Chapter 5
Regulation of Interleukin-10 Expression

Sascha Rutz and Wenjun Ouyang

Abstract Interleukin (IL)-10 is an essential anti-inflammatory cytokine that plays important roles as a negative regulator of immune responses to microbial antigens. Loss of IL-10 results in the spontaneous development of inflammatory bowel disease as a consequence of an excessive immune response to the gut microbiota. IL-10 also functions to prevent excessive inflammation during the course of infection. IL-10 can be produced in response to pro-inflammatory signals by virtually all immune cells, including T cells, B cells, macrophages, and dendritic cells. Given its function in maintaining the delicate balance between effective immunity and tissue protection, it is evident that IL-10 expression is highly dynamic and needs to be tightly regulated. The transcriptional regulation of IL-10 production in myeloid cells and T cells is the topic of this review. Drivers of IL-10 expression as well as their downstream signaling pathways and transcription factors will be discussed. We will examine in more detail how various signals in $CD4^+$ T cells converge on common transcriptional circuits, which fine-tune IL-10 expression in a context-dependent manner.

Keywords Interleukin-10 • Transcriptional regulation • Immune suppression • Inflammation • Tolerance • c-Maf • Blimp-1 • T cell • Myeloid cell

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X. Ma (ed.), *Regulation of Cytokine Gene Expression in Immunity and Diseases*,
Advances in Experimental Medicine and Biology 941,
DOI 10.1007/978-94-024-0921-5_5

5.1 Introduction

Multicellular organisms have developed ever more sophisticated and effective immune systems to defend themselves against a wide variety of pathogens. Equally importantly, the immune system has the capacity to limit its potentially deleterious adverse effects on the host itself by utilizing various strategies, such as self-tolerance and anti-inflammatory pathways. Defects in these strategies can result in the development of autoimmune and inflammatory diseases. The anti-inflammatory cytokine IL-10, identified by Mosmann and colleagues in 1989 [52], is a critical negative regulator of immune responses. Loss of IL-10 leads to inflammatory diseases, most notably the development of IBD [131].

IL-10 is the founding member of the IL-10 family of cytokines, which also includes IL-19, IL-20, IL-22, IL-24, IL-26, and the more distantly related IL-28A, IL-28B, and IL-29 [131, 149]. IL-10 was initially described as a secreted cytokine synthesis inhibitory factor (CSIF) produced by Th2 T cell clones, which inhibits the production of several cytokines from Th1 cells [52]. Since this first characterization it has become clear that IL-10 is in fact expressed by a wide variety of cells of the innate and adaptive arms of the immune system, including macrophages, monocytes, dendritic cells (DCs), mast cells, eosinophils, neutrophils, natural killer (NK) cells, $CD4^+$ and $CD8^+$ T cells, and B cells [119, 131].

IL-10 forms non-covalently linked homodimers, which bind to two receptor chains, IL-10R1 and IL-10R2 [92, 176]. IL-10R1 binds IL-10 with high affinity and is unique to the IL-10 receptor, whereas IL-10R2 is a common component of the receptors for IL-22, IL-26, IL-28A, IL-28B, and IL-29 [131, 149]. While the IL-10R2 chain is ubiquitously expressed, IL-10R1 is mainly present on leukocytes. In fact, IL-10 is the only member of the IL-10 cytokine family, which primarily targets leukocytes [131, 149].

IL-10 signals through the Janus kinase (Jak)/signal transducer and activator of transcription (STAT) signaling pathway (Fig. 5.1). Jak1 and Tyk2 are associated with IL-10R1 and IL-10R2, respectively. Binding of IL-10 to the receptor leads to Jak-dependent phosphorylation of the receptor. This allows for the recruitment of STAT3 and to a lesser extent STAT1. Jak1 and Tyk2 phosphorylate STAT3 on tyrosine 705, leading to its dissociation from the receptor and the formation of an active homodimer [50, 70, 117, 162, 175].

IL-10 has a broad spectrum of anti-inflammatory functions and can suppress immune responses to foreign or self-antigens. It mainly targets antigen-presenting cells, such as monocytes and macrophages, by inhibiting the release of pro-inflammatory mediators, including TNF-α, IL-1β, IL-6, IL-8, G-CSF, and GM-CSF, from these cells [40, 51]. IL-10 also inhibits antigen presentation by reducing the expression of MHC II and co-stimulating (e.g. CD86) and adhesion (e.g. CD54) molecules [34, 41, 194]. Moreover, IL-10 inhibits the production and/or secretion of cytokines required for $CD4^+$ T cell differentiation, such as IL-12 and IL-23 [38, 158]. Apart from these indirect ways of inhibiting T cell responses through the downregulation of APC functions, IL-10 can also directly inhibit both proliferation and cytokine production of $CD4^+$ T cells [65].

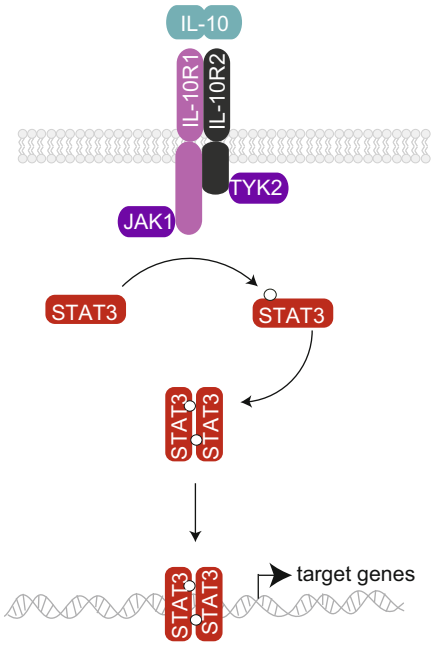

Fig. 5.1 IL-10 signaling. The IL-10 receptor consists of IL-10R1 and IL-10R2 chains. IL-10 binds to the IL-10R1 chain with high affinity. Binding of IL-10 to the receptor leads to phosphorylation and activation of tyrosine kinases Jak1 and Tyk2, which in turn phosphorylate Tyr705 in the C-terminal region of STAT3. This allows STAT3 dimerization and nuclear translocation, and the transcription of IL-10-dependent genes

5.2 IL-10 Function During Homeostasis and Infection

IL-10, like transforming growth factor beta (TGF-β), is a regulatory cytokine with pleiotropic roles in the immune system. However, the prominent function of TGF-β is to maintain T cell tolerance to self or innocuous environmental antigens via its direct effects on differentiation and homeostasis of effector and regulatory T cells (Tregs). Deficiencies in the TGF-β pathway result in hyperactivation and uncontrolled expansion of T cells leading to a lethal multi-organ autoimmune disorder [101]. In contrast, IL-10 functions primarily as a feedback inhibitor of excessive T cell responses to microbial antigens. Most prominently, IL-10-deficient mice spontaneously develop colitis demonstrating that IL-10 has an essential role in maintaining peripheral immune tolerance [95]. Colitis in these mice is mediated by activation and differentiation of effector T cells and is inhibited in IL-10-deficient mice housed under germ-free conditions [95, 160]. This suggests that IL-10 functions to maintain homeostatic T cell tolerance to commensal bacteria in the intestine. IL-10 acts on myeloid cells in order to maintain colonic homeostasis. Mice bearing a deletion of the IL-10 receptor within the myeloid compartment develop colitis indistinguishable from mice with global IL-10 receptor deficiency [165, 203]. The absence of IL-10 signaling results in excessive production of pro-inflammatory cytokines, such as IL-1β, IL-23, and IL-6 by myeloid cells [71]. Deficiency in the signaling adaptor myeloid differentiation primary response gene 88 (MyD88), which abrogates most of Toll-like receptor (TLR) signaling – one of the major pathways by which myeloid cells sense microbial products – blocks the development of colitis in IL-10-deficient

mice [71, 140], further demonstrating that indeed microbial stimuli are required in order to trigger disease.

Various immune cells produce IL-10 in the intestine. IL-10 producing macrophages are abundant in the lamina propria and can induce or expand Tregs in various models [43, 67, 122]. Tregs are critical in the prevention of spontaneous or experimentally induced colitis [145, 183]. Indeed, mice with Tregs that are not able to sense IL-10 develop colitis, although later than IL-10-deficient mice. These mice also exhibit reduced levels of Treg-derived IL-10 [29]. Similarly, macrophage-derived IL-10 is necessary for the Treg-mediated prevention of experimental colitis induced by transferred $CD4^+CD45RB^+$ T cells [122]. However, the selective loss of IL-10 derived from myeloid cells is by itself not sufficient to drive spontaneous colitis [166, 203]. In contrast, Treg-specific IL-10 deficiency does result in spontaneous colitis development demonstrating that indeed Tregs are the critical source for IL-10 in the intestine [145].

Collectively these findings demonstrate that IL-10 is essential to maintain colonic homeostasis by limiting macrophage activation in response to commensal bacteria, which would otherwise trigger detrimental effector T cell responses. The clinical relevance of the pathway is demonstrated by the fact that mutations that block IL-10 function in humans result in the development of severe early onset colitis [64].

Regulatory mechanisms are also essential to properly control and limit inflammatory responses during ongoing immune responses against pathogens. IL-10 is produced as a negative feedback mechanism [33, 129]. Depending on the type of immune response, loss of IL-10 may lead to an enhanced immune reaction and therefore faster pathogen clearance; adversely it can give rise to excessive inflammation resulting in tissue damage or mortality. IL-10 derived from macrophages is critical in order to limit innate responses to certain microbial stimuli, such as the TLR4 ligand lipopolysaccharide (LPS) [166]. Mice with global IL-10 deficiency as well as those with IL-10 deficient macrophages, but not wild-type mice, succumb to septic shock following administration of low doses of LPS [16, 166].

IL-10 production from effector T cells, and to a lesser extent from regulatory T cells, is critical in many infections that trigger an adaptive immune response. During *Toxoplasma gondii* infection, for example, IL-10 produced by Th1 cells is essential to limit an otherwise excessive Th1 cell response [48, 77]. Lack of IL-10 production from T cells in this model is associated with enhanced T cell activation and differentiation. Mice with a T cell-specific deficiency in IL-10 succumb to severe immunopathology upon infection with *T. gondii* akin to mice with global IL-10 deficiency [77, 142]. Similarly, infection with *Plasmodium chabaudi* leads to IL-10 secretion from $CD4^+$ T cells. Deletion of IL-10 from T cells results in decreased survival, greater weight loss, and increased levels of effector cytokines, such as IFN-γ and TNF-α [55]. The contribution of IL-10 derived from B cells during infection is less well understood. However, one report found that B cell-derived IL-10 non-redundantly decreases virus-specific $CD8^+$ T cell responses and plasma cell expansion during murine cytomegalovirus infection [108].

In some cases the IL-10-mediated negative feedback promotes chronic infection as it dampens the immune response enough to prevent efficient pathogen clearance

[33]. This is the case, for example, during infection with the *Leishmania major* strain NIH/S, which induces nonhealing lesions in infected wild-type mice due to IL-10 production from Th1 cells [8]. Certain pathogens, such as the Epstein-Barr virus, even encode IL-10 homologs in their genome as a means to evade the host's immune response [120].

