Review

The Th1 life cycle: molecular control of IFN-γ to IL-10 switching

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Control of IFN-γ-secreting T helper (Th) 1 cells prevents autoimmunity and immunopathology during infection. IL-10-mediated suppression of Th1 cells is achieved not only through IL-10 produced extrinsically, but also through a negative feedback loop that induces ''intrinsic'' IL-10 expression in cells also expressing IFN-γ, during Th1 lineage differentiation. Targeting this Th1 cell IFN-γ to IL-10 switching is a tantalising prospect for developing therapeutics for Th1-mediated diseases. In this review, the molecular pathways that regulate IFN-γ versus IL-10 expression in Th1 cells are examined, with focus on the role of complement regulator and T cell co-stimulatory molecule CD46, and also discussed are challenges and controversies in the field.

The importance of appropriate regulation of T helper (Th) 1 cells

T lymphocyte responses are integral for host defense against pathogens. Human $CD4^+$ effector T cells can differentiate into at least four major subsets, Th1, Th2 [1], Th9 [2] and Th17 [3] cells, depending on the antigen and the cytokine microenvironment encountered during activation. Signature transcription factors and production of hallmark cytokines that mediate specific effector functions characterize each subset. Th1 cells predominantly produce the proinflammatory cytokine IFN-γ and are required for clearance of intracellular pathogens [4]. However, Th1 responses need to be tightly controlled to prevent disease. For example, inappropriate activation of Th1 cells in response to self-antigen or innocuous antigens (derived from food, airborne particulate material or from gut commensals) leads to autoimmune states, such as multiple sclerosis (MS) [5], rheumatoid arthritis (RA) [6], diabetes [7] and lupus erythematosus (SLE) [8], as well as to hypersensitive states in which T cell tolerance to environmental antigens fails – inflammatory bowel disease and allergy, for example [9–11]. Similarly, an unwanted Th1 response against donor-derived antigens is an obstacle in successful graft acceptance following organ transplantation [12]. The ability to induce timely "shut down" of protective Th1 responses during infection is also important, and failure to initiate the Th1 contraction phase after pathogen clearance is characterized by severe tissue immunopathology, and ultimately death in several infection models [13–18].

T cell-mediated chronic inflammatory diseases are progressive, leading to severe organ dysfunction, and are commonly associated with increased mortality hazard ratios [4]. Effective therapies for such diseases are limited, and patient responses can be inadequate, and/or associated with unacceptable toxicity. Thus, therapies that specifically target deregulated Th1 responses are needed, and an understanding of the cellular and molecular processes that regulate Th1 cell differentiation is a priority. This review summarizes the regulation of Th1 cell function by the immunosuppressive cytokine, IL-10, with emphasis on a process that we call intracellular IFN-γ to IL-10 switching – the transition of an IFN-γ only Th1 state into a state characterized by significant decrease of IFN-γ production and gain of IL-10 expression, with concurrent acquisition of (self) regulatory properties. We further highlight recent data that support the role of the complement regulator and T cell co-stimulatory molecule, CD46 [19], in this cytokine switching in Th1 cells.

The central role of IL-10 in Th1 cell regulation

The importance of IL-10 [20] in "tonic" T cell control first became clear when mice deficient for the *Il10* gene were found to develop colitis through an inability to regulate unwanted Th1 immune responses, presumably orchestrated by the gut flora [21,22]. Accordingly, mutations in *IL-10R* are also associated with susceptibility to colitis [23]. IL-10 also prevents/downmodulates Th1 responses against self-antigens, because immunization of IL-10-deficient mice with myelin-derived antigens exacerbates neuro-inflammatory responses, and attenuates the recovery phase in a mouse model of MS [24,25]. The immunoregulatory potency of IL-10 is also manifest through timely contraction of Th1 responses against infectious pathogens: $Il10^{-/-}$ mice clear infections with *Toxoplasma gondii* [13] or *Trypanosoma cruzi* [14] substantially faster, but these animals then die of severe tissue damage due to unbridled Th1 activation and failure to downregulate IFN-γ after pathogen clearance. It should be noted that, in contrast to $CD4^+$ T cells, IL-10 enhances the function of NK cells and cytotoxic $CD8^+$ T cells [20].

Th1 regulation by IL-10-secreting regulatory cells (Tregs)

IL-10 was initially classified as a Th2-derived cytokine [26], but is produced by a range of $CD4^+$ T cell subsets, as well as macrophages, dendritic cells, B cells, eosinophils

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and mast cells [20]. A major source of IL-10 is $CD4^+$ Treg cells. Tregs are divided into two main populations: natural and adaptive/induced [27]. Both are required for regulation of Th1 responses [27]. Natural Treg cells are generated in the thymus, constitutively express Foxp3, and inhibit T cell responses against self-antigens. They can express IL-10 [28], but suppressive activity is mediated mostly via cell/cell-contact and inhibitory surface molecules targeting DC and T cell activation [29]. Adaptive or induced Tregs are generated in the periphery and are categorized based on their suppressive cytokine production: TGF-β-producing Th3 cells, which are thought to be particularly important in mediating oral tolerance [30], and IL-10-secreting T-regulatory type 1 (Tr1) cells [10,31–33]. *In vitro* generated Tr1 cells were first described in the early 1990 s as the cell population that conferred tolerance in severe combined immunodeficiency (SCID) patients successfully transplanted with stem cell allografts [34]. Although Tr1 cells suppress mainly via IL-10 secretion, production of TGF-β, and expression of granzyme B, which confers cytotoxic function, also contribute to their regulatory activity [25,35]. Tr1 may or may not express T-bet [31,36–38], and are anergic unless exposed to high amounts of IL-2, IL-21 or IL-27 [18,31,36,38,39]. Although IL-10 can regulate Th1 cell responses by directly inhibiting their proliferation and IL-2 production [40–43], the major regulatory effect of Tr1 cells seems to root in interfering with DC maturation and IL-12 (a cytokine needed for Th1 induction) secretion by DCs (Figure 1A) [18,20,44]. Our current understanding of Tr1 cell biology is still limited, because: (1) there is no specific marker for Tr1 cells (and therefore Tr1 work is mostly limited to *in vitro*-induced Tr1 cell populations); (2) a lineage-specific transcription factor has yet to be defined that could account for their phenotype and function; and (3) although Tr1 cells can be induced *in vitro*, they proliferate poorly, which has limited their use *in vivo*.

