whether these cells represent distinct populations. Indeed, both cells have been described as double IL-10⁺ IFN- γ ⁺ producers, although the expression of the latter cytokine is not obligatory in Tr1 cells, which can also secrete other cytokines, such as IL-5, IL-4, or transforming growth factor- β .^{28,29} In contrast, IFN- γ production is a key property of IL-10⁺ Th1 cells that derive from conventional T-bet⁺ T cells.²² Indeed, the designation “Tr1” seems to cover a broad range of cells that cannot be characterized by a single transcription factor and do not seem to derive from conventional Th1 cells. In addition, Tr1 cells are typically induced under conditions of multiple Ag stimulation,³⁰ and in some protocols in the presence of IL-10^{31,32} itself leading to a state of anergy/exhaustion. In contrast, IL-10-producing Th1 cells do not require IL-10 for their development

(i.e., as mentioned above they can arise in RAG/IL-10 double-deficient animals after wild-type CD4⁺ T-cell adoptive transfer,²² as well as in infected intact wild-type mice treated with blocking anti-IL-10R monoclonal antibodies (Jankovic *et al.*, unpublished observations)) and show proliferative activity when stimulated with Ag.²² Nevertheless, although representing distinct regulatory CD4⁺ T-lymphocyte populations, it remains possible that the mechanisms regulating IL-10 in both cell types share common elements.

IL-10-PRODUCING Th1 CELLS POSSESS DUAL REGULATORY AND EFFECTOR FUNCTIONS

The finding that host-protective IL-10 in systemic *T. gondii* infection derives from CD4 lymphocytes that have a Th1 effector phenotype seemed at first counterintuitive. Indeed, despite their IL-10 production, these cells were found to express especially high levels of IFN- γ and T-bet. Moreover, when placed in culture with *T. gondii*-infected macrophages, they promoted efficient intracellular killing of the pathogen, indicating that the effector function of these Th1 cells is not compromised by their simultaneous production of IL-10.²² Nevertheless, in the same cultures the secretion of IL-12 was markedly suppressed relative to comparable cultures containing conventional single producing Th1 cells and this effect was abolished by the addition of neutralizing anti-IL-10 monoclonal antibodies.²² Thus, IL-10-producing Th1 cells were able to stimulate infected macrophages to release nitric oxide and mediate intracellular killing of the parasite while blocking their IL-12 release. Although the former observation seems to conflict with the known inhibitory effects of IL-10 on microbicidal activity, simultaneous secretion of IFN- γ may reverse this inhibition, as pre-exposure to IFN- γ has been shown to reprogram several suppressive functions triggered by IL-10 in macrophages.³³ We hypothesized that the suppression of IL-12 by IL-10⁺ Th1 lymphocytes would serve to limit further Th1 differentiation^{34–36} and consequently provide a “brake” for the development of immunopathology. This concept is consistent with previous data showing that uncontrolled IL-12 production is a hallmark of the disease observed in *T. gondii*-infected mice with impaired production of IL-10.^{6,22}

Parallel studies performed at the same time on infection with a nonhealing strain of *Leishmania major* identified a similar population of double IL-10⁺ IFN- γ ⁺ CD4⁺ T lymphocytes.³⁷ However, in this situation in which IL-12 is limiting, these cells were shown to have a detrimental effect, promoting parasite growth and preventing resolution of cutaneous lesions.³⁷ In addition, in mice infected with *L. donovani*, the causative agent of visceral leishmaniasis, a similar population of antigen-specific IL-10- and IFN- γ -coproducing CD4⁺ T cells was shown to expand as the parasite burden increased.³⁸ Thus, IL-10-producing Th1 lymphocytes can have both protective and disease promoting effects in response to pathogens.