5.3 Drivers of IL-10 Expression in Myeloid Cells

IL-10 production in myeloid cells is triggered by microbial products (Fig. 5.2), which are recognized through pattern recognition receptors, including TLRs, C-type lectin receptors, retinoic acid-inducible gene 1 (RIG-I)-like receptors, and nucleotide-binding oligomerization domain (NOD)-like receptors. Several TLRs, including TLR2, TLR4, TLR5, TLR7, and TLR9, have been shown to induce IL-10 production in human and murine macrophages and DCs [2, 18, 27, 30, 44, 47, 51, 56, 59, 68, 69, 80, 143]. TLR2 ligands, such as Pam3cys, appear to be particularly potent inducers of IL-10 production by myeloid cells [44]. Accordingly, TLR2-deficient mice exhibit reduced IL-10 production from macrophages during *Candida albicans* infection [127]. Despite their common expression of TLRs, myeloid cells differ in their ability to produce IL-10 in response to TLR ligation. TLR9 activation, for example, induces IL-10 production more readily from murine macrophages than from DCs [18, 80].

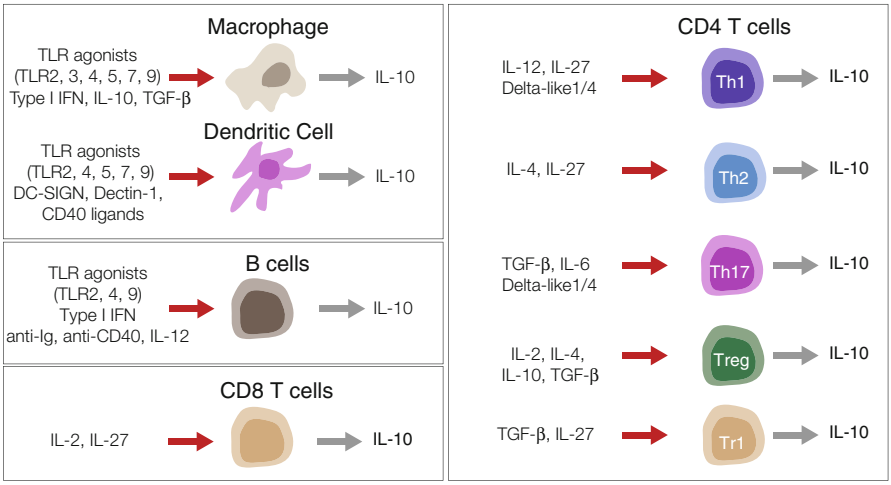

Fig. 5.2 IL-10 expression in different immune cells. Many cell types of the innate and adaptive immune system express IL-10 in response to microbial stimuli or cytokines. Macrophages and dendritic cells produce IL-10 mostly in response to microbial stimuli, such as TLR ligands. Different T cell subsets secrete IL-10 when stimulated by a variety of cytokines. In particular IL-27 and TGF-β broadly induce IL-10 production from various T cell subsets

Myeloid cells also have the capacity to integrate signals received through TLRs with other pathways in order to modulate their IL-10 production. TLR-independent triggers of IL-10 production include the C-type lectin receptors DC-SIGN [59] and Dectin-1 [42, 143]. Co-activation of TLR2 and Dectin-1 enhances IL-10 production in DCs relative to TLR2 or Dectin-1 stimulation alone [42]. Similarly, CD40 ligation together with TLR activation further enhances IL-10 production in DCs [47].

In addition to microbial stimuli, myeloid cells modulate their IL-10 production in response to cytokines secreted by other immune cells. Type I interferon enhances IL-10 production from TLR4-stimulated murine macrophages and human monocytes. In fact, a sustained IL-10 production by macrophages in response to LPS requires an IFN-β-mediated autocrine feedback loop in order to maintain IL-10 transcription [5, 27, 132]. Type I IFN also promotes IL-10 production in *Mycobacterium tuberculosis*-infected macrophages [112, 116]. In contrast, IFN-γ reduces the production of IL-10 in TLR2-activated human macrophages [72].

Phagocytosis of apoptotic cells results in an anti-inflammatory response characterized by IL-10, PGE2, and TGF-β [190]. LPS-activated peripheral blood mononuclear cells in the presence of apoptotic peripheral blood lymphocytes are able to produce higher levels of IL-10 compared to LPS alone [190]. IL-10 may also act to drive its own transcription in macrophages and/or monocytes. Stimulation of monocyte-derived macrophages with IL-10 leads to an increase in IL-10 mRNA [170].

B cells are a source for IL-10 in vitro and in vivo (Fig. 5.2). A combination of anti-Ig and anti-CD40 stimulation induces IL-10 expression, a process that is further enhanced by IL-12 [167]. B cells express a number of TLRs. Agonists of TLR2, TLR4, or TLR9 have all been shown to promote IL-10 production [1, 97, 156]. Similar to macrophages, IFN-α in combination with TLR agonists increases IL-10 production from B cells compared to stimulation with TLR stimulation alone [62, 198].

5.4 Drivers of IL-10 Expression in T Cells

While myeloid cells can directly sense microbial products and produce IL-10 in response, T cells require cytokines or cell-based ligands provided by other immune cells in order to do so. Virtually all T cell subsets (Fig. 5.2), including regulatory T cells, Th1, Th2, Th9, Th17 effector cells, and $CD8^+$ T cells, have the capacity to produce IL-10 [113, 119, 129, 144, 153, 180].

As discussed earlier, IL-10 production from regulatory T cells is essential in order to maintain immune homeostasis in the gut. Accordingly, IL-10 production from Tregs in vivo is largely confined to the intestine under homeostatic conditions [114]. Surprisingly, the signals required to trigger IL-10 expression from Tregs are not well defined. One report concluded that the development of IL-10-competent Tregs does not require IL-10 itself, but is instead dependent on TGF-β [114]. However, another study found that Tregs in the intestine indeed sense IL-10 and that IL-10R-deficient Tregs have reduced levels of IL-10 expression [29]. IL-2 and IL-4 induce IL-10 production from regulatory T cells in vitro and in vivo [13, 35, 96].

In accordance with its function as a negative feedback regulator, effector T cells transiently express IL-10 linked to a state of full activation and effector cytokine production [153, 155]. Although this conclusion is largely based on in vitro studies, in many cases, the induction of IL-10 appears to be an intricate part of the T cell differentiation program, triggered by the same stimuli. Accordingly, strong TCR stimulation together with the Th1-polarizing cytokine IL-12 is required for IL-10 production from Th1 cells, while the Th2-polarizing cytokine IL-4 is essential for IL-10 production from Th2 cells [28, 60, 118, 155, 164]. TGF-β and IL-6 drive the production of IL-10 as part of the Th17 cell differentiation program [173, 195]. The more recently defined subset of IL-9-producing Th9 cells requires IL-4 and TGF-β for its differentiation and IL-10 production [39, 187].

Similar to their role in myeloid cells, type I IFNs promote IL-10 production from $CD4^+$ T cells [5, 32, 100].

5.4.1 IL-10 Induction by IL-27 and TGF-β

IL-27 and TGF-β are potent inducers of IL-10 production in various T cell subsets in vivo (Fig. 5.2). IL-27 is a member of the IL-12 cytokine family and is produced mostly by innate immune cells during infections [73, 84]. IL-27, which is normally not part of T cell differentiation protocols, indeed promotes IL-10 production in $CD4^+$ T cells under neutral and Th1-, Th2-, or Tr1-polarizing conditions in vitro [11, 14, 53, 124, 134, 173]. More importantly, IL-27 limits Th1, Th2, and Th17 cell responses in various infection and autoimmune disease models [9, 53, 73, 84, 172]. For example, IL-10^+IFN-γ^+ Th1 cells are absent in IL-27 receptor-deficient mice, and these mice develop lethal $CD4^+$ T cell-mediated inflammation upon *T. gondii* infection [172, 189]. Acting together with IL-2, IL-27 also induces IL-10 expression in cytotoxic $CD8^+$ T cells [174].

TGF-β, on the other hand, is required for the differentiation and IL-10 production of regulatory T cells [114] and Th17 cells in the intestine. These T cells are generated mainly in response to the commensal microbiota, in the absence of strong pro-inflammatory stimuli, which precludes high levels of IL-27. However, TGF-β does further augment IL-27-induced IL-10 expression in $CD4^+$ T cells in vitro, conditions most commonly used to generate Tr1 cells [11, 173]. In contrast, IL-10 produced by Th17 cells in response to IL-6/TGF-β is not further enhanced by IL-27 [173]. Mechanistically IL-27 and TGF-β rely on distinct but partially overlapping transcriptional programs to induce IL-10 expression [128].

5.4.2 IL-10 Induction by Notch

In addition to secreting cytokines that shape the differentiation of $CD4^+$ T cells, myeloid cells also upregulate Notch ligands in response to microbial stimuli that have been shown to drive T cell polarization and IL-10 production [6, 139].

We found that the Notch pathway is a critical regulator of IL-10 production under pro-inflammatory conditions in Th1 cells. Notch synergizes with IL-12 or IL-27 to strongly enhance IL-10 production in IFN-γ-producing Th1 cells [147]. Activation of the Notch pathway via its ligands Delta-like (Dll) 1 or Dll4 promotes the co-expression of IL-10 and IFN-γ in Th1 cells in vitro [83, 147]. The Notch pathway also promotes IL-10 production from Th17 cells in vitro [131]. Notch-mediated IL-10 induction in vivo critically depends on Dll4, the expression of which is strongly induced on dendritic cells upon TLR stimulation, but is largely restricted to plasmacytoid DCs in the steady state [83].

5.5 The *Il10* Gene Locus and Its Epigenetic Regulation

The *Il10* gene locus in mice exhibits a high degree of homology to the one in human. In both cases *Il10* is located within the *Il10* gene family cluster on chromosome 1 [88], immediately downstream of *Il19*, *Il20*, and *Il24* (Fig. 5.3a). *Il10* exhibits the prototypical genomic organization of all IL-10 family cytokines being comprised of five exons and four introns. Genomic alignments of the mouse and human *Il10* loci have identified a number of highly conserved noncoding sequences (CNS), indicating important roles in the regulation of *Il10* expression (Fig. 5.3b). The highest degree of conservation is observed for CNS-29.8, CNS-26, CNS-20, CNS-9, CNS-4.5, and CNS-0.12 (in kb relative to *Il10* transcriptional start site (TSS)) upstream as well as CNS+1.65, CNS+3, and CNS+6.5 downstream of the TSS [79, 98, 191].

Regions within the *Il10* locus that are hypersensitive to DNase I digestion (HSS) localize mostly to the CNS regions, indicating accessible chromatin in these areas. For instance, bone marrow-derived macrophages stimulated with TLR ligands (LPS, CpG, or zymosan) exhibit five HSS regions located −4.5, −2, and −0.12 kb upstream and +1.65 and +2.98 kb downstream of the TSS [154]. Non-stimulated macrophages also show some degree of sensitivity to DNase I digestion at these sites. This is consistent with the immediate IL-10 expression observed in these cells upon exposure to microbial stimuli. Only HSS −4.5 kb was detected in BM-derived DCs when stimulated with TLR ligands [154]. Again, the reduced accessibility in DCs as compared to macrophages is in line with the weaker IL-10 expression in DCs in response to the same stimuli.

