The regulation of IL-10 production by immune cells

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Abstract | Interleukin-10 (IL-10), a cytokine with anti-inflammatory properties, has a central role in infection by limiting the immune response to pathogens and thereby preventing damage to the host. Recently, an increasing interest in how *IL10* expression is regulated in different immune cells has revealed some of the molecular mechanisms involved at the levels of signal transduction, epigenetics, transcription factor binding and gene activation. Understanding the specific molecular events that regulate the production of IL-10 will help to answer the remaining questions that are important for the design of new strategies of immune intervention.

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The immune response has evolved to protect the host from a wide range of potentially pathogenic microorganisms, but parallel mechanisms to control overexuberant immune responses and prevent reactivity to self are required to limit host damage. Interleukin-10 (IL-10) is an anti-inflammatory cytokine with a crucial role in preventing inflammatory and autoimmune pathologies[1–3]. IL-10-deficient mice[4] develop inflammatory bowel disease following colonization of the gut with particular microorganisms[5] (BOX 1) and show other exaggerated inflammatory responses to microbial challenge. Although the absence of IL-10 leads to better clearance of some pathogens with no enhanced immunopathology[6,7], during other infections the absence of IL-10 can be accompanied by an immunopathology that is detrimental to the host but does not necessarily affect the pathogen load[3,8–11]. This suggests that an absence of IL-10 is not always compensated by other regulatory mechanisms and thus that there is a non-redundant role for IL-10 in limiting inflammatory responses *in vivo*.

To inhibit inflammatory pathologies, IL-10 functions at different stages of an immune response and possibly at different anatomical locations. IL-10 was initially described as a T helper 2 (T_H2)-type cytokine[12], but further evidence suggested that the production of IL-10 was associated with tolerant or regulatory T (T_{Reg}) cell responses[3,13,14]. It is now known that the expression of IL-10 is not specific to T_H2 cells or T_{Reg} cells but instead that it is a much more broadly expressed cytokine (FIG. 1). IL-10 is expressed by many cells of the adaptive immune system, including T_H1, T_H2 and T_H17 cell subsets, T_{Reg} cells, $CD8^+$ T cells and B cells (reviewed in REFS 3,10,11,14–16). It is also expressed by cells of the innate immune system, including dendritic cells (DCs), macrophages, mast cells, natural killer (NK) cells, eosinophils and neutrophils[3] (FIG. 1). Thus, IL-10 production seems to be associated with many immune cells, affirming its crucial role as a feedback regulator of diverse immune responses, not only T_H1 cell responses[10,11] but also T_H2 cell responses to schistosome parasites[17], *Aspergillus* spp.[18] and allergens[19] (reviewed in REF. 1).

Much is known about the function of IL-10. For example, the induction of the anti-inflammatory response mediated through the IL-10 receptor (IL-10R) and activation of signal transducer and activator of transcription 3 (STAT3) is reviewed in REFS 3,20. By acting on DCs and macrophages, IL-10 inhibits the development of T_H1-type responses (reviewed in REF. 3) but also leads to the suppression of T_H2 cell and allergic responses (reviewed in REF. 1). In addition to an autocrine inhibitory effect of IL-10 on macrophages and DCs, and because IL-10 can be produced by T_H1, T_H2 and T_H17 cells, an additional feedback loop exists to limit the innate effector functions of macrophages and DCs and their subsequent activation of T cells. However, IL-10 enhances the differentiation of IL-10-secreting T_{Reg} cells, thus providing a positive regulatory loop for its induction[21] (reviewed in REFS 1,14). In some situations, IL-10 also activates mast cells and enhances the functions of $CD8^+$ T cells, NK cells and B cells (reviewed in REFS 2,3), although these effects have yet to be tested in infection models.

So, IL-10 is a cytokine with important effects on the development of an immune response. An understanding of how *IL10* expression is regulated in different innate

Box 1 | IL-10 expression and gut homeostasis

The intestine is continuously exposed to bacterial flora, dietary antigens and potential pathogens. To prevent chronic intestinal inflammation, various regulatory lymphocyte populations keep the immune response in check. These populations use several regulatory mechanisms, the best characterized of which involves interleukin-10 (IL-10) and transforming growth factor-β (TGFβ)[15,155]. IL-10- or IL-10 receptor-deficient mice do not develop severe autoimmune disorders but develop colitis in the presence of microorganisms[4,156]. Many studies unequivocally identify $CD4^+$ T cell-derived IL-10 as a key mediator of intestinal immune homeostasis[157–161]. Coeliac disease and inflammatory bowel disease (IBD) are the most common causes of non-infectious intestinal inflammation in humans, with recent reports identifying *IL10* as a susceptibility locus for the development of IBD[162]. Polymorphisms in nucleotide-binding oligomerization domain 2 (*NOD2*) have also been associated with IBD in humans[163], which is interesting considering that NOD2 has been associated with IL-10 production[41]. IL-10 seems to function not directly on T cells, but instead on myeloid cell populations in a similar manner to that observed in the immune response to pathogens[3]. *In vivo* IL-10 production by forkhead box P3 ($FOXP3^+$) regulatory T (T_{Reg}) cells and $FOXP3^-$ regulatory T cells in the gut seems to be mediated by TGFβ, independently of endogenous IL-10 (REF. 97). This IL-10 independence is in contrast to that reported *in vitro* for human IL-10-producing regulatory T cells[14]. Retinoic acid was identified as a cofactor for TGFβ in the induction of $FOXP3^+$ T_{Reg} cells[164–166], although retinoic acid itself downregulates the expression of IL-10 by inducible $FOXP3^-$ regulatory T cells[92]. Although the exact mechanisms of IL-10 induction in the intestine remain elusive, the protective role of intestinal T_{Reg} cells mostly depends on their expression of IL-10, suggesting that local IL-10 expression might be a therapy for IBD[2].

and adaptive immune cells is therefore of importance for the development of immune intervention strategies in various pathologies. Several layers of regulation of IL-10 expression exist, and this is a main focus of this Review. First, regulation of IL-10 production involves changes in the chromatin structure at the *IL10* locus. A second layer of regulation involves the enhancement or silencing of *IL10* transcription and is controlled by specific transcription factors activated by discrete signal-transduction pathways. In addition, post-transcriptional mechanisms exist. Many of the molecular events leading to *IL10* expression are common to various IL-10-producing immune cells. However, there are also cell-specific signals and molecular mechanisms that allow IL-10 production by particular immune cells and not by others.