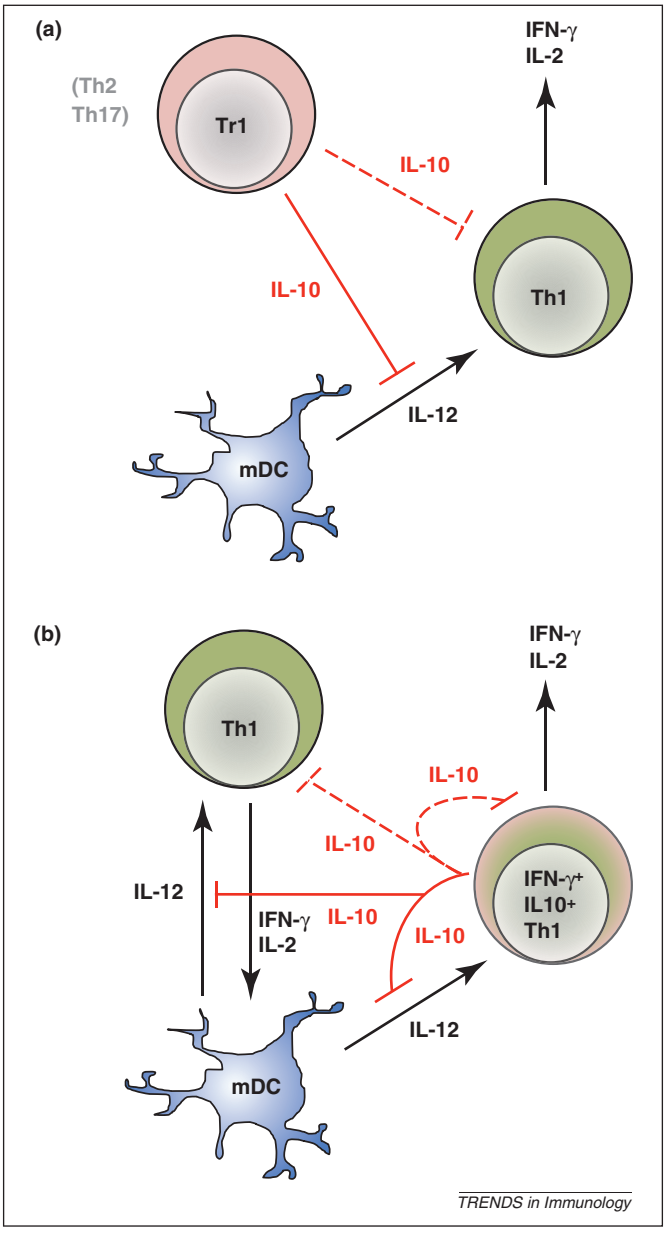

Figure 1. Regulation of Th1 responses via T cell-derived IL-10. Th1 cell activation can be regulated (**a**) ''extrinsically'' by IL-10-secreting, induced Tr1 Tregs, or (**b**) ''intrinsically'' via IL-10 co-expression by Th1 cells. In both cases, IL-10 can suppress T cell activation directly, but probably affects Th1 induction most strongly by inhibiting the maturation of immature DCs (not shown), as well as IL-12 production by mature DCs (mDC) in the vicinity. Other T cell populations producing sufficient amounts of IL-10, such as Th2 and Th17 cells, could also suppress Th1 cell function **(a)**.

Th1 cell autoregulation through co-induction of IL-10 and IFN-γ

Although the importance of IL-10-secreting natural and adaptive Tregs in Th1 regulation is undisputed, a recent concept suggests that Th1 cells themselves are a dominant source of IL-10 that controls immune responses – that is, Th1 effector responses are regulated through a negative feedback loop via the co-induction of IL-10 in addition to IFN-γ in the same cells [4,17,45,46] (Figure 1B). IL-10-secreting Th1 cells, however, seem to be different from Tr1 cells, which are thought currently to not develop via a pro-inflammatory state.

Although IL-10-producing ($Foxp3^-$) Th1 cells were described in the 1990s [47,48], interest in these IFN-γ^+ IL-10^+ double-positive cells has re-emerged, because they regulate immune responses to certain infections. For example, patients with either acute pulmonary tuberculosis, or *Borrelia burgdorferi* or *Leishmania visceralis* infection, express pathogen-specific IL-10^+ IFN-γ^+ Th1 cells in the blood and lung [47–49]. A high proportion of T cell clones derived from the synovium of RA patients express IFN-γ and high levels of IL-10 [50], and single cell analysis has confirmed that RA synovial joint $CD4^+$ T cells are enriched for IFN-γ^+ IL-10^+ cells [51]. An association between low numbers of $CD4^+$ IL-10^+ T cells and synovial hyperplasia and lymphocytic infiltrates provides indirect evidence of autoregulation *in situ* [52], and also points to defective generation of IL-10-producing T cells in chronic inflammatory disease. A mouse model of *T. gondii* infection provided a functional explanation for the existence of double-positive Th1 cells that produce two cytokines with opposing biological effects. This study suggested that IFN-γ produced by antigen-specific IFN-γ^+ IL-10^+ Th1 cells is required for pathogen eradication, and that the concomitant production of IL-10 by these cells is vital for the resolution of the inflammatory response and prevention of tissue

pathology [49]. However, pathogens might exploit high IL-10 expression by Th1 cells to better sustain the infectious process (as has been shown for Tr1 cells [17,18,31,53]). Indeed, during infection with *Leishmania major*, co-expression of IL-10 by *Leishmania*-specific Th1 cells prevented pathogen eradication and contributed to chronic infection [54,55]. A more extreme example is Epstein-Barr virus-derived IL-10 that functions to enhance the survival of newly infected B cells, and to diminish the production of IFN-γ and IL-2 during ongoing immune reactions [56]. Taken together, these studies suggest that the relative amounts or duration of IFN-γ and IL-10 produced by Th1 cells might define pathogen clearance versus pathogen persistence [17,57].

Although IL-10-secreting Th1 cells have been studied mostly in the context of infections, their induction might also be a mechanism by which tolerance is induced in the presence of persistent (e.g. self) antigen [18,58,59] or self-derived antigens of high antigen avidity [60]. Congruent with this notion is that the appearance of mellitin-specific IFN-γ^+ IL-10^+ Th1 cells coincide with the development of immunological tolerance of the host (in this case the bee keeper) to bee venom [58].

The concept of 'cytokine switching' in Th1 cells

To understand cytokine switching, two key issues merit further discussion. The first relates to the precise origin of IFN-γ^+ IL-10^+ T cells. For example, do these cells represent a specific T cell population derived directly from a naïve T cell pool without going through an effector phase, or do they develop from IFN-γ^+ Th1 cells that have received appropriate signals to switch into regulatory IL-10-secreting Th1 cells? The latter concept would be consistent with the growing appreciation that IL-10-secreting Th1 cells might not necessarily represent a lineage, but rather the 'endpoint' of a successful effector response – a cell-intrinsic component of the life cycle of a Th1 cell. Such a switch would have the advantage of producing T cells that express an effector cytokine, while, at the same time, inducing a regulatory cytokine, following recognition of the same antigen. Thus, 'switched' Th1 cells could function independently of 'extrinsic' sources of IL-10. This concurs with the production of IL-10 by Th1, Th2, Th9 and Th17 T cells under certain conditions [61], and indicates that this IL-10-based self-regulatory pathway could apply during the differentiation of effector T cells across a range of lineages.