In contrast, chromatin accessibility in T cells is more dynamic. The *Il10* gene locus in naïve T cells is in a closed, transcriptionally inactive conformation with only one HSS at −8.8 kb [79]. Extensive chromatin remodeling during T cell differentiation leads to the formation of additional, mostly common HSS in Th1 and Th2 cells [74, 79, 154, 191]. Some of these sites are more prominent in Th2 than in Th1 cells (HSS −30.4, −29.8, −21, −17.5, and −0.12 kb and +6.45 kb) [79]. Most described HSS in the *Il10* locus are common between T cells and myeloid cells, apart from HSS −4.5 kb which appears to be specific to macrophage and DCs [154].

The chromatin structure at the *Il10* locus is critical in the regulation of IL-10 expression. Among other mechanisms, chromatin accessibility for the transcriptional

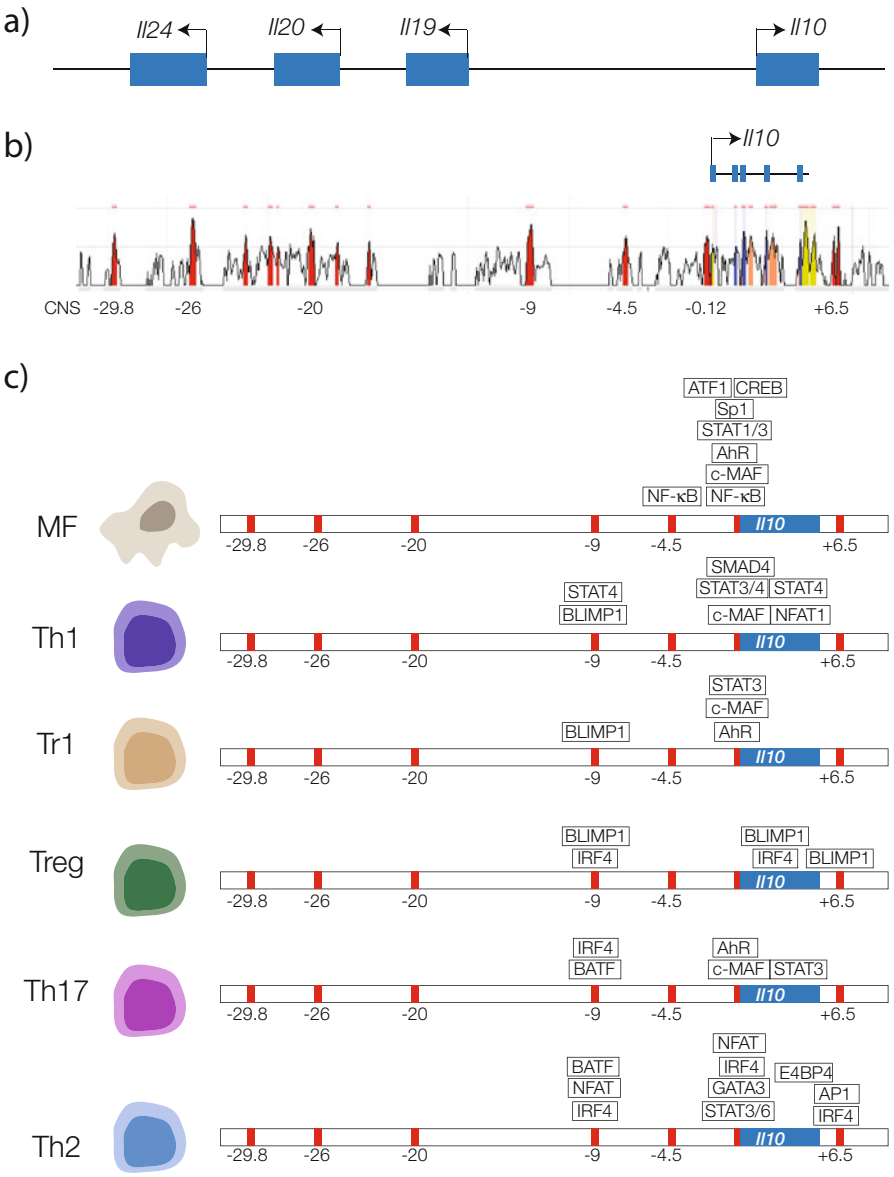

Fig. 5.3 The *Il10* locus and transcriptional regulation of IL-10 expression in various cell types. (**a**) *Il10* is part of the IL-10 cluster on chromosome 1 that also contains *Il19*, *Il20*, and *Il24*. (**b**) Sequence conservation between mouse *Il10* and human *IL10* genes. Highly conserved noncoding sequence (CNS) regions in the *Il10* locus are colored in *red*, and positions are relative to the transcriptional start site. (**c**) Transcription factors implicated in IL-10 regulation in various cell types and their binding within the *Il10* locus

machinery is regulated by different modifications of lysine residues on the tails of histones. For instance, histone 3 K4me3 is a marker for an accessible, transcriptionally competent chromatin, whereas H3K27me3 is associated with inactive chromatin. Chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) has demonstrated that in differentiated Th1 and Th2 cells, the *Il10* locus is in a transcriptionally competent state, characterized by the presence of H3K4me3 and the absence of H3K27me3 marks [192]. The same study found binding of STAT4 in intron 4 in Th1 cells and of STAT6 in the *Il10* promoter region in Th2 cells [192]. H3K4me3 marks are lost and H3K27me3 marks reoccur in STAT4 or STAT6 deficient cells, respectively [128, 192], suggesting that STATs function during IL-10 regulation by increasing the accessibility of the *Il10* gene locus for other transcription factors.

Histone 3 phosphorylation at promoter regions further promotes accessibility and active transcription. Activation of the mitogen-activated protein (MAP) kinase ERK leads to H3 phosphorylation in the *Il10* promoter and facilities the binding of transcription factors, such as the constitutively expressed Sp1 in TLR-stimulated macrophages [104, 199].

Finally, histone acetylation is a hallmark of active transcription. Hyperacetylation of histone H4 was detected at the −4.5 and −1.2 kb HSS regions in IL-10-producing macrophages [154]. The histone deacetylase HDAC11 has been shown to inhibit IL-10 production in macrophages presumably by limiting binding of Sp1, STAT3, and polymerase II to the proximal *Il10* promoter [188]. Ets-1, a member of the ETS family of transcription factors that is highly expressed in resting T cells, negatively regulates IL-10 production in Th1 cells. Ets-1 deficiency significantly decreases recruitment of HDAC1, another histone deacetylase, at HSS −0.12, +1.65, and +2.98 kb [99]. Consistently, Ets1-deficient $CD4^+$ T cells exhibit enhanced IL-10 production when stimulated under Th1-polarizing conditions [99]. The transcription factor E4BP4 also regulates IL-10 production in various $CD4^+$ T cell subsets [121]. At least in Th2 cells, E4BP4 binds to intron 4 and to the 3′ UTR of *Il10* to promote histone acetylation [121].

5.6 Transcriptional Regulation of IL-10 Expression in Myeloid Cells

TLR signaling is the main driver of IL-10 expression in myeloid cells. All TLRs, with the exception of TLR3, bind to the adaptor protein MyD88. TLR3 instead binds the adaptor TRIF (TIR domain-containing adaptor protein inducing IFN-β), while TLR4 recruits both MyD88 and TRIF [4]. Both MyD88- and TRIF-dependent TLR signaling are able to induce IL-10 production [18]. TLR ligation leads to the activation of several downstream pathways, including the MAP kinase pathway, the PI3K/AKT pathway, and the NF-κB pathway [85].

Activation of the MAP3 kinase TPL-2 and downstream ERK1 and ERK2 is critical for IL-10 production in macrophages and DCs in response to TLR2, TLR4, and TLR9 activation [12, 44, 80, 196]. Accordingly, inhibitors of the ERK1/2 pathway reduce IL-10 secretion from macrophages [103]. Similarly, a knockout of TPL-2 reduces TLR-induced IL-10 production [12]. ERK activation is also downstream of Dectin-1-induced IL-10 production in DCs [45, 168]. The degree of ERK activation correlates with the levels of IL-10 production observed in macrophages, mDCs, and pDCs [80] demonstrating the central importance of this pathway. The MAP kinase pathway eventually results in activation of members of the AP1 and ATF transcription factor families. The AP1 factor c-Fos, for instance, has been shown to be involved in TLR-induced IL-10 expression in macrophages and DCs [2, 44, 72, 80].

In addition to ERK, p38 [30, 54, 72, 78, 89, 196] and JNK [26, 30, 72] also contribute to IL-10 production in TLR-stimulated macrophages, monocytes, and DCs. For example, knockout of p38α or treatment with p38α/β inhibitors reduce IL-10 secretion in macrophages [86]. Macrophages deficient for DUSP1, a dual-specificity phosphatase involved in deactivating p38 signaling in response to LPS, display prolonged p38α activation relative to wild-type cells, and this correlates with an increase in IL-10 production [151]. p38γ and -δ also seem to be required for normal LPS-induced IL-10 production, presumably through their regulation of TPL-2 expression. p38γ/δ knockout macrophages have very low TPL-2 levels and fail to activate ERK1/2 downstream of TLR activation [141]. Both ERK and p38 may function cooperatively in their regulation of IL-10 production, through their joined activation of MSK1 and MSK2, mitogen- and stress-activated protein kinases, which promote IL-10 production in TLR4-stimulated macrophages. Downstream of MSK1 and MSK2 the transcription factors CREB and ATF1 bind and trans-activate the *Il10* promoter [7, 133]. The MAPK–MSK1/2–CREB pathway is also downstream of Dectin-1 induced IL-10 expression [49]. Both ERK1/2 and p38α directly phosphorylate Sp1 [37, 177]. Numerous studies using *Il10* promoter reporter genes have shown that Sp1 binding sites are required for the induction of IL-10 transcription [20, 106, 178, 199].

The PI3K/AKT pathway also contributes to IL-10 expression in myeloid cells [109, 130, 193], either by antagonizing GSK3-β, a constitutively active kinase which inhibits the production of IL-10 [125, 130], or through ERK [109] and mTOR [130, 193] activation. mTOR may further regulate IL-10 production through the activation of STAT3 [193].

NF-κB is activated in response to TLR stimulation and contributes to IL-10 regulation. Accordingly, IKK2-deficient macrophages, which have impaired NF-κB activation, show reduced production of IL-10 [82]. In TLR4-stimulated macrophages, the NF-κB subunit p65 is recruited to an NF-κB binding site at HSS −4.5 kb [154]. Its binding to a different site mediates IL-10 production in response to dsRNA [26]. p105 (NF-κB1) is a binding partner for TPL-2. Accordingly, knockout of p105 blocks TLR-induced ERK1/2 activation and downstream IL-10 expression [12]. In addition to binding to TPL-2, p105 can be cleaved to generate the p50 NF-κB subunit. The p50 homodimer is recruited to a site proximal to the *Il10* transcriptional

start site and promotes IL-10 production in macrophages in response to TLR4 activation [22].