In this Review, we discuss our current understanding of the regulation of *IL10* expression at the molecular level in different cell types, from signal transduction pathways to epigenetic regulation and the activation of specific transcription factors involved in IL-10 production. Throughout, we highlight the common and distinct mechanisms of IL-10 regulation that exist in different IL-10-producing immune cells.

Chromatin
Composed of nucleosomes, this is the basic repeating unit of eukaryotic genomes. Nucleosomes consist of 146 base pairs of DNA wound around an octamer of histone proteins.

Plasmacytoid DC
A DC that lacks myeloid markers such as CD11c and CD33 but expresses high levels of HLA-DR and CD123. These cells produce high levels of type I interferons in response to viral infection.

IL-10 production by immune cells

Induction by pathogen-derived products. Pathogen activation of DCs and macrophages involves the recognition of pathogen-derived products by pattern recognition receptors (PRRs), which triggers the expression of cytokines and other factors[22]. Both macrophages[23–27] and DCs[26,28–33] can express IL-10 *in vitro* following activation of specific PRRs (FIG. 2a). In addition, DCs[31,34], macrophages[35] and neutrophils[36] have been reported to express IL-10 *in vivo*.

It has been suggested that Toll-like receptor 2 (TLR2) agonists are specialized in inducing IL-10 expression by antigen-presenting cells (APCs)[29,30,37,38]. For example, TLR2 signalling is crucial for the induction of IL-10 production by macrophages (M. Teixeira-Coelho, J. Carmona, A. G. Castro and M.S., unpublished observations) or by DCs[39] stimulated with *Mycobacterium tuberculosis* or with lipopeptides and the LcrV antigen of *Yersinia pestis*[40]. IL-10 production by macrophages following pneumoccocal cell wall stimulation mainly depends on TLR2; however, in this case a role for nucleotide-binding oligomerization domain 2 (NOD2) signalling, independent of TLR2, has also been described[41]. Significant amounts of IL-10 are also produced by macrophages and myeloid DCs following stimulation with TLR4 and TLR9 ligands[26]. Of note, IL-10 production following TLR3 stimulation was only observed in macrophages[26]. Interestingly, activation of macrophages through TLRs results in high levels of IL-10 production, whereas myeloid DCs only produce intermediate amounts and plasmacytoid DCs (pDCs) do not produce detectable levels of IL-10 (REF. 26) (FIG. 1). In addition, IL-10 can be induced by TLR-independent stimuli, such as the C-type lectins DC-specific ICAM3-grabbing non-integrin (DC-SIGN; also known as CLEC4M)[33] and dectin 1 (also known as CLEC7A)[32] (FIG. 2a). Ligation of CD40 enhances IL-10 production by TLR-stimulated[28] or dectin 1-stimulated DCs[32] and ligation of Fc receptors (FcRs) enhances IL-10 production by TLR-stimulated macrophages[25].

Signalling pathways for innate IL-10 production. Following TLR ligation, signalling cascades are activated through Toll/IL-1 receptor (TIR)-domain-containing adaptor molecules, such as myeloid differentiation primary-response protein 88 (MYD88) and TIR-domain-containing adaptor protein inducing IFNβ (TRIF; also known as TICAM1), leading to the production of IL-10 and pro-inflammatory cytokines[26,30,42]. TLR signalling through MYD88 leads to the activation of mitogen-activated protein kinases (MAPKs) and nuclear factor-κB (NF-κB)[43] (FIG. 2a).

Additional signals that are required for IL-10 production by macrophages have also been reported. Of note, optimal lipopolysaccharide (LPS)-induced IL-10 production by macrophages requires both the activation of the TRIF- and MYD88-dependent pathways[26,27] and the production of and signalling by type I interferons (IFNs)[27]. This secondary induction of IL-10 by type I IFNs has important implications for the use of type I IFNs as potential anti-inflammatory drugs. Moreover, this study is in line with the observation that TNFR-associated factor 3 (TRAF3), an important component of the type I IFN production pathway, is also involved in LPS-induced upregulation of IL-10 expression[42].

The MAPK cascade is composed of three major groups of kinases: extracellular signal-regulated kinases (ERKs) (comprising ERK1 (also known as MAPK3) and ERK2 (also known as MAPK1), which are collectively referred to here as ERK); JUN N-terminal kinases