The second issue relates to the context in which T cells switch from being IFN-γ to IL-10 producers. Several recent observations support the idea that IFN-γtoIL-10 switching in Th 1 cells occurs *in vivo*, and is operational in Th1 cells during induction of tolerance to both self and non-self antigens. Repeated intranasal administration of soluble peptide to myelin basic protein (MBP)-transgenic animals induces the generation of anergic regulatory MBP-specific IFN-γ^+ IL-10^+ Th1 cells that confer tolerance; this population was derived from IFN-γ^+ Th1 cells [59]. Importantly, the initial response of naïve T cells towards peptide administration was marked by IFN-γand IL-2 production, the prototypic proinflammatory Th1 profile. A second administration of antigen was required to induce the switch to IFN-γ^+ IL-2^- IL-10^+ Th1 cells with suppressor function. This observation agrees with the view that peripheral tolerance induction depends on the continuous presence of antigen [58]. The switched IL-10-secreting Th1 cells retained the prototype Th1 transcription factor signature (T-bet^+, $Gata3^-$), while anergy induction was associated with high expression of *egr-2*, a negative regulator of T cell activation [62]. Similar observations have been made in humans where only continuous exposure of non-allergic beekeepers to high doses of bee venom induces the switch from IFN-γ-secreting Th1 cells into IFN-γ- and IL-10-producing Th1 cells, manifest by the decrease of T cell-mediated cutaneous swelling [58]. Once again, these Th1 switched cells maintain T-bet expression. Following antigen withdrawal, bee venom-specific T cells produce high levels IFN-γ, but no IL-10, upon reactivation; however, they then switch to IL-10 production upon repetitive stimulation. Thus, there is evidence of memory in the context of this switch system.

CD46 and IL-2 as environmental cues promoting Th1 IFN-γ to IL-10 switching

A number of conditions required for IL-10 co-expression both in mouse and human Th1 cells have been identified (see Table 1 and for a comprehensive review [18]); however, the exact molecular pathways regulating IFN-γ versus

Table 1. Intracellular signals involved in IL-10 production by $CD4^+$ Th1 and Tr1 cells.

Intracellular signals	IFN-γ^+ IL-10^+ Th1 cells	CD46-med. IL-10 induction in Th1 cells	Tr1 cells
T-bet	+	+	+ [36] and – [38]
Foxp3	+ [28] and – [28]	–	+ [37] and – [37]
STAT1	+	ND	ND
STAT3	+	+	–
STAT4	+	+	+
STAT5	+	+	ND
Erk	+	+	+
c-Maf	+	ND	+
JNK	–	+	ND
p38	–	–	–
SMAD4	ND	ND	+
AhR	ND	ND	+
SPAK	ND	+	ND
Proliferation	+	+ [43] and – [43][a]	–

+, yes; –, no; ND, not determined; numbers in brackets denote source references; [a], CD46-induced IFN-γ^+ IL-10^- and IFN-γ^+ IL-10^+ T cell proliferate strongly, whereas CD46-induced IFN-γ^- IL-10^+ are anergic.

IL-10 expression are largely undefined. Although, IFN-γ and IL-10 co-expressing Th1 cells can now be generated *in vitro* in the presence of IL-27 [63,64] and IL-21 [65], or with high amounts of IL-12 [66], a controlled switch from the IFN-γ^+ IL-10^- to the IFN-γ^+ IL-10^+ state has only recently been reproduced in an *in vitro* system, tractable to in-depth study. Using this system, an intricate link was described between activation of the human complement regulatory protein CD46 and TCR engagement with IFN-γ to IL-10 switching [46]. In humans, CD46 is a widely distributed transmembrane glycoprotein and can express two differentially spliced cytoplasmic domains (designated CYT-1 and CYT-2) with distinct signalling capacities [53,67] (Figure 2A). CD46 inhibits complement activation on host cells by binding and inactivating the opsonins C3b and C4b [53]. CD46 also serves as a receptor for several major human pathogens, including adenovirus, and specific *Neisseria* and *Streptococcal* strains [53]. Further, it functions as a co-stimulatory molecule on T cells, transducing signals through the recruitment to its cytoplasmic tail of the TCR adaptor proteins p120-CBL and LAT, and through subsequent activation of Vav, Rac and Erk [68–70]. CD46 engagement was initially shown to induce low IFN-γ, and high IL-10 and granzyme B expression in naïve CD3-activated $CD4^+$ T cells, a phenotype shared with Tr1 cells [53]. Subsequent studies then showed that CD46 not only induces the switch from proliferating non-suppressive IFN-γ^+ IL-10^- Th1 cells into proliferating suppressive IFN-γ^+ IL-10^+ Th1 cells, but also orchestrates the final transition from the latter to anergic, operationally suppressive IFN-γ^- IL-10^+ Th1 cells [46]. Importantly, the CD46-mediated IFN-γ to IL-10 switch is IL-2-dependant; in the presence of low IL-2 concentrations, TCR and CD46 co-activation induces IFN-γ^+ Th1 cells, with high environmental IL-2 being required for co-induction of IL-10 (Figure 2A). Noteworthy, IFN-γ induction always precedes IL-10 secretion, even in high IL-2 conditions. Further, naïve T cells, in ways similar to that described in the mouse MBP and human 'bee venom tolerance' induction models, require, at the minimum, a second TCR stimulation before they can switch from IFN-γto IL-10 production (with the cells retaining Th1-specific transcription factors) [46]. And, as observed for other switched Th1 cells, CD46-derived IL-10 single-positive T cells produce high amounts of IFN-γ (but only low or no IL-10) after resting and subsequent CD3–CD46-restimulation.