Several transcription factors, including c-Maf, the aryl hydrocarbon receptor (AhR), and STATs, mediate IL-10 production in macrophages as well as in T cells (see below). c-Maf belongs to the Maf family of basic region and leucine zipper transcription factors and has been discussed as somewhat of a master transcription factor for IL-10 in T cells and macrophages [23, 146, 153]. c-Maf binds to the *Il10* promoter and enhances IL-10 production in LPS-stimulated macrophages [23]. Conversely, c-Maf-deficient macrophages exhibit strongly impaired IL-10 production upon LPS stimulation. c-Maf is expressed constitutively in resting monocytes and macrophages and further upregulated by IL-4. The ability of IL-4 to act in combination with LPS to induce IL-10 is blunted in c-Maf knockout cells [23]. The ligand-activated transcription factor AhR is required for optimal IL-10 production in TLR4- but not TLR9-stimulated macrophages, where it forms a complex with STAT1 [90]. LPS-induced IL-10 production is inhibited in AhR-deficient or STAT1-deficient peritoneal macrophages compared to WT cells [90]. The IL-10 enhancing function of type I IFN relies in part on the recruitment of STAT1 or STAT3 to the *Il10* promoter [66, 202]. Inactivation of the STAT-binding motif completely ablates trans-activation by type I IFN.

Type I IFN also activates the transcription factor interferon regulatory factor 1 (IRF1), which enhances IL-10 production [202]. In contrast IRF5, the expression of which is induced downstream of TLR ligation, binds to the *Il10* promoter but functions as a repressor of IL-10 in human GM-CSF differentiated monocytes [94].

5.7 Transcriptional Regulation of IL-10 in T Cells

T cells need to undergo activation and differentiation into effector T cells in order to express IL-10. Accordingly, they integrate signals through their T cell receptor (TCR) and cytokine receptors in regulating IL-10 expression.

5.7.1 TCR Signaling in IL-10 Regulation in T Cells

TCR ligation, which is the minimal requirement for any kind of T cell activation but not sufficient for subsequent effector differentiation, activates several downstream signaling pathways culminating in the activation of transcription factors such as AP1, NFAT, and NF-κB. Similar to its role in macrophages and DCs, the MAP kinase ERK is a common positive regulator of IL-10 expression in different T helper cell subsets [155]. Based on the use of small-molecule kinase inhibitors, the ERK1/2 and p38 MAP kinase signaling pathways also seem to regulate IL-10 production in $CD8^+$ T cells [179]. ERK is activated via Ras downstream of the TCR [46] and eventually leads to the activation of transcription factors of the AP1 family. Several

members of the AP1 family have been shown to promote IL-10 expression in various T cell subsets by binding to AP1 motifs in the *Il10* locus. Early studies found that in Th2, but not in Th1 cells, JunB and to a lesser extent c-Jun bind at HSS +6.45 kb and promote IL-10 expression [79, 191]. Ectopic expression of c-Jun and JunB enhances IL-10 production in activated naïve T cells, whereas a dominant negative c-Jun reduces IL-10 production [191]. The transcription factor IRF4 positively regulates IL-10 expression in Th2 cells and also binds to the *Il10* promoter as well as the CNS+6.45 region [3].

Upon TCR activation, NFAT1 translocates from the cytoplasm into the nucleus where it is known to interact with AP1 and other transcriptional partners to promote cytokine gene transcription [107]. Consistently, NFAT1 binds to the *Il10* promoter in Th2 and to intron 4 in Th1 cell lines [74]. NFAT1/IRF4 co-binding to CNS-9 synergistically enhances IL-10 expression in Th2 cells [98].

More recently the basic leucine zipper transcription factor ATF-like (BATF), another AP1 family member, together with JunB and IRF4 were found to function as "pioneer factors" in T cell differentiation by binding to a multitude of loci, including CNS-9 in the *Il10* locus, early after T cell activation [31, 63, 102, 182]. Mutating either the IRF or AP1 motif within CNS-9 results in a diminished luciferase reporter activity consistent with functional cooperation between these factors in *Il10* gene regulation [102]. Deficiencies in BATF or IRF4 significantly impair Th2 or Th17 differentiation and effector cytokine production demonstrating a much broader function beyond IL-10 regulation [3, 31, 123]. This further demonstrates the close interrelation of IL-10 expression and T cell differentiation. However, the broad function of BATF/IRF4 across different T cell subsets is more consistent with a scenario in which T cell activation provides a framework for both IL-10 expression and T cell differentiation, both of which are then further specified by additional polarizing factors, such as STATs.

5.7.2 STAT/SMAD Pathways in IL-10 Regulation in T Cells

STATs are cytoplasmic transcription factors that translocate to the nucleus to regulate gene expression in response to cytokines and growth factors. In this regard STATs are essential in mediating T cell polarization. STAT4 is downstream of IL-12 in driving Th1 differentiation, and STAT6 downstream of IL-4 mediates Th2 polarization, whereas STAT3 is activated by IL-6 and IL-23 and is critical for Th17 differentiation. All of these STATs have been implicated as critical drivers of IL-10 expression in the respective T cell subsets [28, 147, 155, 195]. STAT3 and to some extent STAT1 downstream of IL-27 and IL-21 have roles in inducing IL-10 beyond a particular T cell subset [14, 53, 115, 169, 170, 173, 195]. Two STAT binding sites have been identified in the murine and human *Il10* promoter [184, 202]. Accordingly, STAT4 and STAT6 bind to the *Il10* promoter in Th1 and Th2 cells, respectively [128, 192]. STAT4 binding has also been detecting in the CNS-9 [128] and intron 4 [192] region in Th1 cells. STAT3 binds to the same intron 4 region in Th17 cells

[102]. However, STAT3 deficiency does not block the ability of TGF-β/IL-6 treatment to promote IL-10 production, although it blocks the effects of IL-27 [195]. In conclusion, STATs downstream of a variety of cytokines critically contribute to IL-10 expression, arguably in some cases as an integral part of the respective differentiation program, but also more broadly in a subset-nonspecific manner.

Similarly, SMADs downstream of TGF-β regulate IL-10 production in T helper cells, although TGF-β in many cases interfers with or alters T helper cell differentiation. For example, Th17 cells generated with IL-6 and TGF-β produce large amounts of IL-10 in addition to the signature cytokine IL-17. In contrast, Th17 cells differentiated in the absence of TGF-β lack IL-10 expression, whereas IL-17 levels are comparable [61]. Even more strikingly, TGF-β potently induces IL-10 production from Th1 cells [91, 128]. Downstream SMAD4 binds and trans-activates the *Il10* promoter in Th1 cells [91]. At the same time, however, TGF-β extinguishes IFN-γ expression in these cells [91, 128]. Similarly, TGF-β, in the presence of IL-4, stirs T cell differentiation toward a Th9 phenotype, away from Th2 [39, 187]. Yet, SMAD3 together with GATA3 positively regulate IL-10 production in response to TGF-β in Th2 cells [17]. TGF-β signaling seems to promote IL-10 expression broadly and mostly independently of the T cell differentiation program.

5.7.3 T Cell Lineage Transcription Factors in Regulation of IL-10 Expression

As discussed previously, IL-10 is not produced by naïve T cells, but instead requires T cell activation following antigen recognition. This activation usually occurs in the context of certain cytokines that favor T cell differentiation. Therefore both processes usually coincide.

One way of dissecting this further is to assess the direct contribution of master transcription factors that are induced during T cell differentiation, and that in fact drive and define this process. T-bet is induced by STAT4 downstream of IL-12 and drives Th1 differentiation. Th2 differentiation requires IL-4 signaling through STAT6 and the expression of GATA binding protein 3 (GATA3). Th17 differentiation is dependent on TGF-β and IL-6, which together induce the expression of RAR-related orphan receptor gamma (RORγt) [201].

The requirement for these master transcription factors for IL-10 expression is probably best studied in the case of GATA3 in Th2 cells. GATA3 is recruited to two locations in the *Il10* locus, but it does not trans-activate the *Il10* promoter. Instead, GATA3 binding facilities chromatin remodeling and leads to histone acetylation at the *Il10* locus [164]. This leads to "epigenetic imprinting," which allows Th2 cells to establish a stable memory for IL-10 expression [28, 87, 164]. However, GATA3 is not required for IL-10 production in differentiated Th2 cells [200]. The role for T-bet in IL-10 expression in Th1 cells has been studied in less detail. However, one of the main functions of T-bet during Th1 polarization is the induction of the

IL-12RβII chain to enable IL-12 signaling [159], which in turn drives IL-10 expression. Similarly, we find that the induction of IL-10 downstream for Notch signaling is not impaired in T-bet-deficient T cells cultured under Th1-polarizing conditions. It is, however, strictly dependent on STAT4 [147]. A detailed recent analysis of the transcriptional network in Th17 cells suggests that RORγt indeed acts as a repressor for *Il10*, whereas it positively regulates the expression of the Th17 signature cytokine *Il17a* [31]. Overall these findings suggest that although IL-10 expression coincides with effector cytokine expression in various T helper cell subsets, it is, at least to some degree, decoupled from the respective differentiation program. The term "master transcription factors" is further called into question by recent findings showing that T-bet, GATA3, and RORγt only regulate the expression of a relatively small subset of genes directly – which includes the signature cytokine genes – whereas STATs affect gene expression much more broadly [31, 185]. Additional factors, first and foremost STATs and SMADs downstream of pro-inflammatory cytokines and TGF-β, determine the expression of IL-10 in response to environmental factors.

5.7.4 The Role of c-Maf in IL-10 Regulation in T Cells

c-Maf expression is induced by several important drivers of IL-10 expression, including IL-27, TGF-β, and ICOS ligand [10, 15, 134, 195]. Indeed, c-Maf has been shown to be critical for IL-10 expression in Th17 and Tr1 cells [10, 134, 195]. But its expression also correlates with IL-10 production in Th1 and Th2 cells [155]. Although its role in Th9 cells has not been studied, it is likely that c-Maf also controls IL-10 production in this T cell subset, given the fact that Th9 cells differentiate in the presence of IL-4 and TGF-β [39, 187], both inducers of c-Maf.

Although c-Maf can trans-activate *Il10* by itself to some extent [10, 195], robust IL-10 expression requires interaction with additional transcriptional regulators. In the case of Tr1 cells generated in the presence of IL-27 or IL-27/TGF-β [134], c-Maf cooperates with AhR in the regulation of IL-10 production in mouse as well as human [10, 58]. As such, knocking down c-Maf or AhR in Tr1 cells decreases *Il10* mRNA expression. Both factors bind and synergistically trans-activate the *Il10* gene promoter [10]. The induction of c-Maf in Tr1 cells is thought to be further regulated by IL-21 and possibly by ICOS, since both IL-21- and ICOS-deficient T cells show reduced IL-10 production and c-Maf expression [135]. In Th17 cells, c-Maf is induced downstream of TGF-β and acts mainly as a suppressor of pro-inflammatory gene expression most likely by antagonizing BATF [31, 148]. However, IL-10 is among the few genes induced by c-Maf in Th17 cells [31, 195]. Although c-Maf expression is low in Th1 cells compared to other T cell subsets, it is driven by IL-12 and can be potently induced by activation of the Notch pathway in order to drive IL-10 expression [128].

