

Regulation of interleukin-10 and interleukin-22 expression in T helper cells

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Interleukin (IL)-10 and IL-22 are crucial regulators of inflammation during immune responses. While IL-10 functions to prevent excessive inflammation by acting on immune cells, IL-22 elicits innate responses from tissue epithelia and promotes wound healing. Although T helper (Th) cells are a major source for both cytokines, IL-10 and IL-22 are rarely co-expressed at high levels in the same cells. Here we discuss a number of common aspects as well as crucial differences in the molecular regulation of both cytokines that might explain their broad, but distinct expression among Th cell subsets.

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Introduction

Interleukin (IL)-10 and IL-22 are the best-studied members of a larger family of cytokines that also includes IL-19, IL-20, IL-24, IL-26, IL-28A, IL-28B and IL-29. IL-10 family cytokines have essential functions in maintaining the integrity and homeostasis of tissue epithelial layers. Some IL-10 family cytokines promote innate immune responses from tissue epithelia thereby limiting the damage caused by viral and bacterial infections, and facilitate the tissue-healing process in injuries [1].

IL-10 itself functions as an anti-inflammatory cytokine, and limits excessive tissue disruption caused by inflammation. It acts on a variety of immune cells, diminishing the production of inflammatory mediators and inhibiting antigen presentation. Monocytes, macrophages, and T helper (Th) cells are the major sources of IL-10, but several other cell types, including natural killer (NK) cells, dendritic cells, cytotoxic T cells, B cells, mast cells, eosinophils, and neutrophils produce IL-10 as well [1,2].

IL-22, on the other hand, mediates the crosstalk between leukocytes and tissue epithelia, with its receptor being expressed mainly on epithelial cells. Hepatocytes, keratinocytes and intestinal epithelial cells are known IL-22 target cells. By eliciting various innate defensive mechanisms from tissue epithelial cells, IL-22 is essential for the host defense against infections of extracellular pathogens, such as bacteria and yeasts [1,3,4]. Cells of both the innate and the adaptive immune system, mainly Th cells, NK cells and lymphoid tissue inducer (LTi) cells, produce IL-22 [1,5].