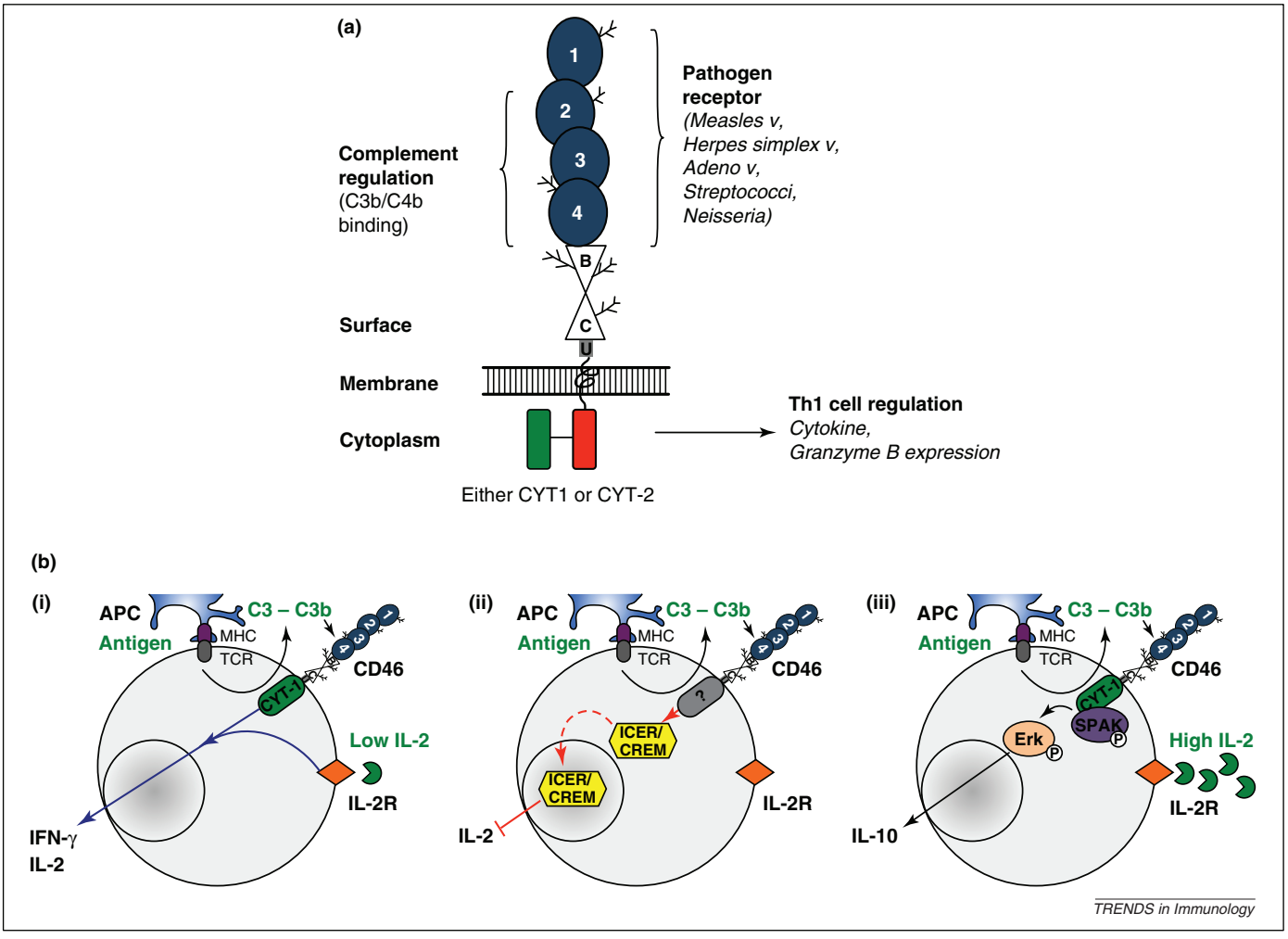

Figure 2. CD46-mediated signals involved in Th1 IFN-γ to IL-10 switching. (**a**) CD46 structure. All CD46 isoforms contain four (1-4) CCPs (complement control protein units), followed by an alternatively spliced highly *O*-glycosylated B or C region, a membrane anchor and one of two possible tails, CYT-1 or CYT-2. (**b**) TCR engagement induces the secretion of C3, which is further processed during antigen presence into the CD46 ligand C3b. (***i***) CD46 activation in the presence of low IL-2 then induces IFN-γ. When environmental IL-2 reaches a certain threshold, CD46-mediated signals induce the (***ii***) inhibition of IL-2 production via the translocation of ICER/CREM to the nucleus and (***iii***) the induction of IL-10 through CYT-1 and SPAK-mediated activation of Erk (and possibly JNK, not shown). The signals responsible for concurrent IFN-γ shut-down are undefined.

The apparent requirement for IL-2 in IL-10 secretion in the CD3CD46-mediated Th1 "switching system" is on one hand compatible with studies showing that deficiencies in IL-2 or components of the IL-2 receptor are associated with autoimmune diseases and hypersensitivity states, including IBD [71]. In addition, in several other model systems, supplementation with exogenous IL-2 also increases Th1-derived IL-10 production [59,72,73]. On the other hand, and somewhat paradoxically, CD46-mediated signals attenuate autocrine IL-2 production when switching from the IFN-γ^+ Th1 phase to the IL-10 (co)-expressing phase. This is coordinated via ICER-CREM (a transcriptional repressor of IL-2 promoter activity [74]) translocation to the nucleus (Figure 2B), in agreement with the finding that IL-2 secretion indeed ceases briefly after activation in Th1 cells through IL-2 transcriptional repression also by T-bet [75]. This active "shut down" of IL-2 and the lack of clear *in vivo* evidence for the necessity of IL-2 in IL-10 co-induction by Th1 cells, makes an autocrine role for IL-2 in Th1 cell cytokine switching unlikely.

If this switching mechanism is fundamental to the control of pro-inflammatory effector responses, one might reasonably anticipate anomalies of IFN-γ to IL-10 switching in disease. Identification of such defects has been confined to studies of human disease, because mice do not express CD46 on somatic cells and a functional homologue has yet to be defined [53,76]. Indeed, it was reported that CD46 and IL-2-induced switching is defective in RA patients. T cells from patients with active disease fail to shut down IFN-γ production upon CD46-activation, and lack the IFN-γ^- IL-10^+ cell subpopulation. As a consequence of this intrinsic cell defect, differentiating Th1 cells produce up to 20 times more IFN-γ compared to T cells from healthy individuals [46]. In addition, and in contrast to T cells from healthy individuals, the IFN-γ^+ IL-10^+ T cells from RA patients lack suppressor functions. The reversibility of this defect is currently under investigation, but will be important to establish, since this has obvious therapeutic implications.

CD3-activated T cells produce the CD46 ligand C3b, and blocking C3bCD46 interactions abrogates IL-10 production in CD3-and CD46-activated, as well as CD3-and CD28-activated T cells [46]. This indicates that the CD46-mediated IFN-γ to IL-10 switching pathway is operational during "conventional" pathways of T cell activation, at least *in vitro*. Thus, CD46-mediated signals appear to be an integral part of Th1 cell biology rather than a 'situation-specific' activation event. This suggests a model by which antigen recognition via the TCR induces the generation of CD46 ligands, which, in turn, provide a means for CD46 activation early during T cell activation. This early phase is marked by high IFN-γ production that will control infection and invading microorganisms at local sites. CD3- and CD46-activated cells then integrate the third signal – high environmental IL-2, indicative of a productive Th1 response – and switch appropriately into the IFN-γ^+ IL-10^+ regulatory phase (Figure 3). Cytokine-switched Th1 cells direct self-regulation, and also inhibit IL-12 production by DC [17,59] in the vicinity, leading to robust contraction/resolution of the Th1 response. This model agrees with the current knowledge about the induction and progression of Th1 responses, the vital contribution of IL-2 in this process [71], and the emerging role of innate signals, including complement activation, as negative regulators of adaptive immunity [53,77].