5.7.5 The Role of Blimp-1 in IL-10 Regulation in T Cells

Blimp-1 encoded by the *Prdm1* gene is a transcriptional repressor that positively regulates IL-10 production in both $CD4^+$ and $CD8^+$ T cells [76, 111, 174]. Blimp-1 expression is limited to highly polarized effector $CD4^+$ T cells and therefore associated with effector cytokine secretion [110, 111].

We recently reported that Blimp-1 is critical for IL-10 production in Th1 cells, where it binds mainly to CNS-9 [128]. Blimp-1 expression in Th1 cells in vitro is dependent on STAT4, which explains its late induction during Th1 polarization. The early phase of Th1 differentiation is IL-12 independent and instead relies on IFN-γ-mediated induction of T-bet [159]. Consistent with the transient nature of IL-10 expression in Th1 cells, Blimp-1 activity is restricted to an effector state and is likely to coincide with high availability of pro-inflammatory cytokines, such as IL-12. Blimp-1-deficient Th1 cells lack IL-10 production in vitro and in vivo [128].

T cell-specific Blimp-1 deficiency results in enhanced inflammation and immunopathology during *T. gondii* infection. IL-27-induced IL-10 production in $CD4^+$ T cells is completely dependent on Blimp-1 [128], further suggesting a broad function of Blimp-1 in IL-10 regulation in T cells. In IL-27-induced Tr1 cells, the transcription factor egr2 is upstream of Blimp-1 [76]. Accordingly, egr2-deficient Tr1 cells have reduced IL-10 production but enhanced secretion of IL-17 or IFN-γ [76]. Although IL-12 and IL-27 seem to use different pathways to induce Blimp-1, IL-12 by signaling through STAT4 and IL-27 by signaling through STAT1/STAT3 [173], their co-expression in many Th1-driven immune responses makes it likely that both cytokines synergize in promoting Blimp-1-dependent IL-10 expression in Th1 cells in vivo.

Similarly, Blimp-1 is critical for IL-10 production in $CD8^+$ T cells, where its expression requires $CD4^+$ T cell help and is limited to effector and memory $CD8^+$ T cells [81, 111, 174]. Blimp-1 is also involved in IL-10 regulation in regulatory T cells and is expressed in an effector regulatory T cell population that is found at the site of inflammation [35]. Blimp-1 deficiency does not prevent Treg development but strongly impairs the production of IL-10 by these cells in response to TCR stimulation. In Treg cells Blimp-1 acts synergistically with IRF4 [35]. Given its expression pattern, Blimp-1 is perfectly suited to restrict IL-10 production to an effector phase at the peak of an acute inflammatory response. However, Blimp-1 is not universally required for IL-10 expression in T cells, as differentiation of Blimp-1-deficient $CD4^+$ T cells into Th2 cells results in normal IL-10 expression [81].

5.7.6 Transcriptional Regulation of IL-10 Expression Through Inflammation

T cells integrate diverse environmental stimuli to appropriately express IL-10. Pro-inflammatory cytokines, such as IL-12 and IL-27, secreted by activated antigen-presenting cells signal ongoing inflammation and eventually the need for

self-limitation of these responses. Blimp-1 is being recognized as the main driver of IL-10 production in pro-inflammatory effector T cells under these conditions [128, 186]. Not only is Blimp-1 essential for IL-10 expression in Th1, Tr1 cells, and $CD8^+$ T cells; it also drives IL-10 production in regulatory T cells that have entered an "effector stage" induced by cytokines such as IL-2, IL-4, and others [36, 128, 174]. All these cells are severely impaired in their capacity to produce IL-10 in the absence of Blimp-1. How does Blimp-1-mediated IL-10 expression relate to c-Maf, another widely expressed transcription factor regulating IL-10 [146]? c-Maf expression can be induced by pro-inflammatory stimuli including IL-27 [134] but also by Notch ligands expressed on APCs in response to TLR stimulation. This process greatly enhances IL-10 production from Th1 cells, but still requires Blimp-1 [128]. Therefore c-Maf acts cooperatively with Blimp-1 in inducing IL-10 under inflammatory conditions.

However, IL-10 production is also required to maintain immune homeostasis in the absence of acute inflammation, mainly in the intestine. TGF-β is an important driver of IL-10 production under those circumstances. While TGF-β is a potent inducer of c-Maf, it strongly antagonizes Blimp-1 expression [10, 128, 134, 150, 195]. In fact, Th17 cells generated in the presence of TGF-β/IL-6 do express high levels of c-Maf but no Blimp-1. The suppressive effect of TGF-β on Blimp-1 is dominant over IL-27, explaining why IL-27 does not further enhance IL-10 from Th17 cells [173]. Ectopic expression of Blimp-1 in Th17 cells indeed strongly increases IL-10 production [128]. Tr1 cells can be generated in vitro with IL-27 in the presence or absence of TGF-β. The addition of increasing concentrations of TGF-β to these cultures shifts IL-10 expression from a Blimp-dependent to a Blimp-independent pathway [128]. In the presence of high levels of TGF-β, in Tr1 cells and Th17 cells, IL-10 expression fully relies on c-Maf interacting with AhR [10, 128]. Collectively, these observations across several T helper cell subsets suggest that IL-10 expression, rather than being regulated in a subset-specific manner as part of the differentiation program, is driven more universally and fine-tuned by the inflammation state.

5.8 Posttranscriptional Regulation of IL-10 Expression

Posttranscriptional control of cytokine production represents an additional layer of regulation that enables the cell to rapidly release cytokines in response to extracellular stimuli but also to shut down this response in a timely manner. Prominent mechanisms of posttranscriptional regulation of cytokines are RNA-binding proteins and microRNAs that control the stability and translational activity of the cytokine RNA [75].

A growing number of studies, mostly conducted in macrophages, has found pathways that regulate IL-10 expression on the posttranscriptional level. The 3′UTR of the IL-10 mRNA contains AU-rich elements (AREs) [136], which are characteristic of short-lived RNAs, that mediate mRNA decay through their recruitment of

ARE-binding proteins [75]. Two studies identified tristetraprolin (TTP) as the ARE-binding protein regulating the stability of IL-10 mRNA in TLR4-stimulated macrophages [57, 171]. As a consequence, TTP-deficient macrophages produce higher levels of IL-10 [57]. A more recent study found higher serum IL-10 levels in LPS-treated mice with a myeloid-specific deficiency in TTP [93]. TTP also targets other cytokine mRNAs for rapid degradation, including IL-6, TNF-α, and GM-CSF [24, 25, 126].

Interestingly, the mRNA-destabilizing function of TTP is inversely regulated by p38 MAPK activity [152, 181], such that after receiving an inflammatory stimulus the TTP-dependent decay is initially limited ensuring IL-10 expression at the height of the inflammatory response [93]. IL-10 signaling itself regulates IL-10 mRNA [21] in a TTP-dependent manner. IL-10 increases TTP expression and activity by antagonizing p38 activity [57, 157].

Another posttranscriptional regulatory circuit involves the RNA-binding protein ARE/poly(U) binding/degradation factor 1 (AUF1), which also binds to the 3′UTR of the IL-10 mRNA and reduces its half-life [19], and the upstream MKP-1, which regulates the translocation of AUF1 from the nucleus to the cytosol [197]. In the absence of MKP-1, both IL-10 mRNA stability and IL-10 secretion are increased [197].

MicroRNAs (miRNAs) target IL-10 and in doing so play important roles in autoimmune and inflammatory diseases such as SLE, reperfusion injury, and asthma [138]. IL-10 can be regulated by several microRNAs, including miR-106a, miR-4661, miR-98, miR-27, let7, and miR-142-3p/5p [138]. While miR-106a binds to the 3′UTR of the IL-10 mRNA and negatively regulates its expression [161], binding of miR-466l to the 3′UTR results in a net increase in the half-life of IL-10 mRNA, by preventing TTP binding [105]. Another miRNA, miR-21, has been shown to indirectly regulate IL-10 via downregulation of the IL-10 inhibitor PDCD4 [163].

A number of viruses exploit the cell-intrinsic control of gene expression by miRNAs for immune evasion by encoding their own viral homologues of miRNAs. For example, Kaposi's sarcoma-associated herpesvirus encodes 17 mature microRNAs, two of which, miR-K12-3 and miR-K12-7, activate transcription and secretion of IL-10 [137].

5.9 Concluding Remarks

Given its crucial roles in maintaining an effective immune response against pathogens while reducing the risk of potentially deleterious excessive inflammation, IL-10 expression has to be finely tuned. As a negative feedback mechanism, it has to be responsive to the degree of inflammation and has to have the ability to be turned on or off quickly in any immune cell. Accordingly, IL-10 expression is regulated at multiple levels, by extracellular stimuli, transcriptionally, through

epigenetics, and posttranscriptionally. A broad understanding of the signals that regulate IL-10 expression has evolved, according to which myeloid cells directly respond to microbial stimuli, most prominently TLR ligands, whereas IL-10 production from T cells requires the release of pro-inflammatory cytokines, such as IL-27 from antigen-presenting cells. These extracellular stimuli then translate into the activation of various signaling pathways and the downstream activation of transcription factors. In T cells this occurs in the context of transcriptional programs of T cell activation and differentiation. Over the past years, we have identified a number of transcription factors, such as c-Maf or Blimp-1, with prominent roles in IL-10 regulation. While we are making progress in elucidating how these factors function individually to enable IL-10 expression, a comprehensive understanding of the transcriptional regulation of IL-10 is only just beginning to emerge. Extensive research will be required to better understand the epigenetic regulation of the *Il10* locus, as it is crucial to a timely expression of IL-10. Currently we only have a rudimentary understanding of how chromatin accessibility at the *Il10* locus is controlled. In particular in T cells, this process appears to be highly dynamic, very different from "effector cytokines" and distinct in various T cell subsets.

While most of our knowledge of the transcriptional regulation of IL-10, in particular in T cells, is derived from the studies of cultured cells, moving forward it will be essential to apply and further develop this knowledge toward an integrated view of IL-10 expression during immune responses in vivo. Only by doing so we will be able to harness the great potential for therapeutic intervention inherent in this fundamental immune-regulatory pathway.