IL-10-PRODUCING Th1 CELLS DO NOT REPRESENT A STABLE CD4 T-CELL SUBSET

In our original description of IL-10-producing Th1 cells in *T. gondii* infection, only a fraction (10–15%) of the total Th1

population were found to express this phenotype.²² This observation suggested that IL-10 expression may be induced in Th1 cells in response to host infection rather than representing the emergence of a specific subset in which cytokine expression is stable. This hypothesis was supported by data in which *T. gondii*-specific IL-10^{neg} Th1 cells were shown to acquire IL-10 expression after restimulation *in vitro*. Interestingly, upon prolonged culture, IL-10⁺ Th1 cells revert back to IL-10^{neg} Th1 cells. Thus, in direct contrast to IFN- γ , *IL-10* gene expression in Th1 lymphocytes seems to be an inducible and transient property of these cells.²² This instability of IL-10 expression may explain why IL-10 was not originally described as a Th1 product in long-term murine T-cell clones but was detected in short-term human Th1 as well as in Th2 clones.³⁹ We believe that the transient expression of IL-10 by Th1 cells endows them with the ability to undergo unrepressed expansion during the initial stages of infection and then compress when pathogen control is achieved and continued inflammatory cytokine production would be host detrimental.

Autocrine production of IL-10 by Ag-specific Th1 lymphocytes may represent a more efficient mechanism of regulation than the paracrine induction of the cytokine from a distinct myeloid or lymphoid cell population. In the case of naturally occurring CD25⁺CD4⁺ Treg cells, IL-10 synthesis seems to be constitutive and primarily functions to control autoreactivity or, when hijacked by certain pathogens, to promote latent infection.^{23,40} Because previous studies have shown an inhibitory effect of Toll-like receptor signaling on natural Treg activity,⁴¹ Th1 lymphocytes may provide a necessary source of IL-10 in the case of pathogens such as *T. gondii* that express potent Toll-like receptor agonists.⁴²

IL-10 IS A COMMON FEATURE OF CD4⁺ EFFECTOR T LYMPHOCYTES

IL-10 production by CD4⁺ T lymphocytes was initially thought to be restricted to Th2 cells. As noted above, this can now be

explained by the fact that IL-10 secretion in Th2 cells is a stable property (**Figure 1**). Induced by IL-4 and mediated by transcriptional factors signal transducers and activators of transcription (STAT)-6 and GATA-3, *IL-10* gene transcription in Th2 cells is associated with epigenetic modifications in chromatin structure that ensure its continued expression after each round of T-cell receptor stimulation without the requirement for additional extrinsic instruction.^{43,44} Although initially thought to selectively cross-regulate Th1 responses, IL-10 production by Th2 cells can also function as an autocrine self-regulatory mechanism, as illustrated by studies in helminth^{9,10} and fungal infection models.⁴⁵

Interestingly, IL-10 secretion has also been recently described as a property of Th17 effector cells (**Figure 1**), and in this case production of the cytokine is conditional rather than constitutive.⁴⁶ Moreover, Th17 cells conditionally express yet another member of the IL-10 cytokine family, IL-22.⁴⁷ Conditional IL-10 production by Th1 and Th17 cells may reflect the strong dependence on proinflammatory cytokine production (IL-12 and IL-6 plus IL-1, respectively) by antigen-presenting cells for the differentiation and maintenance of both subsets and the need to suppress them when they become host detrimental.

IL-10 production has also been described in Th9 cells, which are a putative CD4⁺ T-cell subset defined by their expression of IL-9.^{48,49} Although not yet reported in the literature, we predict that IL-10 expression will also be detected in T follicular helper cells, an additional subset that regulates antibody production by B lymphocytes.^{50–52} The nature and function of IL-10 expression in Th9 and T follicular helper cells are important areas for future investigation. As both Th9^{48,49} and at least some T follicular helper cell populations,⁵² in common with Th2 cells, require IL-4 for their differentiation, it is likely that their production of IL-10 will be found to be imprinted as a constitutive feature of their cytokine production repertoire.