The CD46-mediated intracellular signaling pathway for IL-10 induction

CD46-mediated intracellular signals that induce IL-10 during the IFN-γ "phase" require CYT-1 (but not CYT-2) (Figure 2A), as well as Ste20 (SPS1)-related proline alanine-rich kinase (SPAK) [78]. The necessity for CYT-1 agrees with a study connecting impaired IL-10 production with increased CYT-2 expression upon CD46 activation of $CD4^+$ T cells from patients with MS [79]. SPAK interacts constitutively with CD46 CYT-1 in non-activated T cells, and TCR activation without concurrent CD46 cross-linking leads to CYT-1SPAK dissociation. By contrast, during TCR and CD46 co-activation, SPAK remains bound to CYT-1, leading to phosphorylation of Erk and Erk-dependent induction of IL-10 expression (Figure 2B). Involvement of Erk activation is in line with the notion that Erk is absolutely central for IL-10 production, not only by all effector T cells, but also by DCs and macrophages [18,72]. SPAK silencing abrogates both CD3- and CD46-induced JNK phosphorylation, suggesting that SPAK may also contribute to JNK activation in the CD46-independent pathway, although currently c-Jun activation has only been connected with IL-10 production in Th2 cells. By contrast, p38 phosphorylation in CD3 and CD3CD46-activated T cells remains largely unaffected by SPAK protein knockdown, paralleling observations that p38 activation is indeed not required for IL-10 expression in either Th1, Th2 or Th17 cells [18,72]. The regulation of ICOS, which also participates in IL-10 production by Th1 cells [18], the role of CD46-induced Flt3 [80], and the possible roles of IL-15, IL-21 and/or IL-27 in IFN-γ to IL-10 switching has not yet been evaluated in this system. CD46-induced cytokine switched cells also contain moderate pSTAT5, and high pSTAT4 and T-bet, expression levels (Table 1). There is evidence that T-bet itself may be necessary for IFN-γ to IL-10 switching: prolonged T-bet expression (observed in all IL-10-producing Th1 cells) is required for the induction of the IL-12Rβ chain. IL-12 in turn is essential for STAT4 activation, a key requirement for IL-10 production by human and mouse Th1 cells [17,72]. We speculate that the dependence of IL-10 induction in Th1 cells on the presence of Th1 signature cytokines and key lineage transcription factors ensures that the regulatory phase will not be accidently induced before the inflammatory phase – which could be potentially catastrophic during ongoing infection. In line with this concept, IL-10 expression in Th2 cells is regulated by IL-4, GATA3 and STAT6 [81], and in Th17 cells by STAT3 expression [82].

The Notch system is involved in Th1 versus Th2 lineage decisions [83], and Notch-1 activation induces IL-10 in human Th1 cells [84]. Notch disruption does not affect T cell proliferation, but attenuates IL-10 production after TCR engagement. Further, although Notch is clearly implicated in IL-10 production in Th1 cells, additional signals are required, one of which is IL-12- or IL-27-mediated STAT4 activation [84]. Activated CD46 up-regulates the

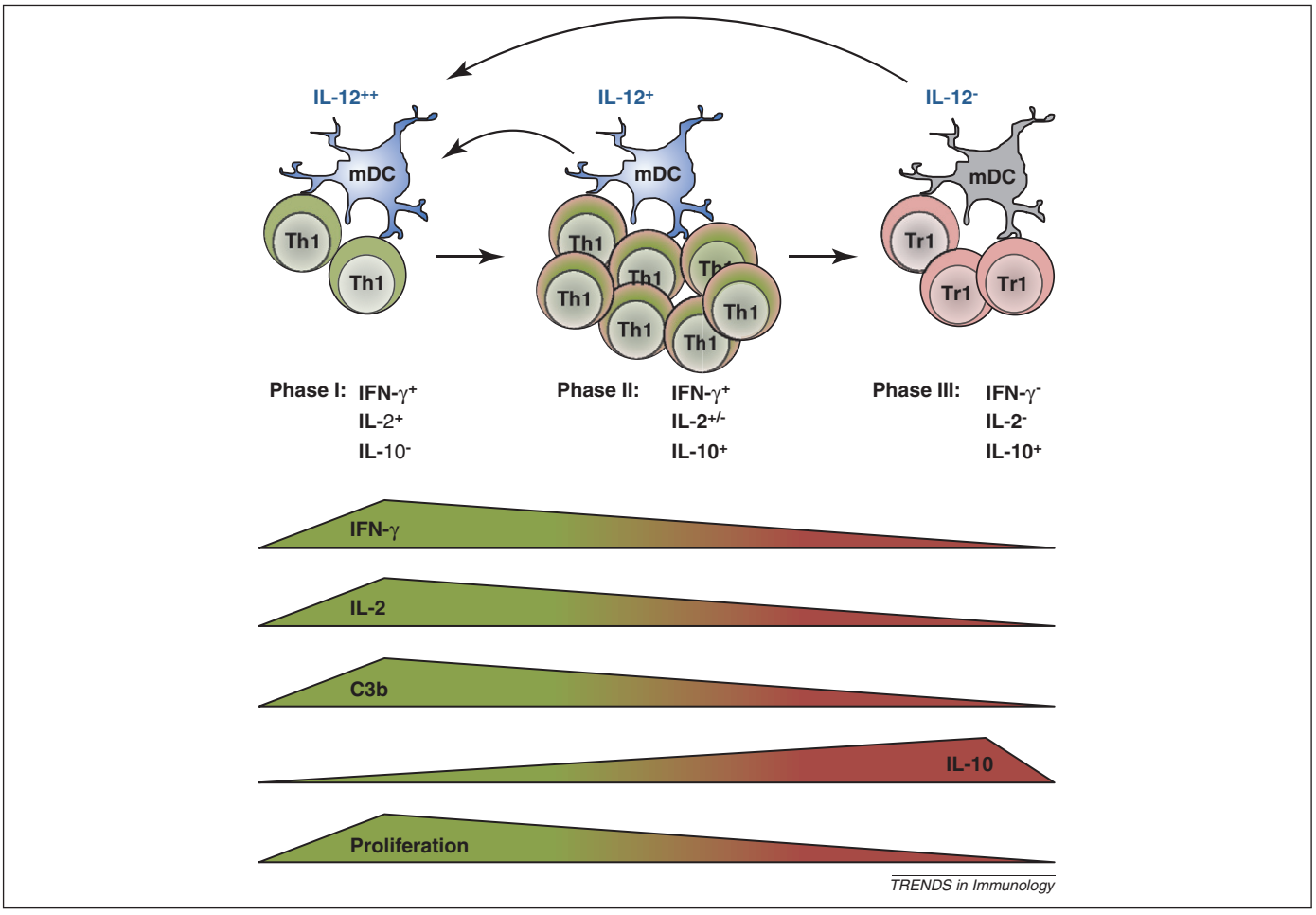

Figure 3. The CD46 and IL-2-driven Th1 life cycle. During the induction of Th1 responses, IL-2 and C3b production are low, and support proliferating, prototypic, IFN-γ secreting Th cells that control infections (phase I). The expansion of Th1 effector cells raises environmental IL-2 sufficiently to induce IL-10 co-expression and the regulatory phases, coinciding with IFN-γ and IL-2 shut-down and suppression of IL-12 production by DCs (phase II and III). Further, the decrease in IL-2 secretion during switching probably induces the loss of proliferation in the IL-10 single-positive cells in the latest phase because these cells require high IL-2 for growth. In line with a Th1 memory cell phenotype, IFN-γ^- IL-10^+ T cells (phase III) will initially produce IFN-γ only (phase I), and then again switch to IL-10 production upon re-stimulation. In addition, Th1 switching from phase II to phase I (without a phase III) has also been observed, particularly in the setting of chronic parasite infection [49].