References

1. Agrawal S, Gupta S. TLR1/2, TLR7, and TLR9 signals directly activate human peripheral blood naive and memory B cell subsets to produce cytokines, chemokines, and hematopoietic growth factors. J Clin Immunol. 2011;31(1):89–98.
2. Agrawal S, et al. Cutting edge: different Toll-like receptor agonists instruct dendritic cells to induce distinct Th responses via differential modulation of extracellular signal-regulated kinase-mitogen-activated protein kinase and c-Fos. J Immunol. 2003;171(10):4984–9.
3. Ahyi A-NN, et al. IFN regulatory factor 4 regulates the expression of a subset of Th2 cytokines. J Immunol. 2009;183(3):1598–606.
4. Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol. 2004;4(7):499–511.
5. Aman MJ, et al. Interferon-alpha stimulates production of interleukin-10 in activated CD4+ T cells and monocytes. Blood. 1996;87(11):4731–6.
6. Amsen D, Antov A, Flavell RA. The different faces of Notch in T-helper-cell differentiation. Nat Rev Immunol. 2009;9(2):116–24.
7. Ananieva O, et al. The kinases MSK1 and MSK2 act as negative regulators of Toll-like receptor signaling. Nat Immunol. 2008;9(9):1028–36.
8. Anderson CF, et al. CD4(+)CD25(-)Foxp3(-) Th1 cells are the source of IL-10-mediated immune suppression in chronic cutaneous leishmaniasis. J Exp Med. 2007;204(2):285–97.
9. Anderson CF, et al. IL-27 regulates IL-10 and IL-17 from CD4+ cells in nonhealing Leishmania major infection. J Immunol. 2009;183(7):4619–27.

10. Apetoh L, et al. The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. Nat Immunol. 2010;11(9):854–61.
11. Awasthi A, et al. A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. Nat Immunol. 2007;8(12):1380–9.
12. Banerjee A, et al. Diverse Toll-like receptors utilize Tpl2 to activate extracellular signal-regulated kinase (ERK) in hemopoietic cells. Proc Natl Acad Sci U S A. 2006;103(9):3274–9.
13. Barthlott T, et al. CD25+ CD4+ T cells compete with naive CD4+ T cells for IL-2 and exploit it for the induction of IL-10 production. Int Immunol. 2005;17(3):279–88.
14. Batten M, et al. Cutting edge: IL-27 is a potent inducer of IL-10 but not FoxP3 in murine T cells. J Immunol. 2008;180(5):2752–6.
15. Bauquet AT, et al. The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and TH-17 cells. Nat Immunol. 2009;10(2):167–75.
16. Berg DJ, et al. Interleukin-10 is a central regulator of the response to LPS in murine models of endotoxic shock and the Shwartzman reaction but not endotoxin tolerance. J Clin Invest. 1995;96(5):2339–47.
17. Blokzijl A, Dijke Ten P, Ibáñez CF. Physical and functional interaction between GATA-3 and Smad3 allows TGF-beta regulation of GATA target genes. Curr Biol. 2002;12(1):35–45.
18. Boonstra A, et al. Macrophages and myeloid dendritic cells, but not plasmacytoid dendritic cells, produce IL-10 in response to MyD88- and TRIF-dependent TLR signals, and TLR-independent signals. J Immunol. 2006;177(11):7551–8.
19. Brewer G, et al. Increased interleukin-10 mRNA stability in melanoma cells is associated with decreased levels of A + U-rich element binding factor AUF1. J Interf Cytokine Res. 2003;23(10):553–64.
20. Brightbill HD, et al. A prominent role for Sp1 during lipopolysaccharide-mediated induction of the IL-10 promoter in macrophages. J Immunol. 2000;164(4):1940–51.
21. Brown CY, et al. Differential regulation of the stability of cytokine mRNAs in lipopolysaccharide-activated blood monocytes in response to interleukin-10. J Biol Chem. 1996;271(33):20108–12.
22. Cao S, et al. NF-kappaB1 (p50) homodimers differentially regulate pro- and anti-inflammatory cytokines in macrophages. J Biol Chem. 2006;281(36):26041–50.
23. Cao S, et al. The protooncogene c-Maf is an essential transcription factor for IL-10 gene expression in macrophages. J Immunol. 2005;174(6):3484–92.
24. Carballo E, Lai WS, Blackshear PJ. Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability. Blood. 2000;95(6):1891–9.
25. Carballo E, Lai WS, Blackshear PJ. Feedback inhibition of macrophage tumor necrosis factor-alpha production by tristetraprolin. Science. 1998;281(5379):1001–5.
26. Chakrabarti A, et al. Protein kinase R-dependent regulation of interleukin-10 in response to double-stranded RNA. J Biol Chem. 2008;283(37):25132–9.
27. Chang EY, et al. Cutting edge: involvement of the type I IFN production and signaling pathway in lipopolysaccharide-induced IL-10 production. J Immunol. 2007;178(11):6705–9.
28. Chang H-D, et al. Expression of IL-10 in Th memory lymphocytes is conditional on IL-12 or IL-4, unless the IL-10 gene is imprinted by GATA-3. Eur J Immunol. 2007;37(3):807–17.
29. Chaudhry A, et al. Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. Immunity. 2011;34(4):566–78.
30. Chi H, et al. Dynamic regulation of pro- and anti-inflammatory cytokines by MAPK phosphatase 1 (MKP-1) in innate immune responses. Proc Natl Acad Sci U S A. 2006;103(7):2274–9.
31. Ciofani M, et al. A validated regulatory network for Th17 cell specification. Cell. 2012;151(2):289–303.

32. Corre B, et al. Type I interferon potentiates T-cell receptor mediated induction of IL-10-producing CD4+ T cells. Eur J Immunol. 2013;43(10):2730–40.
33. Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. J Immunol. 2008;180(9):5771–7.
34. Creery WD, et al. Differential modulation of B7-1 and B7-2 isoform expression on human monocytes by cytokines which influence the development of T helper cell phenotype. Eur J Immunol. 1996;26(6):1273–7.
35. Cretney, E. et al. The transcription factors Blimp-1 and IRF4 jointly control the differentiation and function of effector regulatory T cells. Nat Immunol. 2011;12(4):304–11.
36. Cretney E, Kallies A, Nutt SL. Differentiation and function of Foxp3(+) effector regulatory T cells. Trends Immunol. 2012;34(2):74–80.
37. D'Addario M, Arora PD, McCulloch CA. Role of p38 in stress activation of Sp1. Gene. 2006;379:51–61.
38. D'Andrea A, et al. Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. J Exp Med. 1993;178(3):1041–8.
39. Dardalhon V, et al. IL-4 inhibits TGF-beta-induced Foxp3+ T cells, and together with TGF-beta, generates IL-9+ IL-10+ Foxp3(-) effector T cells. Nat Immunol. 2008;9(12):1347–55.
40. de Waal-Malefyt R, Abrams J, et al. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med. 1991;174(5):1209–20.
41. de Waal-Malefyt R, Haanen J, et al. Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. J Exp Med. 1991;174(4):915–24.
42. Dennehy KM, et al. Reciprocal regulation of IL-23 and IL-12 following co-activation of Dectin-1 and TLR signaling pathways. Eur J Immunol. 2009;39(5):1379–86.
43. Denning TL, et al. Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. Nat Immunol. 2007;8(10):1086–94.
44. Dillon S, et al. A Toll-like receptor 2 ligand stimulates Th2 responses in vivo, via induction of extracellular signal-regulated kinase mitogen-activated protein kinase and c-Fos in dendritic cells. J Immunol. 2004;172(8):4733–43.
45. Dillon S, et al. Yeast zymosan, a stimulus for TLR2 and dectin-1, induces regulatory antigen-presenting cells and immunological tolerance. J Clin Invest. 2006;116(4):916–28.
46. Dong C, Davis R, Flavell R. Map kinases in the immune response. Annu Rev Immunol. 2002;20:55–72.
47. Edwards AD, et al. Microbial recognition via Toll-like receptor-dependent and -independent pathways determines the cytokine response of murine dendritic cell subsets to CD40 triggering. J Immunol. 2002;169(7):3652–60.
48. Eidenschenk C, et al. Role of IL-22 in microbial host defense. Curr Top Microbiol Immunol. 2014;380(Chapter 10):213–36.
49. Elcombe SE, et al. Dectin-1 regulates IL-10 production via a MSK1/2 and CREB dependent pathway and promotes the induction of regulatory macrophage markers. R. Lang, ed. PLoS One. 2013;8(3):e60086.
50. Finbloom DS, Winestock KD. IL-10 induces the tyrosine phosphorylation of tyk2 and Jak1 and the differential assembly of STAT1 alpha and STAT3 complexes in human T cells and monocytes. J Immunol. 1995;155(3):1079–90.
51. Fiorentino DF, et al. IL-10 inhibits cytokine production by activated macrophages. J Immunol. 1991;147(11):3815–22.
52. Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med. 1989;170(6):2081–95.
53. Fitzgerald DC, et al. Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. Nat Immunol. 2007;8(12):1372–9.

54. Foey AD, et al. Regulation of monocyte IL-10 synthesis by endogenous IL-1 and TNF-alpha: role of the p38 and p42/44 mitogen-activated protein kinases. J Immunol. 1998;160(2):920–8.
55. Freitas do Rosário AP, et al. IL-27 promotes IL-10 production by effector Th1 CD4+ T cells: a critical mechanism for protection from severe immunopathology during malaria infection. J Immunol. 2012;188(3):1178–90.
56. Fujita S, et al. Regulatory dendritic cells act as regulators of acute lethal systemic inflammatory response. Blood. 2006;107(9):3656–64.
57. Gaba A, et al. Cutting edge: IL-10-mediated tristetraprolin induction is part of a feedback loop that controls macrophage STAT3 activation and cytokine production. J Immunol. 2012;189(5):2089–93.
58. Gandhi R, et al. Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell-like and Foxp3(+) regulatory T cells. Nat Immunol. 2010;11(9):846–53.
59. Geijtenbeek TBH, et al. Mycobacteria target DC-SIGN to suppress dendritic cell function. J Exp Med. 2003;197(1):7–17.
60. Gerosa F, et al. Interleukin-12 primes human CD4 and CD8 T cell clones for high production of both interferon-gamma and interleukin-10. J Exp Med. 1996;183(6):2559–69.
61. Ghoreschi K, et al. Generation of pathogenic T(H) 17 cells in the absence of TGF-β signalling. Nature. 2010;467(7318):967–71.
62. Giordani L, et al. IFN-alpha amplifies human naive B cell TLR-9-mediated activation and Ig production. J Leukoc Biol. 2009;86(2):261–71.
63. Glasmacher E, et al. A genomic regulatory element that directs assembly and function of immune-specific AP-1-IRF complexes. Science. 2012;338(6109):975–80.
64. Glocker E-O, et al. IL-10 and IL-10 receptor defects in humans. Ann N Y Acad Sci. 2011;1246:102–7.
65. Groux H, et al. Interleukin-10 induces a long-term antigen-specific anergic state in human CD4+ T cells. J Exp Med. 1996;184(1):19–29.
66. Guarda G, et al. Type I interferon inhibits interleukin-1 production and inflammasome activation. Immunity. 2011;34(2):213–23.
67. Hadis U, et al. Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. Immunity. 2011;34(2):237–46.
68. Häcker H, et al. Specificity in Toll-like receptor signalling through distinct effector functions of TRAF3 and TRAF6. Nature. 2006;439(7073):204–7.
69. Higgins SC, et al. Toll-like receptor 4-mediated innate IL-10 activates antigen-specific regulatory T cells and confers resistance to Bordetella pertussis by inhibiting inflammatory pathology. J Immunol. 2003;171(6):3119–27.
70. Ho AS, et al. Functional regions of the mouse interleukin-10 receptor cytoplasmic domain. Mol Cell Biol. 1995;15(9):5043–53.
71. Hoshi N, et al. MyD88 signalling in colonic mononuclear phagocytes drives colitis in IL-10-deficient mice. Nat Commun. 2012;3:1120.
72. Hu X, et al. IFN-gamma suppresses IL-10 production and synergizes with TLR2 by regulating GSK3 and CREB/AP-1 proteins. Immunity. 2006;24(5):563–74.
73. Hunter CA, Kastelein R. Interleukin-27: balancing protective and pathological immunity. Immunity. 2012;37(6):960–9.
74. Im S-H, et al. Chromatin-level regulation of the IL10 gene in T cells. J Biol Chem. 2004;279(45):46818–25.
75. Ivanov P, Anderson P. Post-transcriptional regulatory networks in immunity. Immunol Rev. 2013;253(1):253–72.
76. Iwasaki Y, et al. Egr-2 transcription factor is required for Blimp-1 mediated IL-10 production in IL-27 stimulated CD4(+) T cells. Eur J Immunol. 2013;43(4):1063–73.
77. Jankovic D, et al. Conventional T-bet(+)Foxp3(−) Th1 cells are the major source of host-protective regulatory IL-10 during intracellular protozoan infection. J Exp Med. 2007;204(2):273–83.