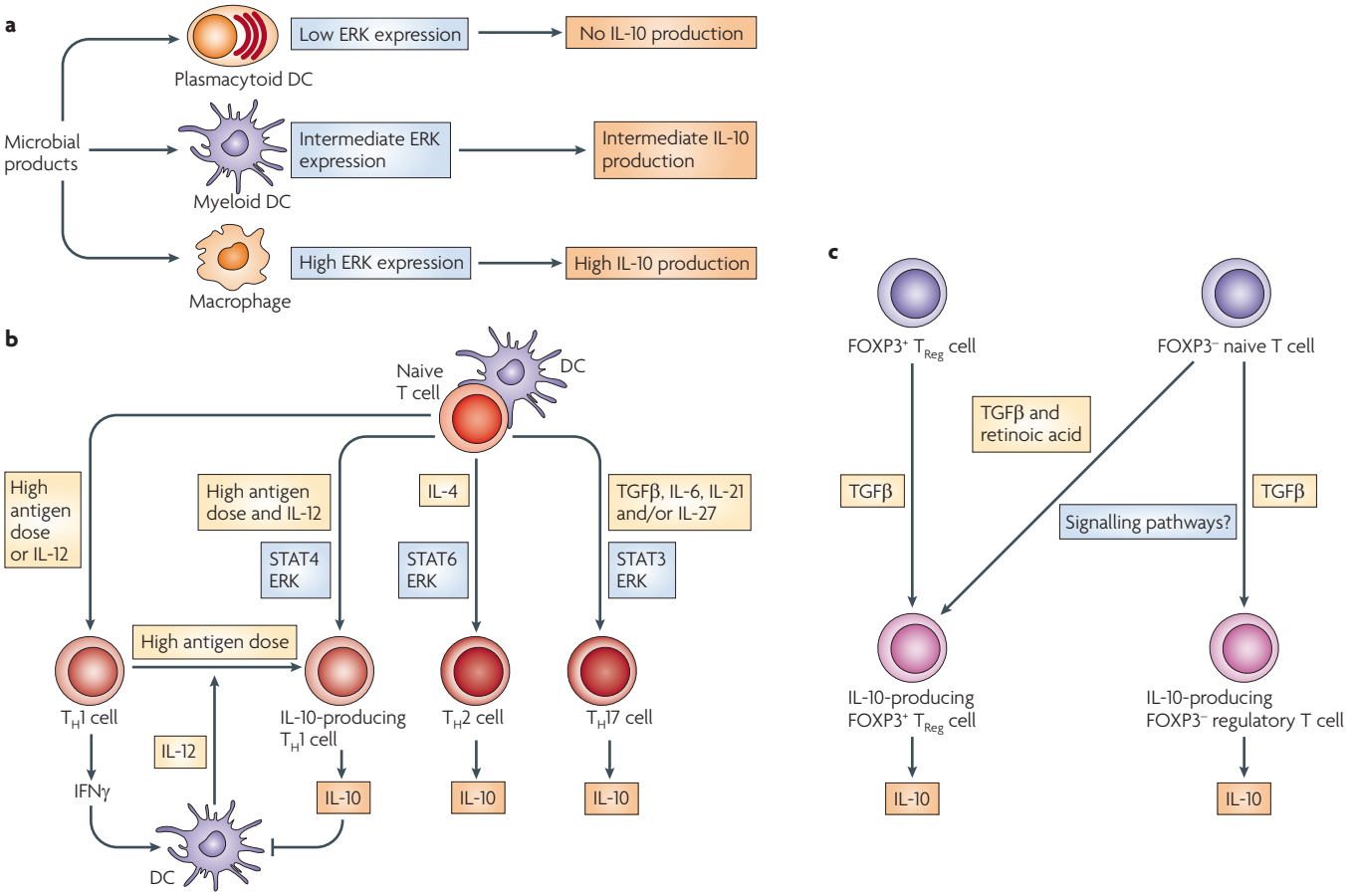

Figure 1 | **Interleukin-10 expression in the immune system. a** | Interleukin-10 (IL-10) is expressed by macrophages and myeloid dendritic cells (DCs), but not by plasmacytoid DCs, in response to microbial products. The extracellular signal-regulated kinase 1 (ERK1) and ERK2 (which are collectively referred to here as ERK) pathway is one of the signalling cascades that is activated in these cells that results in IL-10 expression. For other immune cells, such as B cells, mast cells and eosinophils, the exact signalling pathways that lead to IL-10 production remain elusive. **b** | In T helper (T_H) cells, the expression of IL-10 is accompanied by the expression of the signature cytokines for each subset, with the exception of regulatory T (T_{Reg}) cells, which normally lose the capacity to express other cytokines. Although the differentiation of T_H cells from naive $CD4^+$ T cells requires T cell receptor triggering and the activation of distinct signal transducer and activator of transcription (STAT) pathways, activation of the ERK pathway is a common requirement for IL-10 expression by these cells. High doses of antigen presented by DCs to naive T cells or IL-12 favours the development of T_H1 cells, which produce interferon-γ (IFNγ). IL-10-producing T_H1 cells require high antigen dose and IL-12 and STAT4 signalling for the expression of maximum levels of IL-10 following re-stimulation. In T_H2 cells, IL-4 and STAT6 signalling pathways are required for IL-10 expression. Induction of IL-10-producing T_H17 cells is not well understood, but transforming growth factor-β (TGFβ), IL-6, IL-21 and/or IL-27 and STAT3 signalling are likely to be involved. **c** | TGFβ can induce the production of IL-10 by forkhead box P3 (FOXP3)$^+$ T_{Reg} cells and this cytokine can also promote the development of IL-10-producing FOXP3$^-$ regulatory T cells from naive T cells. Conversely, FOXP3$^+$ IL-10-producing T_{Reg} cells can differentiate from naive T cells *in vitro* in the presence of TGFβ and retinoic acid.

(JNKs) (comprising JNK1 (also known as MAPK8) and JNK2 (also known as MAPK9)); and p38 (REF. 44). Following TLR stimulation, activation of ERK modulates IL-10 expression[30,45–47], and in the presence of chemical inhibitors of ERK[30,45,47] or in ERK-deficient cells[46] IL-10 production by TLR-activated DCs is decreased. Furthermore, the differences in IL-10 production by macrophages, myeloid DCs and pDCs have been shown to correlate with the strength of ERK activation in each of these cell types[47]. Following TLR stimulation, ERK is most highly activated in macrophages, with lower activation of ERK in myeloid DCs and the lowest amount of activated ERK in pDCs[47] (FIG. 1).

Further studies using cells deficient for tumour progression locus 2 (TPL2) or NF-κB1 (also known as p105) support the role of ERK in the induction of IL-10. TPL2 is an upstream activator of ERK and, following TLR stimulation, TPL2 dissociates from the TPL2–NF-κB1 complex and activates ERK. In the absence of NF-κB1, TPL2 is rapidly degraded in the cell and, as a consequence, ERK activation by TPL2 is compromised[48]. In TPL2-deficient macrophages and myeloid DCs the amounts of TLR-induced IL-10 were lower than in wild-type cells owing to the absence of ERK activation[47]. Similarly, NF-κB1-deficient macrophages have lower levels of IL-10 expression than control cells