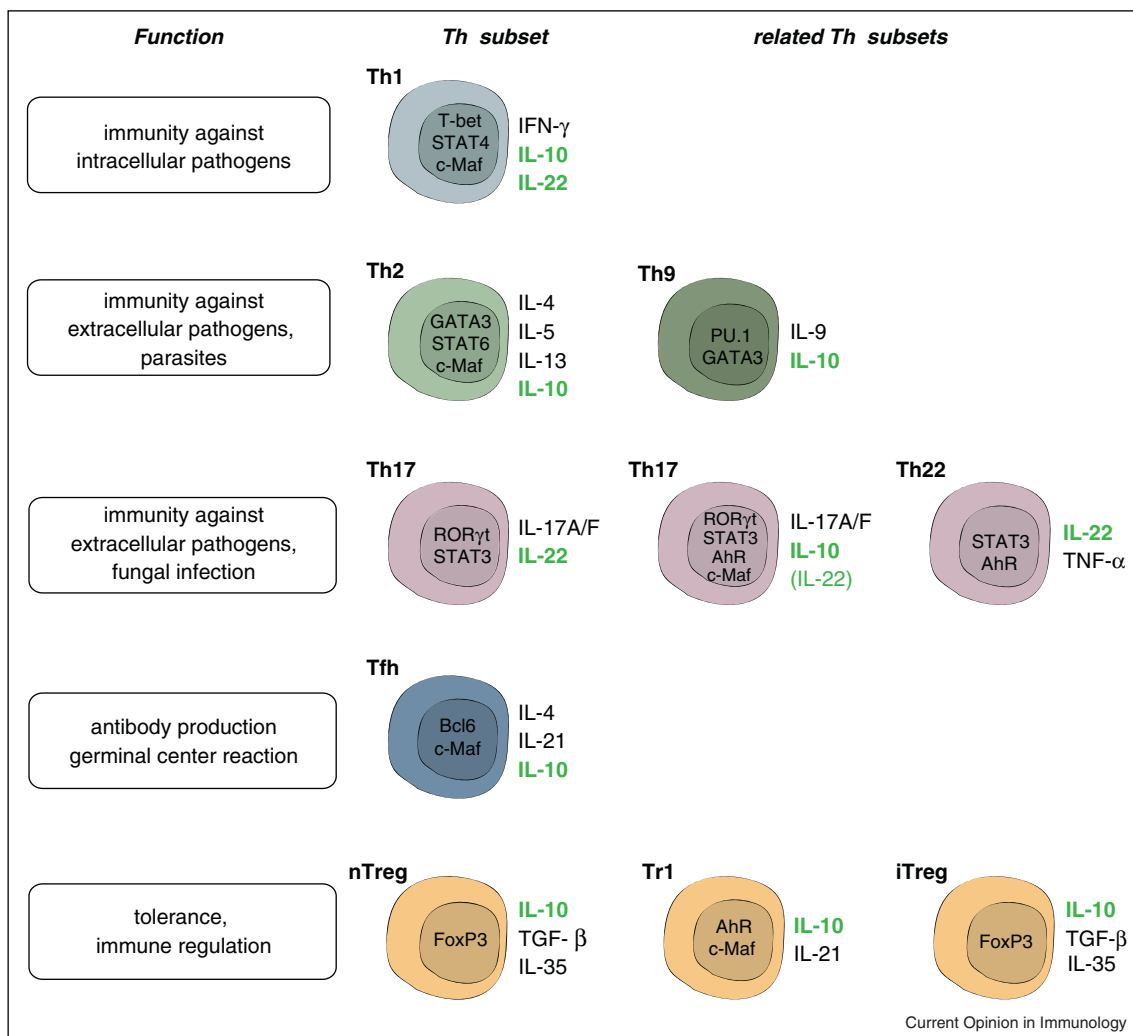
The stimuli and pathways controlling IL-10 and IL-22 production in Th cells, which are a major source of both cytokines, will be the focus of this review. Whereas IL-10 regulation has been studied extensively in various Th cell subsets, IL-22 is mostly viewed as an effector cytokine of one particular subset, Th17 cells. However, IL-22 and the Th17 signature cytokine IL-17 are not always co-expressed and their molecular regulation is different. Here we will attempt a comparison of IL-10 and IL-22, we will highlight recently identified common aspects in the regulation of both cytokines. We will also discuss factors, such as TGF- β , ICOS, or IL-27 that promote IL-10 production while potentially suppressing IL-22, which might account for the very distinct expression pattern of both cytokines among Th cell subsets.

Expression of IL-10 and IL-22 in Th cell subsets

Upon encounter with their cognate antigen, naïve CD4⁺ cells differentiate into a number of different subsets, which are conceptually defined by their biological functions and production of characteristic effector cytokines. Th1, Th2, Th17, T follicular helper (Tfh) and T regulatory (Treg) cells are phenotypically classified by their production of IFN- γ , IL-4, IL-17, IL-21, and IL-10/TGF- β , respectively. In recent years, there is a trend to classify T cells with any unique cytokine signature into new Th subsets, for example, Th9 cells expressing IL-9, and in humans, Th22 cells producing IL-22, but no IL-17 or IFN- γ . In addition, sets of key transcription factors required for driving and maintaining Th differentiation are used to further characterize Th cell subsets (recently reviewed in [6]).