78. Jarnicki AG, et al. Attenuating regulatory T cell induction by TLR agonists through inhibition of p38 MAPK signaling in dendritic cells enhances their efficacy as vaccine adjuvants and cancer immunotherapeutics. J Immunol. 2008;180(6):3797–806.
79. Jones EA, Flavell RA. Distal enhancer elements transcribe intergenic RNA in the IL-10 family gene cluster. J Immunol. 2005;175(11):7437–46.
80. Kaiser F, et al. TPL-2 negatively regulates interferon-beta production in macrophages and myeloid dendritic cells. J Exp Med. 2009;206(9):1863–71.
81. Kallies A, Hawkins E, Belz G, Metcalf D, Hommel M, Corcoran L, Hodgkin P, Nutt S. Transcriptional repressor Blimp-1 is essential for T cell homeostasis and self-tolerance. Nat Immunol. 2006;7(5):466–74.
82. Kanters E, et al. Inhibition of NF-kappaB activation in macrophages increases atherosclerosis in LDL receptor-deficient mice. J Clin Invest. 2003;112(8):1176–85.
83. Kassner N, et al. Cutting edge: plasmacytoid dendritic cells induce IL-10 production in T cells via the delta-like-4/Notch axis. J Immunol. 2010;184(2):550–4.
84. Kastelein RA, Hunter CA, Cua DJ. Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. Annu Rev Immunol. 2007;25:221–42.
85. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol. 2010;11(5):373–84.
86. Kim C, et al. The kinase p38 alpha serves cell type-specific inflammatory functions in skin injury and coordinates pro- and anti-inflammatory gene expression. Nat Immunol. 2008;9(9):1019–27.
87. Kim JI, et al. The transcription factor c-Maf controls the production of interleukin-4 but not other Th2 cytokines. Immunity. 1999;10(6):745–51.
88. Kim JM, et al. Structure of the mouse IL-10 gene and chromosomal localization of the mouse and human genes. J Immunol. 1992;148(11):3618–23.
89. Kim L, et al. p38 MAPK autophosphorylation drives macrophage IL-12 production during intracellular infection. J Immunol. 2005;174(7):4178–84.
90. Kimura A, et al. Aryl hydrocarbon receptor in combination with Stat1 regulates LPS-induced inflammatory responses. J Exp Med. 2009;206(9):2027–35.
91. Kitani A, et al. Transforming growth factor (TGF)-beta1-producing regulatory T cells induce Smad-mediated interleukin 10 secretion that facilitates coordinated immunoregulatory activity and amelioration of TGF-beta1-mediated fibrosis. J Exp Med. 2003;198(8):1179–88.
92. Kotenko SV, et al. Identification and functional characterization of a second chain of the interleukin-10 receptor complex. EMBO J. 1997;16(19):5894–903.
93. Kratochvill F, et al. Tristetraprolin-driven regulatory circuit controls quality and timing of mRNA decay in inflammation. Mol Syst Biol. 2011;7(1):560.
94. Krausgruber T, et al. IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. Nat Immunol. 2011;12(3):231–8.
95. Kühn R, et al. Interleukin-10-deficient mice develop chronic enterocolitis. Cell. 1993;75(2):263–74.
96. de La Rosa M, et al. Interleukin-2 is essential for CD4+CD25+ regulatory T cell function. European J Immunol. 2004;34(9):2480–8.
97. Lampropoulou V, et al. TLR-activated B cells suppress T cell-mediated autoimmunity. J Immunol. 2008;180(7):4763–73.
98. Lee C-G, et al. A distal cis-regulatory element, CNS-9, controls NFAT1 and IRF4-mediated IL-10 gene activation in T helper cells. Mol Immunol. 2009;46(4):613–21.
99. Lee C-G, et al. Interaction of Ets-1 with HDAC1 represses IL-10 expression in Th1 cells. J Immunol. 2012;188(5):2244–53.
100. Levings MK, et al. IFN-alpha and IL-10 induce the differentiation of human type 1 T regulatory cells. J Immunol. 2001;166(9):5530–9.
101. Li MO, Flavell RA. Contextual regulation of inflammation: a duet by transforming growth factor-beta and interleukin-10. Immunity. 2008;28(4):468–76.

102. Li P, et al. BATF-JUN is critical for IRF4-mediated transcription in T cells. Nature. 2012;490(7421):543–6.
103. Liu Y-W, et al. Lipopolysaccharide-induced transcriptional activation of interleukin-10 is mediated by MAPK- and NF-kappaB-induced CCAAT/enhancer-binding protein delta in mouse macrophages. Cell Signal. 2006;18(9):1492–500.
104. Lucas M, et al. ERK activation following macrophage FcgammaR ligation leads to chromatin modifications at the IL-10 locus. J Immunol. 2005;175(1):469–77.
105. Ma F, et al. MicroRNA-466l upregulates IL-10 expression in TLR-triggered macrophages by antagonizing RNA-binding protein tristetraprolin-mediated IL-10 mRNA degradation. J Immunol. 2010;184(11):6053–9.
106. Ma W, et al. The p38 mitogen-activated kinase pathway regulates the human interleukin-10 promoter via the activation of Sp1 transcription factor in lipopolysaccharide-stimulated human macrophages. J Biol Chem. 2001;276(17):13664–74.
107. Macian F. NFAT proteins: key regulators of T-cell development and function. Nat Rev Immunol. 2005;5(6):472–84.
108. Madan R, et al. Nonredundant roles for B cell-derived IL-10 in immune counter-regulation. J Immunol. 2009;183(4):2312–20.
109. Martin M, et al. Role of the phosphatidylinositol 3 kinase-Akt pathway in the regulation of IL-10 and IL-12 by Porphyromonas gingivalis lipopolysaccharide. J Immunol. 2003;171(2):717–25.
110. Martins G, Calame K. Regulation and functions of Blimp-1 in T and B lymphocytes. How TCRS bind MHCS, peptides, and coreceptors. Annu Rev Immunol. 2008;26:133–169.
111. Martins GA, et al. Transcriptional repressor Blimp-1 regulates T cell homeostasis and function. Nat Immunol. 2006;7(5):457–65.
112. Mayer-Barber KD, et al. Innate and adaptive interferons suppress IL-1α and IL-1β production by distinct pulmonary myeloid subsets during Mycobacterium tuberculosis infection. Immunity. 2011;35(6):1023–34.
113. Maynard C, Weaver C. Diversity in the contribution of interleukin-10 to T-cell-mediated immune regulation. Immunol Rev. 2008;226:219–33.
114. Maynard CL, et al. Regulatory T cells expressing interleukin 10 develop from Foxp3+ and Foxp3- precursor cells in the absence of interleukin 10. Nat Immunol. 2007;8(9):931–41.
115. McGeachy MJ, et al. 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. 2007;8(12):1390–7.
116. McNab FW, et al. TPL-2-ERK1/2 signaling promotes host resistance against intracellular bacterial infection by negative regulation of type I IFN production. J Immunol. 2013;191(4):1732–43.
117. Meraz MA, et al. Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. Cell. 1996;84(3):431–42.
118. Meyaard L, et al. IL-12-induced IL-10 production by human T cells as a negative feedback for IL-12-induced immune responses. J Immunol. 1996;156(8):2776–82.
119. Moore K et al. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001;19:683–765.
120. Moore KW, et al. Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein-Barr virus gene BCRFI. Science. 1990;248(4960):1230–4.
121. Motomura Y, et al. The transcription factor E4BP4 regulates the production of IL-10 and IL-13 in CD4+ T cells. Nat Immunol. 2011;12(5):450–9.
122. Murai M, et al. Interleukin 10 acts on regulatory T cells to maintain expression of the transcription factor Foxp3 and suppressive function in mice with colitis. Nat Immunol. 2009;10(11):1178–84.
123. Murphy TL, Tussiwand R, Murphy KM. Specificity through cooperation: BATF-IRF interactions control immune-regulatory networks. Nat Rev Immunol. 2013;13(7):499–509.
124. Murugaiyan G, et al. IL-27 is a key regulator of IL-10 and IL-17 production by human CD4+ T cells. J Immunol. 2009;183(4):2435–43.