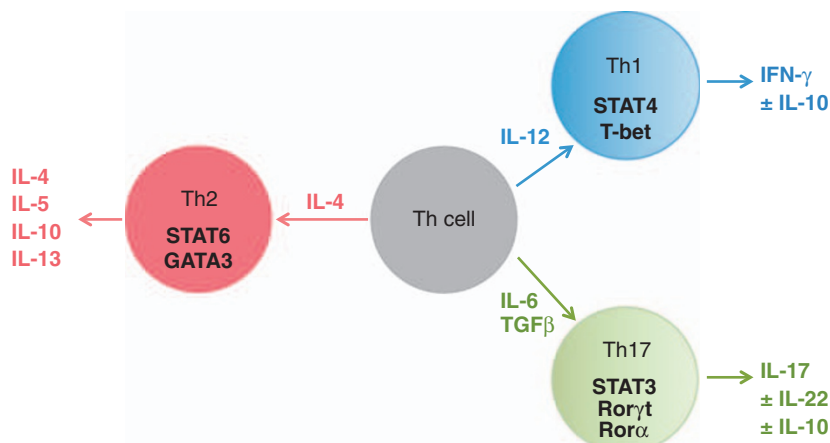


Figure 1 Stable vs. conditional expression of interleukin (IL)-10 in Th effector subsets. Antigen-activated naive CD4⁺ T lymphocytes can adopt at least three distinct effector phenotypes as directed by cytokines within the local environment. In the presence of IL-4 they differentiate into Th2 cells, which in addition to IL-4, -5, and -13 also stably express IL-10 (without the requirement for IL-4 instruction during subsequent stimulation). IL-12 promotes Th1 differentiation by inducing interferon- γ (IFN- γ) synthesis. Although Th1 lymphocytes can also express IL-10, their production of the cytokine is not stable. Similarly, Th17 effectors, generated in the presence of IL-6 and transforming growth factor- β (TGF- β) and characterized by IL-17 expression (with or without IL-22), can secrete IL-10 under the correct set of conditions.

MECHANISMS DETERMINING IL-10 EXPRESSION IN CD4 T CELLS

A topic that has received considerable attention is the pathway by which IL-10 expression is triggered in Th1 and Th17 cells. Three basic mechanisms have been proposed.

Instruction by DCs

In the case of Tr1 cells, which as noted above closely resemble Th1 lymphocytes but are anergic, IL-10 expression was shown to depend on exposure of precursors to a subset of DC that itself expresses the cytokine.³¹ However, in more recent work, DCs from IL-10-deficient mice have been shown to promote the generation of a similar population of IL-10⁺ IFN- γ ⁺ Tr1 cells through their production of IL-27.⁵³ In the latter case, DCs were characterized with a plasmacytoid-like CD11c^{int} CD11b^{lo} CD8 α ⁻ CD45RB^{hi} B220^{hi} surface phenotype. In agreement, the induction of IL-10 in CD4⁺ T cells seems to be more closely associated with plasmacytoid DCs (pDCs) than myeloid DCs. The former are thought to be tolerogenic as they suppress rather than stimulate CD4 T effector function.^{54,55}

It is not clear yet whether this is due to IL-10, as low production of the cytokine by pDCs was shown in some,^{53,56} but not all experimental settings.⁵⁷ Nevertheless, pDCs have been shown to promote IL-10 secretion from CD4⁺ and CD8⁺ T cells through inducible costimulatory molecule (ICOS) ligand-induced expression on these DCs during their maturation.⁵⁸ Moreover, in a recent report pDCs have also been implicated in the induction of IL-10⁺ Th1 effectors.⁵⁹ In the latter study, IL-10 expression was linked with the presence of high levels of delta-like-4 ligand on pDCs that activate Notch receptor on CD4 T lymphocytes. Nevertheless, our preliminary data suggest that pDCs, which are known to become activated during *T. gondii* infection,⁶⁰ do not have an important role in the generation of IL-10⁺ Th1 cells.