While classical effector cytokines, such as IFN- γ , IL-4 or IL-17, are rather confined to one subset, both IL-10 and IL-22 are more widely expressed among Th cells (Figure 1). Nonetheless, both cytokines are rarely

Figure 1



IL-10 and IL-22 production among Th cell subsets. Upon activation by their cognate antigen and depending on the cytokines present in the microenvironment, naïve T cells can differentiate into various Th cell subsets. These subsets are defined based on their production of characteristic effector cytokines and the expression of key transcription factors, which drive and maintain their differentiation. In contrast to classical effector cytokines, IL-10 and IL-22 are more broadly expressed among Th cells. Nonetheless, co-expression of IL-10 and IL-22 at high levels by the same T cell is rare.

co-expressed at high levels at the same time, which reflects their very different functions during an immune response. IL-10 is produced by all regulatory T cells, including Tr1 cells [7] and Treg [8], and is one of the main mediators of the immunosuppressive potential of these cells. Regulatory T cells have not been described to produce IL-22. IL-10-producing Th1 cells are present in a variety of infections [9,10]. Th1 cells also express IL-22. In fact, in humans the expression of IL-22 in T cells correlates best with IFN- γ production [11^{••}]. However, the extent to which Th1 cells co-express IL-10 and IL-22 at any given stage is not well studied. Th2 cells produce large amounts of IL-10. Originally, IL-10 had been described as a Th2 cytokine [12]. On the other hand,

Th2 cells produce little or no IL-22 [13]. In mice IL-22 expression is mainly associated with IL-17 [13,14], and therefore IL-22 is widely regarded as a Th17 cytokine. However, TGF- β , which together with IL-6 is one of the critical cytokines needed to induce Th17 differentiation [15–17], is a potent inhibitor of IL-22 production [11^{••},13,18[•]]. At the same time TGF- β drives IL-10 production by Th17 cells [19^{••}]. Therefore, depending on the concentration of TGF- β , Th17 cells produce either high amounts of IL-10 and little IL-22 or vice versa. Consistently a recent report finds high IL-22 production in Th17 cells differentiated with IL-6/IL-23/IL-1 β , but high IL-10 levels in Th17 cells generated with IL-6/TGF- β [20^{••}]. Th9 cells produce high amounts of IL-10

but were not shown to make IL-22 [21,22]. In contrast, Th22 cells in humans are defined by their IL-22 production but express little, though detectable, levels of IL-10 [23,24]. Collectively these findings suggest a fundamentally different regulation of both cytokines.

Transcriptional regulation of IL-10 and IL-22 in Th cells

The transcriptional regulation of IL-10 expression has been extensively studied in macrophages but also in T cells, and involves several transcription factors, including Sp1, C/EBPs, IRF-1, AP-1, NF- κ B, and CREB (reviewed in [2]). A number of reports highlight the role of the transcription factor c-Maf as essential for IL-10 expression both in T cells as well as in macrophages [19[•],25[•],26,27[•],28[•]]. IL-10 induction in T cells also critically depends on the STAT pathway, in particular STAT3, at least partially due to the downstream induction of c-Maf [19[•],29].

The regulation of IL-22 is less well understood and has mostly been studied as part of the Th17 differentiation process. However, it is clear that IL-22 is also expressed independently of IL-17, and that TGF- β , a major driver of Th17 development, suppresses IL-22 [13]. Nonetheless, various transcription factors known to drive Th17 differentiation, such as STAT3 [30,31], retinoic acid receptor-related orphan receptor ROR γ t [32], the basic leucine zipper transcription factor ATF-like (BATF) [33] or the aryl hydrocarbon receptor (AhR) [34], have been implicated as positive regulators of IL-22. This is evidenced by the fact that mice deficient in either of those factors show a severe impairment in their ability to produce IL-17 and IL-22. The precise molecular mechanisms by which these factors regulate IL-22 expression are largely unknown. Binding to the *Il22* promoter has only been demonstrated for BATF [33]. It is likely that most of the transcription factors associated with IL-22 function indirectly in a cooperative way, because for instance, ROR γ t [35,36] or AhR [36] over-expression alone is not sufficient to drive IL-22 production.