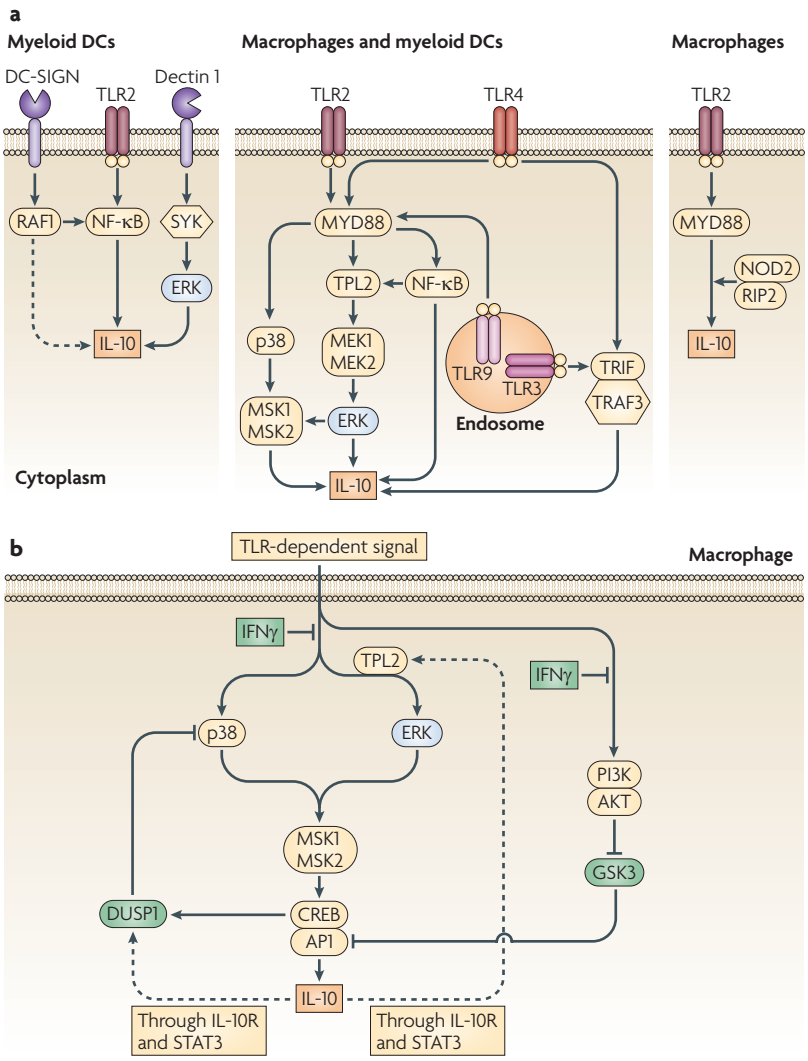

Figure 2 | **Signals that induce interleukin-10 expression by cells of the innate immune response. a** | The expression of interleukin-10 (IL-10) can be induced by Toll-like receptor (TLR) or non-TLR signalling in macrophages and myeloid dendritic cells (DCs). Activation of TLRs and their adaptor molecules — myeloid differentiation primary-response protein 88 (MYD88) and TIR-domain-containing adaptor protein inducing IFNβ (TRIF) — results in the activation of the extracellular signal-regulated kinase 1 (ERK1) and ERK2 (which are collectively referred to here as ERK), p38 and nuclear factor-κB (NF-κB) pathways. Activation of these pathways results in the induction of IL-10 expression, in addition to pro-inflammatory cytokines. In myeloid DCs, non-TLR signals through DC-specific ICAM3-grabbing non-integrin (DC-SIGN) and RAF1 can augment TLR2-induced IL-10 production. Furthermore, activation of dectin 1 and the signalling molecules spleen tyrosine kinase (SYK) and ERK results in IL-10 production. In macrophages, a role for nucleotide-binding oligomerization domain 2 (NOD2) signalling in IL-10 induction, in crosstalk with TLR2, has been described. **b** | Positive and negative feedback loops for IL-10 regulation in macrophages. The p38 and ERK pathways leading to IL-10 expression by macrophages are tightly controlled by interferon-γ (IFNγ) and IL-10 itself. IL-10 feeds back to induce the expression of dual-specificity protein phosphatase 1 (DUSP1), which negatively regulates p38 phosphorylation and thus limits IL-10 production. IL-10 can also positively feed back to upregulate tumour progression locus 2 (TPL2) expression, thus providing a positive amplification loop for its own production. In addition, IFNγ can also interfere with the phosphoinositide 3-kinase (PI3K)–AKT pathway, releasing glycogen synthase kinase 3 (GSK3). As GSK3 normally blocks IL-10 expression by acting on the transcription factors cAMP response element-binding protein (CREB) and activator protein 1 (AP1), IL-10 production is inhibited by IFNγ through its effects on PI3K. IL-10R, IL-10 receptor; MEK, MAPK/ERK1 kinase; MSK, mitogen- and stress-activated protein kinase; RIP2, receptor-interacting protein 2; STAT3, signal transducer and activator of transcription 3; TRAF3, TNFR-associated factor 3.

following TLR activation[49]. IL-10 expression was only partially restored following rescue of ERK activation in these cells[49], indicating that NF-κB-mediated regulation of IL-10 production involves both ERK-dependent and ERK-independent mechanisms, as had been suggested by previous studies[50,51]. Furthermore, pathogen triggering of DC-SIGN in human DCs resulted in the activation of RAF1, leading to acetylation of the NF-κB p65 subunit and to prolonged and increased *IL10* transcription[52]. This effect was only observed after TLR-dependent NF-κB activation, suggesting that activation of DC-SIGN can modulate TLR-induced IL-10 production[52].

The regulation of IL-10 production in response to dectin 1 ligation depends on spleen tyrosine kinase (SYK)[32]. SYK is recruited to phosphorylated dectin 1 (REF. 53) and initiates a signalling cascade that induces IL-2 and IL-10 production[32]. IL-10 production downstream of dectin 1 also requires signalling through the ERK pathway, despite being independent of TLR activation[54] (FIG. 2a). Furthermore, IL-10 production by FcR ligation in the presence of TLR signals in macrophages can also lead to ERK activation[55]. Therefore, ERK activation is common to several signalling pathways upstream of *IL10* in macrophages and DCs.