Notch-1 and -2 receptors and the Notch ligand jagged-1, and is associated with reduced delta-1 expression [85]. Regulation of the Notch system by CD46 might also involve a positive regulatory loop, because Notch not only induces IL-10, but IL-10 also upregulates Notch [86]. Inhibiting CD46-mediated regulation of Notch signaling leads to dysregulation of IFN-γ versus IL-10 production [85], to an extent similar to that recently documented in RA patients.

IL-10-secreting Th1 cells and Tr1 cells: common or distinct?

Although there is evidence that IL-10-producing Th1 cells – at least in humans – are generated via CD46-mediated cytokine switching, the relationship between IL-10-producing Th1 cells and Tr1 cells is less intuitive. Tr1 cells have been generated only *in vitro*, and, common to IL-10-secreting Th1 cells, they seem to utilize IL-2 and/or IL-12 cytokine family members for induction. However, there are important differences between Tr1 cells and IL-10-producing Th1 cells: Tr1 cells require IL-10 for their induction and are anergic [33], whereas IL-10-secreting Th1 cells are independent of IL-10, and proliferate vigorously [although CD46-switched Th1 cells also lose their proliferative capacity at the IL-10 single positive stage (Table 1)] [46]. Also, Tr1 cells may or may not express T-bet [36,38] while T-bet expression is clearly retained in IL-10-secreting Th1 cells (Table 1) [58]. It is now understood, however, that T cell plasticity is not only much greater than acknowledged previously, but also desirable for optimal responses to micro-environmental cues [87]. Thus, IL-10-producing T cells and Tr1 cells might be derived from a single precursor population, but acquire distinctive features depending on 'incoming' signals (supporting this, Tr1 cells vary phenotypically, depending on the induction protocol; for example they can be IFN-γ- and/or Foxp3- and/or granzyme B-negative or positive [25]). *In vitro* induction models leading to IL-10 production by $CD4^+$ T cells do not preclude 'physiological' CD46 engagement, because C3b and C4b are secreted in abundance during TCR engagement [46,88]. According to this model, Tr1 cells might not derive from a unique lineage, but instead reflect a state of terminally switched Th1 (or even Th2, Th17 or Th9) cells. Tr1 cells are abundant at the host/environment interface, such as the skin, gut and lung [22], immunological niches that are continuously exposed to tissue-specific environmental factors including vitamin D (in skin) and retinoic-acid (in the gut) that can 'lock' Th1

cells into a regulatory or Tr1 state. The issue of a common versus distinct IL-10-secreting precursor cell remains to be resolved. Identifying a Tr1-specific lineage marker would help to address this question, and analysis of the epigenetic changes and gene expression profiles among existing IL-10-producing T cell populations could be rewarding.

Concluding remarks and future outlook

The ability to manipulate therapeutically the IFN-γ to IL-10 Th1 cell switch would be invaluable, but will depend to a large extent on the identification of the molecular checkpoints that regulate each phase of the Th1 life cycle (from the IFN-γ to IL-10 single positive state), and how tractable these pathways will be to therapeutic manipulation. It is conceivable that perturbations of any component of TCR, CD46 or IL-2R signaling could influence the dynamics of the Th1 life cycle. For example, allelic variants associated with a wide range of autoimmune diseases might perturb IFN-γ to IL-10 switching through effects on thresholds of TCR signaling (as in the case of *PTPN22*), the integrity of the IL-2-IL-2R pathway (through variants at *IL-2-IL-21*, *IL2RA*, *IL2RB* loci), as well as through inherited or acquired deficiencies of complement factors (e.g. C3/C4 deficiency associated with SLE). The possibility that analogous defects in a switch from IL-17- to IL-17$^+$/IL-10$^+$-producing cells in Th17-mediated inflammatory disease exists, merits further attention. Conversely, low intrinsic thresholds for CD46- and IL-2-mediated IL-10 production might protect from autoimmunity at the price of an increased risk of persistent infections or malignancies.

The inherent plasticity in the pathways of T helper differentiation could pose a major obstacle for the use of IL-10-secreting Th1 suppressor cells as a potential therapeutic strategy. Tregs generated in a controlled *in vitro* environment may re-acquire a pro-inflammatory Th1 phenotype *in vivo* after injection into patients. Identifying the molecular signatures and pathways that characterize both the effector and regulatory phases of CD46-induced Th1 differentiation might provide a framework for actively "inducing and locking" these cells into the desired functional state in the clinical setting. If reversal of the defects in IFN-γ to IL-10 switching that we have recently observed in RA patients after induction of clinical remission, turns out to be a more general phenomenon, applicable to other inflammatory diseases, then therapeutic manipulation of this pathway could be a realistic goal.