More recently it became clear that the molecular regulation of IL-10 and IL-22 shares some common aspects, which are independent of one particular Th subset differentiation program. First of all, the STAT pathway, in particular STAT3 downstream of IL-12 family cytokines and IL-6, is critical for both IL-10 and IL-22 induction [19[•],30,31,37] (Figure 2a). Secondly, the Notch [38,39,40[•]] and the AhR pathways [28[•],34,36,41[•]] have been identified as common positive regulators of both cytokines (Figure 2b/2c). Thirdly, TGF- β , ICOS and IL-27 were shown to affect both IL-10 and IL-22 expression in various T cell subsets. Interestingly, those factors exert quite contrasting effects on IL-10 and IL-22, providing an explanation for the observation that rarely both cytokines are expressed at high levels by the same cell. While TGF-

β , ICOS and IL-27 drive IL-10 expression, all of them are potent inhibitors of IL-22 [11[•],13,27[•],37,42,43[•],44]. Interestingly, c-Maf is downstream of all of these factors (Figure 2c).

STAT signaling is essential for IL-10 and IL-22 production

STATs are cytoplasmic transcription factors that translocate to the nucleus to regulate gene expression in response to cytokines and growth factors. In particular, STAT3 activation has been identified as a common denominator in IL-10 as well as IL-22 production in various Th cell subsets. All cytokines shown to induce IL-22 production, that is IL-6, IL-21, and IL-23, signal mainly through STAT3, which explains why STAT3 is crucial for IL-22 expression. Over-expression of a constitutively active STAT3 enhances IL-23R and IL-22 expression [31]. STAT3-deficient T cells, on the other hand, are strongly impaired in their expression of IL-22, as well as IL-17, IL-23R, and ROR γ t [30,31].

STAT3 is also critical for IL-10 production in various Th cell subsets. Th1, Th2 and Th17 cells produce IL-10 in response to IL-27/IL-6 stimulation in a STAT3-dependent process [37]. The induction of IL-10 in Th17 cells by a combination of TGF- β /IL-6 [19[•],42] was also found to require STAT3 activation [19[•]].