IL-10 expression can also be compromised by inhibition of p38 signalling in LPS- or CpG-activated macrophages[45,56–58], primary DCs[59] and human peripheral blood monocytes[60]. Primary cells lacking the p38 regulator dual-specificity protein phosphatase 1 (DUSP1) have prolonged p38 activation and increased levels of IL-10 expression following TLR stimulation[61–63]. This could be reversed by chemically inhibiting p38 signalling[61,62].

Interestingly, abrogation of either ERK or p38 activation leads to a reduction, but not abrogation, of IL-10 expression, which suggests that these two pathways might cooperate in TLR-induced IL-10 production. Supporting this hypothesis, inhibition of both the ERK and p38 pathways in LPS- or CpG-stimulated macrophages leads to an almost complete abrogation of IL-10 production (A.O'G., unpublished observations). Furthermore, deficiency of mitogen- and stress-activated protein kinase 1 (MSK1; also known as RPS6Kα5) and MSK2 (also known as RPS6Kα4), which are activated downstream of the p38 and ERK pathways, correlated with a loss of IL-10 expression by LPS-stimulated macrophages[64].

The production of IL-10 by macrophages and DCs is also regulated by the activation of certain inhibitory pathways. ERK- and p38-dependent IL-10 production is inhibited by IFNγ[38] (FIG. 2b). In addition to directly blocking TLR-induced MAPK activation, IFNγ induces the release of glycogen synthase kinase 3 (GSK3) by antagonizing phosphoinositide 3-kinase (PI3K)–AKT activation. This leads to inhibition of TLR-induced IL-10 production by suppressing the binding of activator protein 1 (AP1) to the *Il10* promoter[38]. Another negative feedback loop controlling IL-10 production by macrophages is mediated by IL-10 itself. IL-10 induces the expression of DUSP1, which negatively regulates p38 phosphorylation and thus limits IL-10 production[65]. By contrast, IL-10 positively feeds back to upregulate *Tpl2* expression[66], thus providing a positive amplification

loop for its own production. IL-10 was also described to induce its own transcription in human monocytes in a STAT3-dependent manner[67], which may result from its upregulation of TPL2 and thus ERK activation[66]. However, the mechanisms dictating the balance between IL-10-mediated negative and positive feedback loops are currently not clear. Furthermore, the inhibition of IL-10 production by NK cell- or T cell-derived IFNγ *in vivo* will also influence these loops.

Various pathogen-derived products induce *IL10* expression by macrophages and DCs through the activation of signalling cascades that, although common to various stimuli and different cells, have distinct thresholds of activation depending on the cell type, which reflect the distinct amounts of IL-10 produced by these cells.

IL-10 production by T_H cells. IL-10 production was first described in T_H2 cells[12,68], where its expression accompanies that of the T_H2-type cytokines IL-4, IL-5 and IL-13. T_H1 cells can also be induced to produce IL-10, but, in contrast to T_H2 cells, only under certain conditions[10,11,15,69–76] (FIG. 1). Furthermore, T_H17 cells have recently been shown to produce IL-10 (REFS 72, 77–79). The fact that T_H1, T_H2 and T_H17 cells are dependent on DC- and macrophage-derived factors that are downregulated by IL-10, but these subsets can all be induced to produce IL-10, is indicative of a negative feedback loop that ensures that effector T cell responses do not result in immunopathology. It is of interest to note that IL-9-producing T_H cells, which have recently been suggested to be a unique T_H cell subset (T_H9 cells), also express IL-10 (REF. 80).

Molecular signals for IL-10 induction in T_H cells. IL-10-inducing signalling cascades have been studied less thoroughly in T_H cells than in macrophages and DCs. IL-10-producing T_H1 cells have been described in infectious diseases, human $CD4^+$ T cell clones and mouse $CD4^+$ T cells. T_H1 cells that produce both IFNγ and IL-10 can be generated by inducing T cells to proliferate with high levels of antigen-specific or polyclonal stimulation in the presence of IL-12 (REFS 10,11,69,70) (FIG. 1). However, until recently the signals that determine whether T_H1 cells produce IL-10 were not known. Strong T cell receptor (TCR) triggering (high antigen dose)[71] and endogenous IL-12 have now been shown to be essential for the differentiation of IL-10-producing T_H1 cells, as well as for maximal expression of IL-10 following re-stimulation of these cells[72]. IL-10 induction in T_H1 cells is STAT4 and ERK dependent[72] (FIG. 1). Notch signalling can also induce IL-10 expression by T_H1 cells, a process that requires STAT4 (REF. 81). In T_H2 cells, IL-10 production seems to be regulated by the main T_H2 type-associated signalling pathways and transcription factors: IL-4, STAT6 and GATA binding protein 3 (GATA3)[82–84]. IL-10 expression by T_H17 cells seems to occur in a STAT3- and, in some cases, STAT1-dependent manner[79,85] (FIG. 1). Thus, to induce IL-10 expression, T_H1, T_H2 and T_H17 cells require the same signals needed for each T_H cell differentiation programme. However, IL-10 production by all these subsets requires ERK activation[72], indicating that a common molecular mechanism exists for IL-10 production by T_H cells. Chemical inhibition of the p38 signalling pathway did not compromise the production of IL-10 by T_H1, T_H2 or T_H17 cells[72], suggesting that in T_H cells the role of ERK is dominant over that of p38. This observation is in contrast to a joint role for ERK and p38 in IL-10 induction in macrophages and DCs.

IL-21 can enhance IL-10 expression by $CD4^+$ T cells in the context of different stimuli[86], and IL-27 enhances IL-10 expression by T_H1, T_H2 and T_H17 cells[78,79,85,87,88]. By contrast, IL-27 attenuates TLR-induced *IL10* expression by human monocytes[89]. Of interest, it has recently been shown that both IL-21 and IL-27 induce ERK activation[90,91], but it is currently not clear whether this explains the ability of these cytokines to upregulate IL-10 production. Also, both IL-21 and IL-27, in addition to ERK, activate STAT3, which seems to be involved in IL-27-mediated IL-10 upregulation by T cells[79].

Undoubtedly, all T cell subsets can produce IL-10, as well as their hallmark cytokines, following TCR triggering, but this depends on the environmental context and strength of stimulus[10,11,69–73,92–94].