125. Nandan D, et al. Myeloid cell IL-10 production in response to leishmania involves inactivation of glycogen synthase kinase-3β downstream of phosphatidylinositol-3 kinase. J Immunol. 2012;188(1):367–78.
126. Neininger A, et al. MK2 targets AU-rich elements and regulates biosynthesis of tumor necrosis factor and interleukin-6 independently at different post-transcriptional levels. J Biol Chem. 2002;277(5):3065–8.
127. Netea MG, et al. Toll-like receptor 2 suppresses immunity against Candida albicans through induction of IL-10 and regulatory T cells. J Immunol. 2004;172(6):3712–8.
128. Neumann C, et al. Role of Blimp-1 in programing Th effector cells into IL-10 producers. J Exp Med. 2014;204(2):285.
129. O'Garra A, Vieira P. T(H)1 cells control themselves by producing interleukin-10. Nat Rev Immunol. 2007;7(6):425–8.
130. Ohtani M, et al. Mammalian target of rapamycin and glycogen synthase kinase 3 differentially regulate lipopolysaccharide-induced interleukin-12 production in dendritic cells. Blood. 2008;112(3):635–43.
131. Ouyang W, et al. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annu Rev Immunol. 2011;29(1):71–109.
132. Pattison MJ, MacKenzie KF, Arthur JSC. Inhibition of JAKs in macrophages increases lipopolysaccharide-induced cytokine production by blocking IL-10-mediated feedback. J Immunol. 2012;189(6):2784–92.
133. Platzer C, et al. Cyclic adenosine monophosphate-responsive elements are involved in the transcriptional activation of the human IL-10 gene in monocytic cells. Eur J Immunol. 1999;29(10):3098–104.
134. Pot C, et al. 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. 2009;183(2):797–801.
135. Pot C, et al. Induction of regulatory Tr1 cells and inhibition of T(H)17 cells by IL-27. Semin Immunol. 2011;23(6):438–45.
136. Powell MJ, et al. Posttranscriptional regulation of IL-10 gene expression through sequences in the 3′-untranslated region. J Immunol. 2000;165(1):292–6.
137. Qin Z, et al. Pivotal advance: Kaposi's sarcoma-associated herpesvirus (KSHV)-encoded microRNA specifically induce IL-6 and IL-10 secretion by macrophages and monocytes. J Leukoc Biol. 2010;87(1):25–34.
138. Quinn SR, O'Neill LA. The role of microRNAs in the control and mechanism of action of IL-10. Curr Top Microbiol Immunol. 2014;380(Chapter 7):145–55.
139. Radtke F, Macdonald HR, Tacchini-Cottier F. Regulation of innate and adaptive immunity by Notch. Nat Rev Immunol. 2013;13(6):427–37.
140. Rakoff-Nahoum S, Hao L, Medzhitov R. Role of toll-like receptors in spontaneous commensal-dependent colitis. Immunity. 2006;25(2):319–29.
141. Risco A, et al. p38γ and p38δ kinases regulate the Toll-like receptor 4 (TLR4)-induced cytokine production by controlling ERK1/2 protein kinase pathway activation. Proc Natl Acad Sci U S A. 2012;109(28):11200–5.
142. Roers A, et al. T cell-specific inactivation of the interleukin 10 gene in mice results in enhanced T cell responses but normal innate responses to lipopolysaccharide or skin irritation. J Exp Med. 2004;200(10):1289–97.
143. Rogers NC, et al. Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. Immunity. 2005;22(4):507–17.
144. Roncarolo MG, et al. Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. Immunol Rev. 2006;212:28–50.
145. Rubtsov YP, et al. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity. 2008;28(4):546–58.
146. Rutz S, Ouyang W. Regulation of interleukin-10 and interleukin-22 expression in T helper cells. Curr Opin Immunol. 2011;23(5):605–12.

147. Rutz S, et al. Notch regulates IL-10 production by T helper 1 cells. Proc Natl Acad Sci U S A. 2008;105(9):3497–502.
148. Rutz S, et al. Transcription factor c-Maf mediates the TGF-β-dependent suppression of IL-22 production in T(H)17 cells. Nat Immunol. 2011;12(12):1238–45.
149. Rutz S, Wang X, Ouyang W. The IL-20 subfamily of cytokines – from host defence to tissue homeostasis. Nat Rev Immunol. 2014;14(12):783–95.
150. Salehi S, et al. B lymphocyte-induced maturation protein-1 contributes to intestinal mucosa homeostasis by limiting the number of IL-17-producing CD4+ T cells. J Immunol. 2012;189(12):5682–93.
151. Salojin KV, et al. Essential role of MAPK phosphatase-1 in the negative control of innate immune responses. J Immunol. 2006;176(3):1899–907.
152. Sandler H, Stoecklin G. Control of mRNA decay by phosphorylation of tristetraprolin. Biochem Soc Trans. 2008;36(Pt 3):491–6.
153. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. Nat Rev Immunol. 2010;10(3):170–81.
154. Saraiva M, et al. Identification of a macrophage-specific chromatin signature in the IL-10 locus. J Immunol. 2005;175(2):1041–6.
155. Saraiva M, et al. Interleukin-10 production by Th1 cells requires interleukin-12-induced STAT4 transcription factor and ERK MAP kinase activation by high antigen dose. Immunity. 2009;31(2):209–19.
156. Sayi A, et al. TLR-2-activated B cells suppress Helicobacter-induced preneoplastic gastric immunopathology by inducing T regulatory-1 cells. J Immunol. 2011;186(2):878–90.
157. Schaljo B, et al. Tristetraprolin is required for full anti-inflammatory response of murine macrophages to IL-10. J Immunol. 2009;183(2):1197–206.
158. Schuetze N, et al. IL-12 family members: differential kinetics of their TLR4-mediated induction by Salmonella enteritidis and the impact of IL-10 in bone marrow-derived macrophages. Int Immunol. 2005;17(5):649–59.
159. Schulz EG, et al. Sequential polarization and imprinting of type 1 T helper lymphocytes by interferon-gamma and interleukin-12. Immunity. 2009;30(5):673–83.
160. Sellon RK, et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun. 1998;66(11):5224–31.
161. Sharma A, et al. Posttranscriptional regulation of interleukin-10 expression by hsa-miR-106a. Proc Natl Acad Sci U S A. 2009;106(14):5761–6.
162. Shaw MH, et al. Tyk2 negatively regulates adaptive Th1 immunity by mediating IL-10 signaling and promoting IFN-gamma-dependent IL-10 reactivation. J Immunol. 2006;176(12):7263–71.
163. Sheedy FJ, et al. Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. Nat Immunol. 2010;11(2):141–7.
164. Shoemaker J, Saraiva M, O'Garra A. GATA-3 directly remodels the IL-10 locus independently of IL-4 in CD4+ T cells. J Immunol. 2006;176(6):3470–9.
165. Shouval DS, et al. Interleukin-10 receptor signaling in innate immune cells regulates mucosal immune tolerance and anti-inflammatory macrophage function. Immunity. 2014;40(5):706–19.
166. Siewe L, et al. Interleukin-10 derived from macrophages and/or neutrophils regulates the inflammatory response to LPS but not the response to CpG DNA. Eur J Immunol. 2006;36(12):3248–55.
167. Skok J, Poudrier J, Gray D. Dendritic cell-derived IL-12 promotes B cell induction of Th2 differentiation: a feedback regulation of Th1 development. J Immunol. 1999;163(8):4284–91.
168. Slack EC, et al. Syk-dependent ERK activation regulates IL-2 and IL-10 production by DC stimulated with zymosan. Eur J Immunol. 2007;37(6):1600–12.

169. Spolski R, et al. IL-21 mediates suppressive effects via its induction of IL-10. J Immunol. 2009;182(5):2859–67.
170. Staples KJ, et al. IL-10 induces IL-10 in primary human monocyte-derived macrophages via the transcription factor Stat3. J Immunol. 2007;178(8):4779–85.
171. Stoecklin G, et al. Genome-wide analysis identifies interleukin-10 mRNA as target of tristetraprolin. J Biol Chem. 2008;283(17):11689–99.
172. Stumhofer JS, et al. Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system. Nat Immunol. 2006;7(9):937–45.
173. Stumhofer JS, et al. Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. Nat Immunol. 2007;8(12):1363–71.
174. Sun J, et al. CD4+ T cell help and innate-derived IL-27 induce Blimp-1-dependent IL-10 production by antiviral CTLs. Nat Immunol. 2011;12(4):327–34.
175. Takeda K, et al. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. Immunity. 1999;10(1):39–49.
176. Tan JC, et al. Characterization of interleukin-10 receptors on human and mouse cells. J Biol Chem. 1993;268(28):21053–9.
177. Tan NY, Khachigian LM. Sp1 phosphorylation and its regulation of gene transcription. Mol Cell Biol. 2009;29(10):2483–8.
178. Tone M, et al. IL-10 gene expression is controlled by the transcription factors Sp1 and Sp3. J Immunol. 2000;165(1):286–91.
179. Trandem K, et al. Highly activated cytotoxic CD8 T cells express protective IL-10 at the peak of coronavirus-induced encephalitis. J Immunol. 2011;186(6):3642–52.
180. Trinchieri G. Interleukin-10 production by effector T cells: Th1 cells show self control. J Exp Med. 2007;204(2):239–43.
181. Tudor C, et al. The p38 MAPK pathway inhibits tristetraprolin-directed decay of interleukin-10 and pro-inflammatory mediator mRNAs in murine macrophages. FEBS Lett. 2009;583(12):1933–8.
182. Tussiwand R, et al. Compensatory dendritic cell development mediated by BATF-IRF interactions. Nature. 2012;490(7421):502–7.
183. Uhlig HH, et al. Characterization of Foxp3+CD4+CD25+ and IL-10-secreting CD4+CD25+ T cells during cure of colitis. J Immunol. 2006;177(9):5852–60.
184. Unterberger C, et al. Role of STAT3 in glucocorticoid-induced expression of the human IL-10 gene. Mol Immunol. 2008;45(11):3230–7.
185. Vahedi G, et al. STATs shape the active enhancer landscape of T cell populations. Cell. 2012;151(5):981–93.
186. Vasanthakumar A, Kallies A. IL-27 paves different roads to Tr1. Eur J Immunol. 2013;43(4):882–5.
187. Veldhoen M, et al. Transforming growth factor-beta "reprograms" the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. Nat Immunol. 2008;9(12):1341–6.
188. Villagra A, et al. The histone deacetylase HDAC11 regulates the expression of interleukin 10 and immune tolerance. Nat Immunol. 2009;10(1):92–100.
189. Villarino A, et al. The IL-27R (WSX-1) is required to suppress T cell hyperactivity during infection. Immunity. 2003;19(5):645–55.
190. Voll RE, et al. Immunosuppressive effects of apoptotic cells. Nature. 1997;390(6658):350–1.
191. Wang Z-Y, et al. Regulation of IL-10 gene expression in Th2 cells by Jun proteins. J Immunol. 2005;174(4):2098–105.
192. Wei L, et al. Discrete roles of STAT4 and STAT6 transcription factors in tuning epigenetic modifications and transcription during T helper cell differentiation. Immunity. 2010;32(6):840–51.

193. Weichhart T, et al. The TSC-mTOR signaling pathway regulates the innate inflammatory response. Immunity. 2008;29(4):565–77.
194. Willems F, et al. Interleukin-10 inhibits B7 and intercellular adhesion molecule-1 expression on human monocytes. Eur J Immunol. 1994;24(4):1007–9.
195. Xu J, et al. c-Maf regulates IL-10 expression during Th17 polarization. J Immunol. 2009;182(10):6226–36.
196. Yi A-K, et al. Role of mitogen-activated protein kinases in CpG DNA-mediated IL-10 and IL-12 production: central role of extracellular signal-regulated kinase in the negative feedback loop of the CpG DNA-mediated Th1 response. J Immunol. 2002;168(9):4711–20.
197. Yu H, et al. MKP-1 regulates cytokine mRNA stability through selectively modulation subcellular translocation of AUF1. Cytokine. 2011;56(2):245–55.
198. Zhang X, et al. Type I interferons protect neonates from acute inflammation through interleukin 10-producing B cells. J Exp Med. 2007;204(5):1107–18.
199. Zhang X, Edwards J, Mosser D. Dynamic and transient remodeling of the macrophage IL-10 promoter during transcription. J Immunol. 2006;177(2):1282–8.
200. Zhu J et al. Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. Nat Immunol. 2004;5(11):1157–65.
201. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations (*). Annu Rev Immunol. 2010;28:445–89.
202. Ziegler-Heitbrock L, et al. IFN-alpha induces the human IL-10 gene by recruiting both IFN regulatory factor 1 and Stat3. J Immunol. 2003;171(1):285–90.
203. Zigmond E, et al. Macrophage-restricted interleukin-10 receptor deficiency, but not IL-10 deficiency, causes severe spontaneous colitis. Immunity. 2014;40(5):720–33.