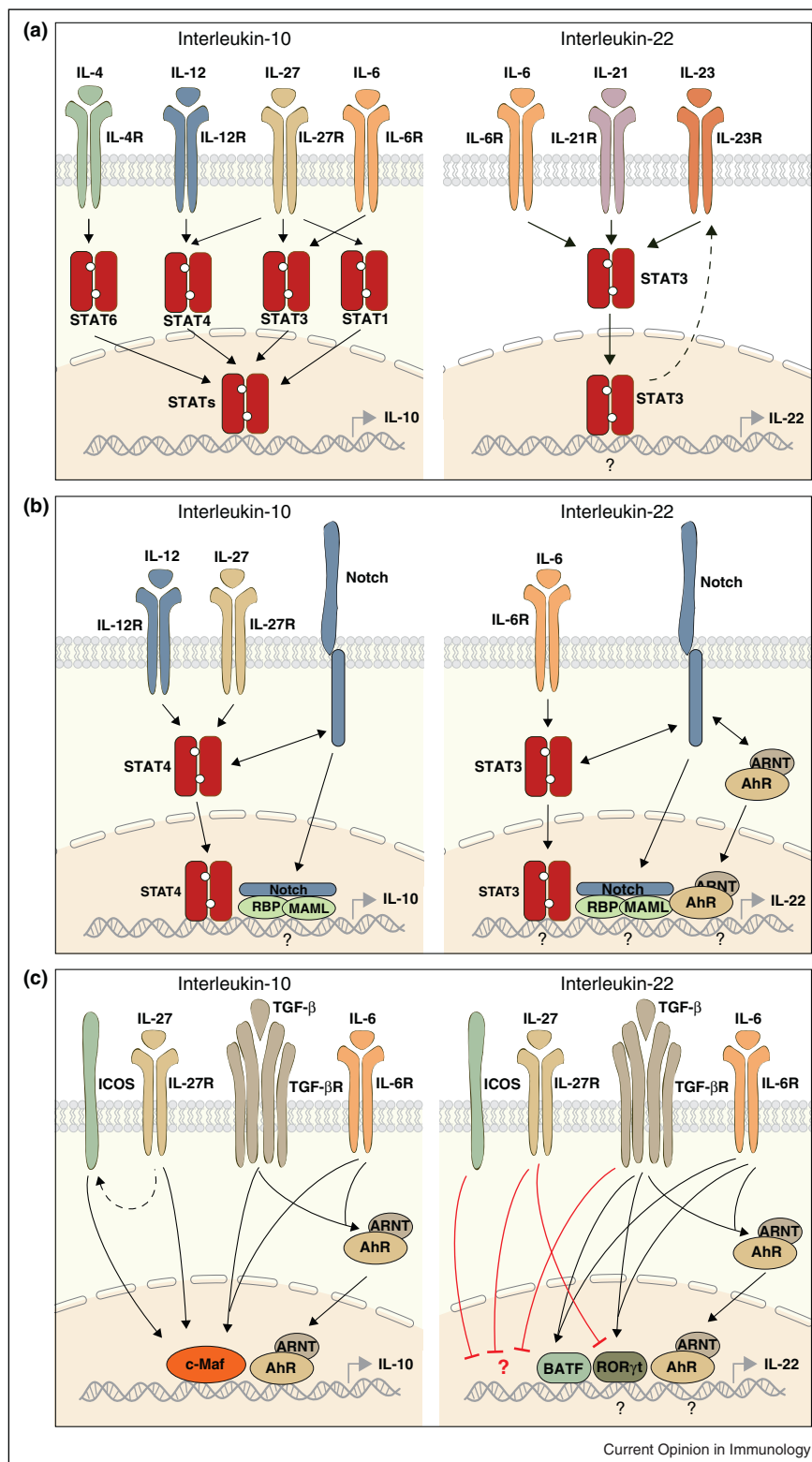
In addition to STAT3, other STATs can also drive IL-10 in certain Th cell subsets. At least two studies find a critical role for STAT1, in IL-27-induced IL-10 in Th1 cells [45,46]. IL-12, which signals through STAT4, has been shown to promote IL-10 expression by Th1 cells [25[•],38,47,48]. Continuous STAT4 activation is required to maintain IL-10 expression in these cells [25[•],48]. In Th2 cells, IL-10 expression is induced by STAT6 downstream of the Th2-driving cytokine IL-4, and requires GATA3 in addition [48,49].

AhR promotes IL-10 and IL-22 production in T cells

The cytosolic transcription factor aryl hydrocarbon receptor, AhR, recognizes numerous small xenobiotic and natural molecules, such as dioxin or the tryptophan photometabolite 6-formylindolo(3,2-b)carbazole (FICZ), and thus represents a link between the adaptive immune system and environmental factors. More recently AhR was shown to influence certain immune responses. Among other functions, AhR can affect Th cell differentiation [50].

AhR participates in the generation of FoxP3⁺ Tregs [41[•],51], and IL-10 producing Tr1 cells [28[•],41[•]]. During Tr1 cell differentiation, AhR is upregulated by IL-27, and acts synergistically with c-Maf to promote the development of Tr1 cells. AhR binds the transcription factor c-Maf, known to be essential for IL-10 expression in T

Figure 2



Common factors in the transcriptional regulation of IL-10 and IL-22. **(a)** STATs are crucial regulators of IL-10 and IL-22 expression. STAT3 activation downstream of IL-6 and IL-27 induces IL-10 production in various T cell subsets. In Th1 cells, STAT4 downstream of IL-12, and in Th2 cells, STAT6 downstream of IL-4, drive IL-10 expression. IL-22 expression critically depends on STAT3 activation downstream of IL-6, IL-23 or IL-21. **(b)** The Notch pathway promotes IL-10 and IL-22 production. The Notch ligand Delta-like-4 acts in synergy with STAT4 downstream of IL-12 or IL27 to drive IL-10

cells, and together they promote the transactivation of *Il10* and *Il21* in Tr1 cells [28^{••}].