IL-10 and regulatory T cells. T_{Reg} cells, which are characterized by their specific expression of the transcription factor forkhead box P3 (FOXP3), do not express IL-10 following stimulation directly after *ex vivo* isolation[95,96], unless isolated from the gut[97] (BOX 1). Although $FOXP3^+$ T_{Reg} cells inhibit naive T cell proliferation *in vitro* independently of IL-10, in some cases, T_{Reg} cells mediate their regulatory function *in vivo* through IL-10 (reviewed in REFS 1,14,16,98–100). Therefore, T_{Reg} cells must receive signals *in vivo* to induce the expression of this suppressive cytokine. Both IL-2 and IL-4 have been shown to induce IL-10 production after culture of T_{Reg} cells *in vitro*[101,102]. However, in these studies, the T_{Reg} cell population analysed might have contained some effector T cells and therefore the source of IL-10 cannot be confirmed. So far the signals that induce IL-10 expression by $FOXP3^+$ T_{Reg} cells remain elusive, although transforming growth factor β (TGFβ) has been shown to be required *in vivo*[97] (FIG. 1).

Several populations of antigen-driven $FOXP3^-$ IL-10-producing T cells with regulatory activity that are distinct from naturally occurring T_{Reg} cells have been described (reviewed in REFS 1,2,13,14). These cells produce IL-10, but not IL-2, IL-4 or IFNγ, and can be generated *in vitro* using various stimuli, such as cytokine cocktails (TGFβ, IL-10 and IFNα) or immunosuppressive drugs (vitamin D3 and dexamethasone)[1,2,13,14,21], or *in vivo* by repeated stimulation with soluble antigen[71,103]. Additional signals for IL-10 expression by these $FOXP3^-$ regulatory T cells include co-stimulation through CD2 or CD46 and stimulation with type I IFNs or with immature DCs (reviewed in REFS 1,14,104). Signals delivered through inducible T cell co-stimulator (ICOS) have also been suggested to induce IL-10 expression by $FOXP3^-$ regulatory T cells[105,106]; a similar effect has been observed in T_H2 cells[107,108], suggesting that, although it is involved in the induction of IL-10, ICOS is not a cell type-specific inducer of IL-10.

Notch
A signalling system comprising highly conserved transmembrane receptors that regulate cell fate choice in the development of many cell lineages. Therefore, they are crucial in the regulation of embryonic differentiation and development.

DNaseI hypersensitive sites
Sites of nuclease sensitivity in the nuclei on exposure of cells to limiting concentrations of DNaseI. The digested regions of DNA correspond to sites of open DNA, which might be factor-binding sites or areas of altered nucleosome conformation.

Chromatin remodelling
Alterations that are induced in chromatin by enzymes that modify the extent of acetylation, methylation or other covalent modifications of histones.

Acetylation
A post-translational modification of chromatin components, particularly histones. It correlates with actively transcribed chromatin.

It will be of interest to determine whether IL-10-producing FOXP3$^-$ regulatory T cells differentiate directly from naive T cells or are derived from T_H1, T_H2, T_H9 or T_H17 cells that have lost expression of their effector T cell cytokines but have maintained IL-10 expression. It is possible that FOXP3$^-$ regulatory T cells that only make IL-10 have originally differentiated along a T_H1 cell pathway through repeated high-level antigenic stimulation, which results in IL-10 production and ultimately in the downregulation of T_H1 cell production of IFNγ by feedback inhibition of IL-12 production by DCs and macrophages[71].

Additional cell types that produce IL-10. In addition to macrophages, DCs and CD4$^+$ T cells, other cells of the immune system are also known to express IL-10. CD8$^+$ T cells express IL-10 following TCR activation or interaction with CD40 ligand expressed by activated pDCs[109–111], and this IL-10 production can be enhanced by IL-21 (REF. 86). Stimulation of B cells with autoantigens, TLR4 and TLR9 ligands or vitamin D3 also leads to IL-10 production[112–117]. Finally, mast cells can express IL-10 following TLR4 activation or during skin allergic or damage responses[1,118,119]. Recently, neutrophils were reported to produce IL-10 in response to TLR and C-type lectin co-activation through MYD88 and SYK, respectively[36]. These IL-10-producing neutrophils were shown to be recruited to the lung during mycobacterial infections and to regulate local immune inflammatory responses[36]. It is currently not clear whether the molecular mechanisms required for the induction of IL-10 by these cells are regulated by the common factors that regulate IL-10 production by T_H cells, macrophages and DCs.

Box 2 | **Epigenetic control of *IL10* expression**

Several studies suggest that the expression of interleukin-10 (IL-10) is regulated by changes in the structure of the chromatin at the *IL10* locus[51,133–135] (FIG. 3). Various DNaseI hypersensitive sites (HSSs) were found in the mouse *Il10* locus, most of which are common to IL-10-producing T cells, macrophages and dendritic cells (DCs). However, a macrophage-specific regulatory element (HSS–4.5), which is absent in T cells, was also found[51]. Although chromatin remodelling seems to be one of the initial events leading to *IL10* expression, additional signals are required to allow high rates of *IL10* transcription. Epigenetic imprinting of the *Il10* locus in mice, as measured by histone acetylation, was observed in high-IL-10-producing T helper 2 (T_H2 cells)[84] and macrophages[51] but not in low-IL-10-producing T_H1 cells[84], despite the open conformation of the *IL10* locus observed for all these cell types. In macrophages, the histones at the *Il10* locus were also reported to be hyperphosphorylated[55,167]. Furthermore, by interacting with the distal segment of the *IL10* promoter, histone deacetylase 11 negatively regulated the expression of this cytokine in human and mouse antigen-presenting cells[168].

Several studies have identified GATA binding protein 3 (GATA3) as a possible initiator of chromatin remodelling and histone acetylation of the *Il10* locus in mice[83,133]. However, as GATA3 is only expressed by T_H2 cells and not in other IL-10-producing T_H cells, macrophages or DCs, other mechanisms must operate in these cells to induce chromatin remodelling at the *Il10* locus. A clue may come from the finding that, in macrophages, remodelling of the *Il10* locus occurs following TLR stimulation[51] or Fc receptor binding in an extracellular signal-regulated kinase (ERK)-dependent manner[55].