Induction by IL-12 family cytokines

As IL-12 has a major role in promoting Th1 development, it is not unexpected that this cytokine is also intimately involved in promoting IL-10⁺ Th1 effectors. Using *in vitro* cultured naive transgenic CD4⁺ T cells, O'Garra and colleagues⁶¹ showed that both high Ag dose and high IL-12 levels selectively trigger a population of Th1 lymphocytes that contain double-producing IL-10⁺ IFN- γ ⁺ CD4 T cells. Moreover, they showed that the presence of both of these two conditions is necessary to sustain the latter phenotype. In this system, Erk phosphorylation was shown to be required for IL-10 expression. Taken together, the above findings argue that IL-10 production by Th1 lymphocytes may be a consequence of high-level activation of these cells under strong Th1 promoting conditions and may represent a stage in the natural differentiation of Th1 effectors. This conclusion is consistent with our finding that IL-10-producing Th1 cells are induced in *T. gondii* infection *in vivo*, in which both high Ag load and high IL-12 levels are present.²² Nevertheless, IL-12 itself may be a redundant signal *in vivo* as *T. gondii*-immunized IL-12-deficient mice were still able to generate IL-10⁺ IFN- γ ⁺ CD4⁺ T lymphocytes.⁶²

A second IL-12 family cytokine implicated in IL-10 production by Th1 effectors is IL-27. Indeed, early studies by Hunter and colleagues⁶³ established that *T. gondii*-infected IL-27R (WSX-1)-deficient mice have an early death phenotype that closely resembles that observed in IL-10 KO animals. In more recent work, this group has shown that IL-27 can exert an effect directly on CD4⁺ T lymphocytes to trigger IL-10 expression and that *T. gondii*-infected IL-27R KO mice show a decreased frequency of IL-10⁺ Th1 cells despite unimpaired IL-12 production.⁶⁴ Interestingly, IL-27 has also been shown to trigger IL-10 production in Th17 cells,⁶⁴ suggesting the involvement of a parallel mechanism. Indeed, c-maf induction was found to be associated with IL-10 expression in both Th1 and Th17 cells.^{60,65} Nevertheless, in a parallel work it was shown that IL-10 induction by Th17 cells can be triggered by IL-6, in a STAT-3-dependent manner independently of IL-27.⁴⁶ Moreover, this IL-6-dependent pathway is also associated with c-maf expression.⁶⁵ Indeed c-maf induction seems to be a common mechanism for IL-10 induction by IL-12, IL-27, and IL-6, and in this regard an active binding site (MARE) for c-maf has been identified in the IL-10 promoter.⁶⁵ Interestingly, c-maf has also been implicated in IL-10 regulation in macrophages. Although c-maf seems to be a major determinant of conditional IL-10 expression in CD4⁺ T cells, it is likely that other, as yet to be defined, transcriptional regulators also have a role in this process.

Other signals

In the case of Th1 cells, both Stat-1 and Stat-3 have been implicated in the induction of IL-10 expression. Stat-1 is a signal transduction element that is downstream of both the IL-27 and IFN- γ receptors. In this regard, IFN- γ signaling in antigen-presenting cells has been shown to be required for IL-10 secretion by *T. gondii*-primed Th1 lymphocytes after *in vitro* restimulation and to be associated with the increased expression of the costimulatory molecule ICOS ligand on non-T cells.⁶⁵ Previous work had implicated ICOS–ICOS ligand interaction in the induction of IL-10 in T cells,⁵⁷ and in recent studies the same costimulatory trigger has been shown to be required for optimal IL-27-induced IL-10 production in Tr1 cells.⁶⁶ On the basis of this type of evidence, it has been proposed that Th1 IFN- γ production through the Stat-1-dependent induction of ICOS ligand could trigger autocrine IL-10 expression as a negative feedback mechanism.