AhR has also been identified as a crucial factor in the development of Th17 [34,36,51,52]. Deficiency in AhR or treatment with an AhR antagonist impairs, but does not abrogate Th17 development [34,36,52]. More importantly, AhR has been described to be essential for IL-22 expression in Th17 cells [34,36], $\gamma\delta$ -T cells [53], and human Th22 cells [18[•],23[•]]. Th cells from AhR-deficient mice, while still being able to develop Th17 responses, fail to produce IL-22 [34]. The exact mechanism by which AhR promotes IL-22 production remains elusive. The core nucleotide sequence, to which the nuclear AhR complex binds, also termed the 'xenobiotic responsive element', occurs frequently in the mammalian genome. It is present not only in the *Il22* locus but also in the *Il17a*, *Il17f*, and *Rorc* loci. So far, however, binding of AhR to the *Il22* promoter or other regulatory elements has not been confirmed experimentally. Given the fact that TGF- β is a potent suppressor of IL-22 [13,18[•]] it is puzzling that the expression of AhR in Th17 cells requires a combination of TGF- β /IL-6 or TGF- β /IL-21 [34,51,52]. While neither IL-6 nor IL-21 alone is sufficient to induce AhR, T cells cultured with either cytokine in the absence of TGF- β express the highest levels of IL-22 [13], suggesting that AhR is dispensable for high level expression of IL-22, but rather functions to partially overcome the suppressive effect of TGF- β . It will be interesting to see whether this process also requires the interaction with c-Maf.

The Notch pathway promotes IL-10 and IL-22 production in T cells

Earlier work, using over-expression of Notch ligands in antigen-presenting cells or of active Notch receptors in T cells, had demonstrated an induction of IL-10 both in murine and human T cells under tolerogenic conditions [54]. However, the contribution of endogenous Notch to IL-10 induction under these conditions is not well understood. We have identified that the Notch pathway is a critical regulator of IL-10 production under pro-inflammatory conditions in Th1 cells. Notch synergizes with STAT4, activated by either IL-12 or IL-27, to strongly enhance IL-10 production and anti-inflammatory capacity in IFN- γ -producing Th1 cells [38]. *In vivo*, Notch-mediated IL-10 induction is critically dependent on the Notch ligand Delta-like-4, the expression of which is strongly induced on dendritic cells upon TLR stimulation, but largely restricted to plasmacytoid DC (pDC) in the steady state [39[•]]. pDCs are known to potently induce

IL-10 production in T cells, a process that involves the Notch and ICOS pathways [39[•],55].

More recently, Notch ligands of the Delta-like family were also shown to promote IL-22 production in T cells under Th17-polarizing conditions *in vitro* [40[•],56]. T cells from RBP-J κ deficient mice, which are deficient in downstream Notch signaling, are largely impaired in their ability to produce IL-22. The mice are highly susceptible to the detrimental immunopathology associated with ConA-induced hepatitis, but can be protected by exogenous IL-22 [40[•]]. The Notch ligand driving IL-22 production *in vivo* has not been identified. In humans, however, pDC have been shown to mediate Th22 differentiation [18[•]], which might imply a role for Delta-like-4.

The molecular mechanism behind the Notch-mediated IL-10 and IL-22 induction has not been fully resolved yet. It has been suggested that Notch promotes the production of endogenous ligands for AhR [40[•]], providing a link to a well-established regulator of IL-22 [34], and also IL-10 expression [28^{••}]. Notch also upregulates ROR γ t expression by directly binding to the *Rorc* promoter [56], which might contribute to IL-22 expression. Notch strongly induces IL-22 under Th17-polarizing conditions [40[•]], while having a minor effect on IL-22 under Th1, Th2 and Th0 conditions as well. This strongly suggests a synergistic effect between Notch and STAT3 downstream of IL-6 or IL-23, reminiscent of the role of STAT4 in Notch-mediated IL-10 induction in Th1 cells. Indeed, a direct crosstalk between both pathways has been described [57].