Overall, analysis of the chromatin conformation at the *IL10* locus may help to explain the different levels of *IL10* expression and the different factors involved in this expression by different cell types, although many questions still remain unanswered.

Understanding the molecular pathways leading to IL-10 production by different immune cells might provide valuable information on possible targets for IL-10 manipulation. This will be useful in the design of intervention strategies to modulate IL-10 production and ultimately the immune response.

Transcription factors that regulate IL-10

Activating the* IL10 *promoter. The structure of the human and mouse *IL10* promoters is similar and both contain a TATA box and a CCAAT box (CCAGT in mice). The human and mouse *IL10* promoters have a high level of homology, particularly around certain putative binding sites for transcription factors. However, the presence of a conserved putative binding site in a gene promoter does not guarantee transcription factor binding.

In addition to epigenetic control (BOX 2), the expression of *IL10* depends on transcription factor binding. The transcription factors specific protein 1 (SP1)[120], SP3 (REF. 121), CCAAT/enhancer binding protein-β (C/EBPβ)[122,123], IFN-regulatory factor 1 (IRF1) and STAT3 (REF. 124) have been proposed to bind to and transactivate *IL10* in macrophage and T cell lines of mouse or human origin (FIGS 3, 4). Also, binding of the NF-κB p50 subunit to the *IL10* promoter in a human T cell lymphoma cell line has been described[125]. Moreover, some of these findings depend on the cell type and on the stimulus used. For example, whereas one study indicates that *IL10* promoter activity relies on an SP1 site located between positions –636 and –631 relative to the initiation site[56], another report shows that *IL10* promoter activity relies on the C/EBP5 motif positioned between the TATA box and the translation start point[122]. Although both studies were carried out using the human promonocytic cell line THP1, the type of stimulation was different (LPS versus cyclic AMP (cAMP), respectively), which might account for the differences observed.

Studies using mouse primary cells have shown that homodimers of NF-κB p50 bind to the proximal *Il10* promoter, activating *Il10* transcription in primary macrophages[126], and p50-deficient macrophages have an impaired expression of IL-10 following LPS stimulation compared with wild-type macrophages[126]. Furthermore, IL-10 induction in response to double-stranded RNA stimulation and viral infection of mouse macrophages was described to be protein kinase R (PKR) dependent and to be regulated by binding of NF-κB to a distinct site in the *Il10* promoter[127]. In addition to NF-κB, a role for C/EBPβ in cAMP-mediated IL-10 production was confirmed in mouse primary macrophages; nuclear accumulation and DNA binding of C/EBPβ was involved in IL-10 production in response to adenosine and *Escherichia coli* infection, and C/EBPβ-deficient macrophages failed to produce IL-10 (REF. 128).

Recently, two cofactors of the homeobox (HOX) family, pre-B-cell leukaemia transcription factor 1 (PBX1) and PBX-regulating protein 1 (PREP1), were implicated in inducing IL-10 expression by mouse macrophages[129]. In this study, the expression of IL-10 was triggered by the interaction of macrophages with apoptotic cells and depended on p38; in human cells, transcription of *IL10* cells was mediated by the binding of PBX1 and

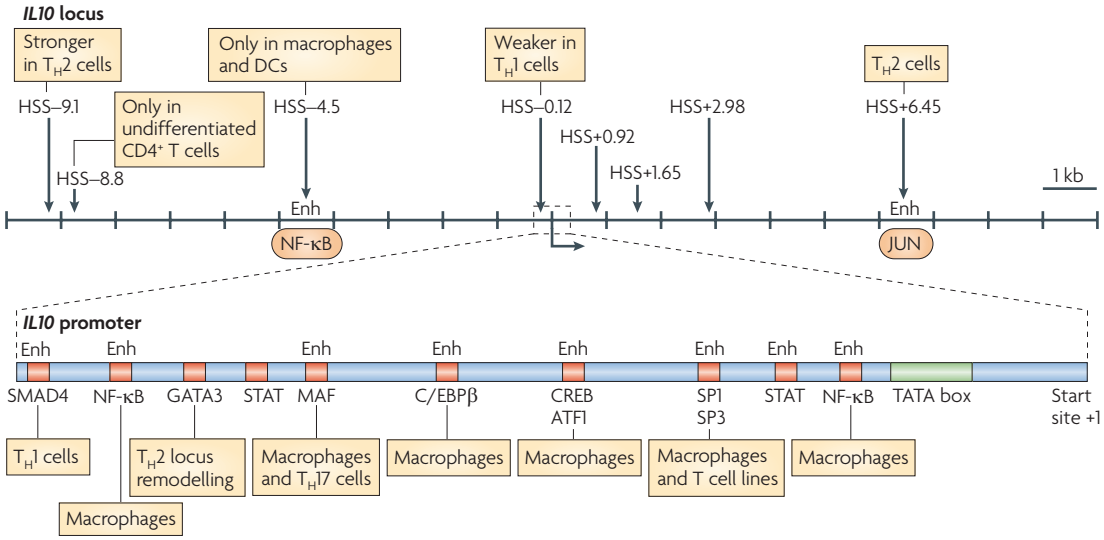

Figure 3 | **Molecular regulation of interleukin-10 expression: the interleukin-10 locus and promoter.** The mouse interleukin-10 (*Il10*) locus (top panel) and *Il10* promoter (bottom panel) are represented here. Several DNaseI hypersensitive sites (HSSs), and their relative position to the *Il10* starting site (+1), are indicated. Most of these HSSs are common to all cells, although some cellular specificity is also observed. Two of these sites (HSS–4.5 and HSS+6.45) have been studied in more detail, and their role in *Il10* regulation has been described. HSS–4.5 contains hyperacetylated histones and binds nuclear factor-κB (NF-κB) in macrophages, whereas HSS+6.45 binds JUN proteins in T helper 2 (T_H2) cells. Both HSS–4.5 and HSS+6.45 were shown to enhance the *Il10* promoter activity in reporter assays. The biological role for the other HSSs needs to be further clarified. The proximal elements that regulate the expression of IL-10, including the *Il10* promoter, have been well studied. Several transcription factors have been shown to bind to the *Il10* promoter and to enhance *Il10* transcription in various cell types. In T_H2 cells, GATA binding protein 3 (GATA3) functions as a master regulator for *Il10* expression by binding to sites in the *Il10* locus (including to the promoter) and inducing locus remodelling. Also represented are putative signal transducer and activator of transcription (STAT) binding sites in the mouse *Il10* promoter. ATF1, activating transcription factor 1; C/EBPβ, CCAAT/enhancer binding protein-β; CREB, cAMP-responsive-element-binding protein; DC, dendritic cell; Enh, enhancer; GATA3, GATA binding protein 3; SMAD4, mothers against decapentaplegic homologue 4; SP, specific protein.