As mentioned above, in addition to Stat-1, Stat-3 has also been shown to participate in the induction of IL-10 in Th1 cells. In common with IL-27 and IL-6, IL-21 signals through Stat-3.⁶⁷ IL-21 is now known to be produced by Th1, Th2, and Th17 CD4⁺ T cells and is believed to have broad immunoregulatory functions. This property has been attributed to the ability of IL-21 to induce IL-10 expression in CD4⁺ T lymphocytes.⁶⁸ Although Th1 priming *in vitro* in the presence of IL-21 leads to increased frequencies of IL-10⁺ IFN- γ ⁺ CD4⁺ T cells, mice deficient in IL-21 receptor do not show increased susceptibility or acute tissue pathology after *T. gondii* infection,⁶⁹ suggesting that IL-21 is not required for generation of these cells *in vivo* but may have an amplifying role. This concept is consistent with

in vitro studies that have shown that IL-21 signaling is required for maximal induction of IL-10 by IL-6 in Th17 cells⁶⁸ or by IL-27 in Tr1 lymphocytes.⁷⁰ Thus, in common with IFN- γ , IL-21 could serve as an autocrine factor that feeds back on CD4 T-cell function through the induction of IL-10.

Together, these paracrine DC and autocrine T-cell pathways for conditional induction of IL-10 production in Th1 and Th17 lymphocytes form a crisscrossing regulatory network as schematically depicted in **Figure 2**.

IL-10-PRODUCING Th1 CELLS IN HUMANS

Even before IL-10 production by Th1 cells was described in mice, cells with this phenotype had been characterized among recently derived purified protein derivative-specific CD4⁺ T-cell clones isolated from human peripheral blood.³⁹ In a related study, similar clones were obtained from bronchiolar lavages of patients with active tuberculosis.⁷¹ In the case of visceral leishmaniasis, IL-10 has been implicated in the profound immunosuppression occurring in diseased individuals and IL-10-producing CD25^{neg} Foxp3^{neg} CD4⁺ T lymphocytes were found to be the major source of the cytokine in such patients.⁷²

More recently, Foxp3^{neg} CD4⁺ T cells with an IL-10⁺ IFN- γ ⁺ phenotype have been identified in peripheral blood mononuclear cells of children infected with acute malaria infections and interestingly, their frequency correlated inversely with the severity of disease.⁷³ In addition, in the absence of known infectious stimuli, effector-like IL-10⁺ IFN- γ ⁺ CD4⁺ T lymphocytes can be isolated from the peripheral blood mononuclear cells of normal donors after brief polyclonal stimulation *in vitro*.⁷⁴ These cells, however, do not proliferate *in vitro* and are able to cause bystander suppression of other T cells in an IL-10-dependent manner.

At present, the molecular mechanism governing IL-10 expression in human T cells is even less well understood than the corresponding pathway in the mouse. However, several different single-nucleotide polymorphisms have been described in the human IL-10 promoter region that are known to control the levels at which the cytokine is secreted and are associated with different risk factors for a number of infectious diseases and autoimmune disorders.^{75,76} Interestingly, when IL-10-deficient mice were genetically engineered to express a human IL-10 bacterial artificial chromosome (BAC) containing a single-nucleotide polymorphism associated with low IL-10 production in humans, they failed to develop IL-10-secreting Th1 cells and showed enhanced resistance after *L. donovani* infection.⁷⁷ In contrast to these observations with T lymphocytes, human IL-10 was expressed in normal levels in myeloid cells and these animals were protected against lethal endotoxin shock. Moreover, when stimulated *in vitro* with IL-27, T cells from the hIL-10 BAC transgenic animals on an IL-10-sufficient background upregulated mouse, but not human, IL-10 production. Although the possibility cannot be excluded that one or more of the regulatory elements necessary for human T-cell-specific IL-10 expression were located outside of the