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References

1 Mosmann, T.R. *et al.* (1986) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 136, 2348–2357
2 Veldhoen, M. *et al.* (2008) Transforming growth factor-beta 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. *Nat. Immunol.* 9, 1341–1346
3 Harrington, L.E. *et al.* (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
4 Chen, J. and Liu, X. (2009) The role of interferon gamma in regulation of CD4+ T-cells and its clinical implications. *Cell. Immunol.* 254, 85–90
5 Kuchroo, V.K. *et al.* (1993) Cytokines and adhesion molecules contribute to the ability of myelin proteolipid protein-specific T cell clones to mediate experimental allergic encephalomyelitis. *J. Immunol.* 151, 4371–4382
6 Harris, E.D., Jr (1990) Rheumatoid arthritis. Pathophysiology and implications for therapy. *N. Engl. J. Med.* 322, 1277–1289
7 Haskins, K. and McDuffie, M. (1990) Acceleration of diabetes in young NOD mice with a CD4+ islet-specific T cell clone. *Science* 249, 1433–1436
8 Viallard, J.F. *et al.* (1999) Th1 (IL-2, interferon-gamma (IFN-gamma)) and Th2 (IL-10, IL-4) cytokine production by peripheral blood mononuclear cells (PBMC) from patients with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol.* 115, 189–195
9 Baecher-Allan, C. and Hafler, D.A. (2006) Human regulatory T cells and their role in autoimmune disease. *Immunol. Rev.* 212, 203–216
10 Jager, A. and Kuchroo, V.K. (2010) Effector and regulatory T-cell subsets in autoimmunity and tissue inflammation. *Scand. J. Immunol.* 72, 173–184
11 Wing, K. and Sakaguchi, S. (2009) Regulatory T cells exert checks and balances on self tolerance and autoimmunity. *Nat. Immunol.* 11, 7–13
12 Spoerl, S. and Li, X.C. (2011) Regulatory T cells and the quest for transplant tolerance. *Discov. Med.* 11, 25–34
13 Gazzinelli, R.T. *et al.* (1996) In the absence of endogenous IL-10, mice acutely infected with *Toxoplasma gondii* succumb to a lethal immune response dependent on CD4+ T cells and accompanied by overproduction of IL-12, IFN-gamma and TNF-alpha. *J. Immunol.* 157, 798–805
14 Hunter, C.A. *et al.* (1997) IL-10 is required to prevent immune hyperactivity during infection with *Trypanosoma cruzi*. *J. Immunol.* 158, 3311–3316
15 Deckert, M. *et al.* (2001) Endogenous interleukin-10 is required for prevention of a hyperinflammatory intracerebral immune response in *Listeria monocytogenes* meningoencephalitis. *Infect. Immun.* 69, 4561–4571
16 Schopf, L.R. *et al.* (2002) IL-10 is critical for host resistance and survival during gastrointestinal helminth infection. *J. Immunol.* 168, 2383–2392
17 O'Garra, A. and Vieira, P. (2007) T(H)1 cells control themselves by producing interleukin-10. *Nat. Rev. Immunol.* 7, 425–428
18 Saraiva, M. and O'Garra, A. (2010) The regulation of IL-10 production by immune cells. *Nat. Rev. Immunol.* 10, 170–181
19 Liszewski, M.K. *et al.* (1991) Membrane cofactor protein (MCP or CD46): newest member of the regulators of complement activation gene cluster. *Annu. Rev. Immunol.* 9, 431–455
20 Moore, K.W. *et al.* (2001) Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol.* 19, 683–765
21 Kuhn, R. *et al.* (1993) Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 75, 263–274
22 Rubtsov, Y.P. *et al.* (2008) Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. *Immunity* 28, 546–558
23 Franke, A. *et al.* (2008) Sequence variants in IL10, ARPC2 and multiple other loci contribute to ulcerative colitis susceptibility. *Nat. Genet.* 40, 1319–1323
24 Jager, A. *et al.* (2009) Th1, Th17, and Th9 effector cells induce experimental autoimmune encephalomyelitis with different pathological phenotypes. *J. Immunol.* 183, 7169–7177
25 Pot, C. *et al.* (2010) Molecular pathways in the induction of interleukin-27-driven regulatory type 1 cells. *J. Interferon Cytokine Res.* 30, 381–388
26 Fiorentino, D.F. *et al.* (1989) Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med.* 170, 2081–2095
27 Sakaguchi, S. *et al.* (2008) Regulatory T cells and immune tolerance. *Cell* 133, 775–787