This crosstalk with pro-inflammatory cytokines in the regulation of IL-10 and IL-22 expression puts Notch in a unique position as a key regulator of inflammation during immune responses.

TGF- β , ICOS and IL-27 differentially regulate IL-10 and IL-22 production in T cells

TGF- β , ICOS, and IL-27 all promote IL-10 production in T cells [19^{••},27^{••},37,42,45,55,58], while also being potent inhibitors of IL-22 [11^{••},13,18[•],43[•],44]. The transcription factor c-Maf, a cellular homolog of a viral oncogene, which belongs to the Maf family of basic region and leucine zipper transcription factors, is induced by all of these factors [19^{••},27^{••},28^{••},59].

In fact, c-Maf has been implicated in the regulation of IL-10 production in various T cell subsets, including Th1, Th2, Th17, and Tr1 [19^{••},25^{••},27^{••},28^{••}], as well as in

(Figure 2 Legend Continued) production in Th1 cells. Notch also enhances IL-22 production in Th17 cells. STAT3 and/or AhR activation are required in that process. (c) TGF- β , IL-27 and ICOS differentially regulate IL-10 and IL-22 expression. By inducing c-Maf, a crucial transcriptional activator of IL-10 expression, TGF- β , IL-27 or ICOS promote IL-10 production in a variety of T cell subsets. On the other hand, all three factors potentially suppress, via a yet unknown mechanism, the expression of IL-22. AhR has been shown to interact with c-Maf to induce IL-10 production. AhR is also a positive regulator of IL-22. Dotted lines indicate up-regulation of expression. Question marks indicate the binding of the respective factor to the *Il10* or the *Il22* promoter has not been demonstrated.

macrophages [26]. The induction of c-Maf in response to IL-6/TGF- β or IL-27/TGF- β in Th17 cells is STAT3-dependent and drives IL-10 production in these cells [19^{••}]. c-Maf has also been shown to be downstream of IL-27-induced IL-10 production in Tr1 cells [27^{••}]. IL-27 drives the expansion and differentiation of IL-10-producing murine Tr1 cells by inducing c-Maf, which in turn transactivates IL-21 production. IL-21 acts as an autocrine growth factor for the expansion and/or maintenance of IL-10 producing Tr1 cells [27^{••}]. Although its role in Th9 cells has not been studied, it is likely that c-Maf also controls IL-10 production in this Th cell subset, given the fact that Th9 cells differentiate in the presence of IL-4 and TGF- β [21,22], both inducers of c-Maf.

c-Maf directly transactivates *Il10* through binding to a MARE (Maf recognition element) motif in the *Il10* promoter [19^{••},28^{••}]. Collectively, these findings uniquely position c-Maf as a master regulator of IL-10 expression.

It is presently not known how TGF- β , ICOS or IL-27 suppresses IL-22 production. Whereas IL-27 might simply interfere with the Th17 differentiation program and as a consequence inhibit IL-22 as well as IL-17 production [44], the functions of ICOS and TGF- β appear to be more specific for IL-22, because both factors were shown to further increase ROR γ t and IL-17 expression [13,43[•]]. It will be particularly interesting to determine the role of c-Maf in the suppression of IL-22.

Conclusion

In contrast to effector cytokines which function to tailor an immune response to most effectively attack a specific threat out of a wide variety of different pathogens, the role of IL-10 and IL-22 lies in the regulation of the inflammation inherent in every immune response. Consequently, IL-10 and IL-22 are much more broadly expressed among Th cells. The regulation of their expression is largely independent of any specific T cell differentiation program, despite their co-expression with certain effector cytokines in particular settings. Given their very different mode of action, IL-10 and IL-22 also have to be expressed at different stages and in different microenvironments during an immune response. Therefore, although a number of common aspects have been described lately, their molecular regulation is very different and consequently their expression at times mutually exclusive. Whereas great progress has been made over the years in elucidating the regulation of IL-10 expression, unfortunately our understanding of the exact mechanisms that promote or suppress IL-22 production are still very limited and require further investigation.

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