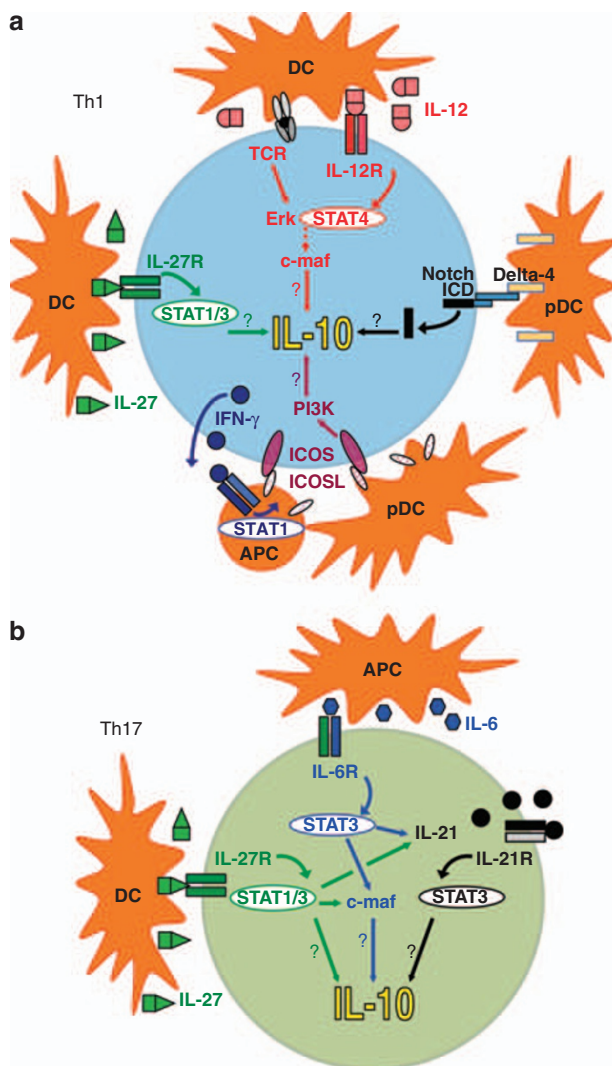


Figure 2 Extracellular signals and intracellular pathways regulating expression of *interleukin (IL)-10* gene in Th1 and Th17 cells. **(a)** The diagram depicts four different mechanisms of IL-10 induction in Th1 cells proposed in the literature and discussed in this review. Although not mutually exclusive, they together reveal the involvement of extracellular factors such as antigen (Ag) concentration, the cytokines IL-12 and IL-27, as well as expression of Notch and inducible costimulatory molecule (ICOS) ligands on dendritic cells (DCs). In each mechanism, distinct signal transducers and activators of transcription (STAT) proteins and/or intracellular signaling cascades have been implicated, suggesting that IL-10 expression in Th1 cells may be achieved through multiple non-redundant pathways. Nevertheless, in each case our incomplete understanding of the downstream DNA-binding regulatory elements that determine *IL-10* gene activation (as indicated with question marks) leaves open the possibility for an as yet undefined unifying mechanism. **(b)** In Th17 cells expression of IL-10 can be induced by IL-6, IL-21, and IL-27, all of which signal through STAT3. Interestingly, IL-6 and IL-21 are known to promote Th17 differentiation, whereas IL-27 inhibits the development of Th17 cells. Although STAT3 may have a critical role, it is not yet clear whether additional signaling pathways known to be activated by each of three cytokines contribute to conditional IL-10 expression. Nevertheless, the transcriptional factor c-maf, which binds to the MARE sequence in the IL-10 promoter, seems to be a common component in all three pathways, suggesting a consensus mechanism of IL-10 expression in Th17 effectors that is currently lacking for Th1 lymphocytes (see **a** above).

region included in the tested BAC, the development of additional hIL-10-BAC mice bearing the transgenes from donors with different IL-10 promoter haplotypes should provide a powerful tool for testing whether any of these polymorphisms specifically control expression of IL-10 in human T cells.