28 Maynard, C.L. *et al.* (2007) Regulatory T cells expressing interleukin 10 develop from Foxp3+ and Foxp3- precursor cells in the absence of interleukin 10. *Nat. Immunol.* 8, 931–941
29 Sakaguchi, S. *et al.* (2010) FOXP3+ regulatory T cells in the human immune system. *Nat. Rev. Immunol.* 10, 490–500
30 Miller, A. *et al.* (1992) Suppressor T cells generated by oral tolerization to myelin basic protein suppress both *in vitro* and *in vivo* immune responses by the release of transforming growth factor beta after antigen-specific triggering. *Proc. Natl. Acad. Sci. U.S.A.* 89, 421–425
31 Roncarolo, M.G. *et al.* (2006) Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunol. Rev.* 212, 28–50
32 Zou, W. (2006) Regulatory T cells, tumour immunity and immunotherapy. *Nat. Rev. Immunol.* 6, 295–307
33 Groux, H. *et al.* (1997) A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 389, 737–742
34 Bacchetta, R. *et al.* (1994) High levels of interleukin 10 production *in vivo* are associated with tolerance in SCID patients transplanted with HLA mismatched hematopoietic stem cells. *J. Exp. Med.* 179, 493–502
35 Grossman, W.J. *et al.* (2004) Differential expression of granzymes A and B in human cytotoxic lymphocyte subsets and T regulatory cells. *Blood* 104, 2840–2848
36 Apetoh, L. *et al.* (2010) The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. *Nat. Immunol.* 11, 854–861
37 Vieira, P.L. *et al.* (2004) IL-10-secreting regulatory T cells do not express Foxp3 but have comparable regulatory function to naturally occurring CD4+CD25+ regulatory T cells. *J. Immunol.* 172, 5986–5993
38 Pot, C. *et al.* (2009) Cutting edge: IL-27 induces the transcription factor c-Maf, cytokine IL-21, and the costimulatory receptor ICOS that coordinately act together to promote differentiation of IL-10-producing Tr1 cells. *J. Immunol.* 183, 797–801
39 Gandhi, R. *et al.* (2010) Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell-like and Foxp3$^+$ regulatory T cells. *Nat. Immunol.* 11, 846–853
40 Taga, K. and Tosato, G. (1992) IL-10 inhibits human T cell proliferation and IL-2 production. *J. Immunol.* 148, 1143–1148
41 Taylor, A. *et al.* (2009) IL-10 suppresses CD2-mediated T cell activation via SHP-1. *Mol. Immunol.* 46, 622–629
42 de Waal Malefyt, R. *et al.* (1993) Direct effects of IL-10 on subsets of human CD4+ T cell clones and resting T cells. Specific inhibition of IL-2 production and proliferation. *J. Immunol.* 150, 4754–4765
43 Kemper, C. *et al.* (2003) Activation of human CD4+ cells with CD3 and CD46 induces a T-regulatory cell 1 phenotype. *Nature* 421, 388–392
44 Fiorentino, D.F. *et al.* (1991) IL-10 inhibits cytokine production by activated macrophages. *J. Immunol.* 147, 3815–3822
45 Trinchieri, G. (2007) Interleukin-10 production by effector T cells: Th1 cells show self control. *J. Exp. Med.* 204, 239–243
46 Cardone, J. *et al.* (2010) Complement regulator CD46 temporally regulates cytokine production by conventional and unconventional T cells. *Nat. Immunol.* 11, 862–871
47 Assenmacher, M. *et al.* (1994) Flow cytometric determination of cytokines in activated murine T helper lymphocytes: expression of interleukin-10 in interferon-gamma and in interleukin-4-expressing cells. *Eur. J. Immunol.* 24, 1097–1101
48 Del Prete, G. *et al.* (1993) Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. *J. Immunol.* 150, 353–360
49 Jankovic, D. *et al.* (2007) Conventional T-bet$^+$Foxp3$^-$ Th1 cells are the major source of host-protective regulatory IL-10 during intracellular protozoan infection. *J. Exp. Med.* 204, 273–283
50 Cohen, S.B. *et al.* (1995) High level of interleukin-10 production by the activated T cell population within the rheumatoid synovial membrane. *Arthritis Rheum.* 38, 946–952
51 Morita, Y. *et al.* (1998) Flow cytometric single-cell analysis of cytokine production by CD4+ T cells in synovial tissue and peripheral blood from patients with rheumatoid arthritis. *Arthritis Rheum.* 41, 1669–1676
52 Yudoh, K. *et al.* (2000) Reduced expression of the regulatory CD4+ T cell subset is related to Th1/Th2 balance and disease severity in rheumatoid arthritis. *Arthritis Rheum.* 43, 617–627
53 Kemper, C. and Atkinson, J.P. (2007) T-cell regulation: with complements from innate immunity. *Nat. Rev. Immunol.* 7, 9–18
54 Anderson, C.F. *et al.* (2007) CD4$^+$CD25$^-$Foxp3$^-$ Th1 cells are the source of IL-10-mediated immune suppression in chronic cutaneous leishmaniasis. *J. Exp. Med.* 204, 285–297
55 Jankovic, D. *et al.* (2010) IL-10 production by CD4+ effector T cells: a mechanism for self-regulation. *Mucosal Immunol.* 3, 239–246
56 Moore, K.W. *et al.* (1990) Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein-Barr virus gene BCRFI. *Science* 248, 1230–1234
57 O'Garra, A. and Vieira, P. (2004) Regulatory T cells and mechanisms of immune system control. *Nat. Med.* 10, 801–805
58 Meiler, F. *et al.* (2008) *In vivo* switch to IL-10-secreting T regulatory cells in high dose allergen exposure. *J. Exp. Med.* 205, 2887–2898
59 Gabrysova, L. *et al.* (2009) Negative feedback control of the autoimmune response through antigen-induced differentiation of IL-10-secreting Th1 cells. *J. Exp. Med.* 206, 1755–1767
60 Gabrysova, L. and Wraith, D.C. (2010) Antigenic strength controls the generation of antigen-specific IL-10-secreting T regulatory cells. *Eur. J. Immunol.* 40, 1386–1395
61 McGeachy, M.J. *et al.* (2007) TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. *Nat. Immunol.* 8, 1390–1397
62 Safford, M. *et al.* (2005) Egr-2 and Egr-3 are negative regulators of T cell activation. *Nat. Immunol.* 6, 472–480
63 Stumhofer, J.S. *et al.* (2007) Negative regulation of Th17 responses. *Semin. Immunol.* 19, 394–399
64 Batten, M. *et al.* (2008) Cutting edge: IL-27 is a potent inducer of IL-10 but not FoxP3 in murine T cells. *J. Immunol.* 180, 2752–2756
65 Spolski, R. *et al.* (2009) IL-21 mediates suppressive effects via its induction of IL-10. *J. Immunol.* 182, 2859–2867
66 Meyaard, L. *et al.* (1996) IL-12-induced IL-10 production by human T cells as a negative feedback for IL-12-induced immune responses. *J. Immunol.* 156, 2776–2782
67 Le Friec, G. and Kemper, C. (2009) Complement: coming full circle. *Arch. Immunol. Ther. Exp. (Warsz)* 57, 393–407
68 Astier, A. *et al.* (2000) Cutting edge: CD46, a new costimulatory molecule for T cells, that induces p120CBL and LAT phosphorylation. *J. Immunol.* 164, 6091–6095
69 Zaffran, Y. *et al.* (2001) CD46/CD3 costimulation induces morphological changes of human T cells and activation of Vav, Rac, and extracellular signal-regulated kinase mitogen-activated protein kinase. *J. Immunol.* 167, 6780–6785
70 Hawkins, E.D. and Oliaro, J. (2010) CD46 signaling in T cells: Linking pathogens with polarity. *FEBS Lett.* 584, 4838–4844
71 Malek, T.R. and Castro, I. (2010) Interleukin-2 receptor signaling: at the interface between tolerance and immunity. *Immunity* 33, 153–165
72 Saraiva, M. *et al.* (2009) Interleukin-10 production by Th1 cells requires interleukin-12-induced STAT4 transcription factor and ERK MAP kinase activation by high antigen dose. *Immunity* 31, 209–219
73 Tsuji-Takayama, K. *et al.* (2008) The production of IL-10 by human regulatory T cells is enhanced by IL-2 through a STAT5-responsive intronic enhancer in the IL-10 locus. *J. Immunol.* 181, 3897–3905
74 Bodor, J. *et al.* (2007) Regulatory T cell-mediated suppression: potential role of ICER. *J. Leukoc. Biol.* 81, 161–167
75 Szabo, S.J. *et al.* (2000) A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 100, 655–669
76 Inoue, N. *et al.* (2003) Disruption of mouse CD46 causes an accelerated spontaneous acrosome reaction in sperm. *Mol. Cell. Biol.* 23, 2614–2622
77 Ricklin, D. *et al.* (2010) Complement: a key system for immune surveillance and homeostasis. *Nat. Immunol.* 11, 785–797
78 Delpire, E. and Gagnon, K.B. (2008) SPAK and OSR1: STE20 kinases involved in the regulation of ion homoeostasis and volume control in mammalian cells. *Biochem. J.* 409, 321–331
79 Astier, A.L. *et al.* (2006) Alterations in CD46-mediated Tr1 regulatory T cells in patients with multiple sclerosis. *J. Clin. Invest.* 116, 3252–3257
80 Astier, A.L. *et al.* (2010) RNA interference screen in primary human T cells reveals FLT3 as a modulator of IL-10 levels. *J. Immunol.* 184, 685–693
81 Shoemaker, J. *et al.* (2006) GATA-3 directly remodels the IL-10 locus independently of IL-4 in CD4+ T cells. *J. Immunol.* 176, 3470–3479

82 Stumhofer, J.S. *et al.* (2007) Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. *Nat. Immunol.* 8, 1363–1371
83 Amsen, D. *et al.* (2009) The different faces of Notch in T-helper-cell differentiation. *Nat. Rev. Immunol.* 9, 116–124
84 Rutz, S. *et al.* (2008) Notch regulates IL-10 production by T helper 1 cells. *Proc. Natl. Acad. Sci. U.S.A.* 105, 3497–3502
85 Le Friec, G. *et al.* (2010) CD46: Just a Notch up your complement regulator. *Mol. Immunol.* 47, 2229
86 Chen, J. and Liu, X.S. (2009) Development and function of IL-10 IFN-gamma-secreting CD4(+) T cells. *J. Leukoc. Biol.* 86, 1305–1310
87 Murphy, K.M. and Stockinger, B. (2010) Effector T cell plasticity: flexibility in the face of changing circumstances. *Nat. Immunol.* 11, 674–680
88 Strainic, M.G. *et al.* (2008) Locally produced complement fragments C5a and C3a provide both costimulatory and survival signals to naive CD4+ T cells. *Immunity* 28, 425–435