CONCLUSIONS

This review has focused on the concept that lymphoid effector cells can control their own function through the production of a “master” downregulatory cytokine, IL-10. This mechanism was discovered in studies on CD4 Th lymphocyte subsets; however, in more recent work, it has become clear that the same auto-regulatory process may be used by other lymphocytic effector cells. Thus, murine CD8 T lymphocytes, in addition to Th1 cells, have been shown to simultaneously produce IFN- γ and IL-10 after influenza infection,¹⁶ and IL-10-producing CD8 cells have also been described in humans infected with hepatitis C virus⁷⁸ or *L. donovani*.⁷⁹ Similarly, the B-1 subset of B lymphocytes has been known for many years to secrete IL-10 upon activation.⁸⁰ More recently, conventional B cells have been shown to produce IL-10 that can modestly suppress B-cell responses after anti-immunoglobulin D challenge⁸¹ as well as CD8⁺ T-cell expansion after murine cytomegalovirus infection⁸¹ and can protect against allergic hypersensitivity.⁸² Finally, both human^{83,84} and murine^{85,86} natural killer cells are known to be capable of IL-10 synthesis. In the case of murine natural killer cells, this IL-10 can suppress IL-12 production *in vivo* and modify the outcome of parasitic infection.^{85,86} Although the IL-10 produced by these non-CD4 effector cells has been shown in only few situations to exert autocrine effects, we predict that further studies will reveal more examples of this type of self-regulation.

Implicit in the discovery of IL-10-producing effector cells is the idea that these cells should exert an effect to regulate immune function in non-lymphoid tissues. Although this hypothesis has not yet been extensively examined, it is already supported by several recent studies. For example, during acute *T. gondii* infection, high frequencies of IL-10⁺ Th1 cells can be found in peritoneal infection site²² and later in brain tissue⁶⁴ as well as in gut after peroral infection.⁸⁷ In non-healing murine *L. major* infection, similar cells were isolated from skin lesions³⁷ and in influenza infection from lungs.¹⁶ Although known to protect the host against systemic immunopathology in acute infection, IL-10-producing effector cells may accumulate in peripheral tissues at the site of persistent infection to prevent local damage or as a mechanism used by the pathogen to promote its own survival as has been proposed for Treg.⁴⁰ In this regard, it has been shown that IL-10 expression in Th1 cells correlates with the selective loss of chemokine receptors involved in lymphatic recirculation.⁸⁸ Alternatively, the peripheral accumulation of IL-10-producing effector cells may reflect the function of as yet to be defined tissue-specific mediators that enhance (stabilize) IL-10 expression in these infiltrating cell populations. It has been previously shown that immature DCs,⁸⁹ such as those present in peripheral tissues, can promote IL-10 production by CD4⁺ T cells. These antigen-presenting cells are known to be more sensitive targets of IL-10 inhibition than the mature DCs^{3,90} in

lymphoid organs, and thus would be efficient regulators of CD4 effector function in these sites.

With the growing realization that the cytokine secretion profiles of Th effector cells are more plastic than originally thought,⁹¹ an important remaining challenge in the case of IL-10 is to elucidate the molecular mechanisms that control its conditional expression in different Th subsets. Despite the fact that regulatory functions of IL-10 have been extensively characterized, the promoter region of the *IL-10* gene is still poorly defined.⁹² Although the list of factors that can induce IL-10 expression in T cells *in vitro* is rapidly increasing, their physiological roles *in vivo* in many cases remain unclear. Finally, we are still at the early stages in mapping the pathways that link extracellular signals with the activation of the transcriptional factors that regulate IL-10 expression. With the realization that IL-10 production by effector cells is an inducible property, understanding the signaling pathways involved could lead to important new strategies for intervention in inflammatory disorders, infectious disease, and malignancy.

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DISCLOSURE

The authors declared no conflict of interest.

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