PREP1 to the apoptotic cell-response element (ACRE) in the *IL10* promoter[129]. The transcription factors cAMP-responsive-element-binding protein (CREB) and activating transcription factor 1 (ATF1) have been shown to be activated by MSKs in LPS-stimulated mouse macrophages and to bind to the *Il10* promoter, thus suggesting a direct effect of the kinases MSK1 and MSK2 in the regulation of IL-10 induction[64] (FIG. 2b). There is also evidence for TGFβ1-induced SMAD4 binding to and activating the *Il10* promoter in mouse T_H1 cells[130]; however, it is worth noting that TGFβ1 inhibits the development of T_H1 and T_H2 cells *in vitro* and thus their production of IL-10 (REF. 72).

The transcription factor GATA3 was shown to be a master regulator of IL-10 expression in mouse T_H2 cells by binding to and initiating changes in the chromatin structure at the *Il10* locus[83,84]. Although one of the binding sites for GATA3 is located in the *Il10* promoter, GATA3 alone does not transactivate the *Il10* promoter[83]. GATA3 may thus be responsible for remodelling the *Il10* locus in T_H2 cells, with other factors being necessary to induce high levels of IL-10 expression in other cell types such as T_H1 cells that do not express GATA3 (REFS 69,72).

Originally described as a T_H2 cell-specific factor[131], the transcription factor MAF has been shown to bind to the *Il10* promoter and have a role in the transcriptional regulation of IL-10 in mouse macrophages stimulated with LPS and IL-4, although MAF alone is not sufficient to induce *Il10* expression in these cells[132]. Other recent reports have also implicated MAF in the expression of IL-10 by T_H17 cells, showing binding of this transcription factor to MAF recognition elements in the *Il10* promoter[85], and in the differentiation of IL-10-producing $FOXP3^-$ regulatory T cells[88]. Finally, MAF expression is also detectable in T_H1, T_H2 and T_H17 cells, correlating with IL-10 production, and MAF expression depends on ERK activation in T_H1 and T_H17 cells, similar to IL-10 expression[72]. Taken together, these reports suggest that MAF may be a universal transcription factor for the regulation of IL-10 production, important in all IL-10-producing cells of both the innate and the adaptive immune systems.

As discussed, various transcription factors have been described to bind to and activate the *IL10* promoter (FIGS 3, 4). The activity of one transcription factor or another to regulate *IL10* expression seems to depend on the cell type and the type of stimuli. Furthermore, multiple studies suggest that IL-10 is not regulated in a simple and linear manner, which is in accord with its function to keep diverse immune responses in check.

***Enhancing* IL10 *transcription*.** Following the description of several distal regulatory elements in *IL10*[51,133–135] (FIG. 3), the search for transcription factors with a role in regulating *IL10* expression has been expanded to those that bind regions of the locus outside of the promoter region.

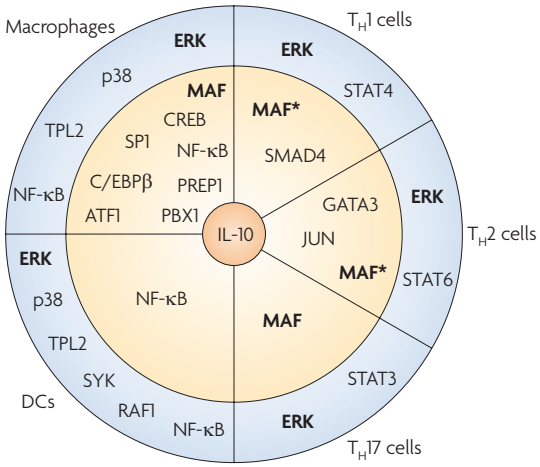

Figure 4 | **Transcription factors that control interleukin-10 expression by CD4⁺ T cells and antigen-presenting cells.** Many transcription factors have been found to regulate the expression of interleukin-10 (IL-10) both in antigen-presenting cells and in CD4⁺ T cells. Represented here are the signalling molecules (in the outer circle) and transcription factors (inner circle) involved in IL-10 regulation with a role validated by promoter studies (mutagenesis or chromatin immunoprecipitation) or by studies in genetically modified mice. Part of these studies were carried out in cell lines, although recently the study of IL-10 regulation has involved primary cells. Some of the indicated transcription factors are cell specific and others seem to have a wider role, as discussed in detail in the text. The transcription factors in bold are common to various cell types in regulating IL-10 production. Transcription factors marked with * are those that have not been shown to bind the promoter or the *Il10* locus. ATF1, activating transcription factor 1, C/EBPβ, CCAAT/enhancer binding protein-β; CREB, cAMP-responsive-element-binding protein; DC, dendritic cell; ERK, extracellular signal-regulated kinase; GATA3, GATA binding protein 3; NF-κB, nuclear factor-κB; PBX1, pre-B-cell leukaemia transcription factor 1; PREP1, PBX-regulating protein 1; SMAD4, mothers against decapentaplegic homologue 4; SP1, specific protein 1; STAT, signal transducer and activator of transcription; SYK, spleen tyrosine kinase; T_H, T helper; TPL2, tumour progression locus 2.

The NF-κB p65 subunit binds to a newly described κB site located 4.5 kb upstream of the *Il10* start site and has a role in enhancing IL-10 expression by LPS-stimulated mouse macrophages[51] (FIG. 3). This is in keeping with a report that mice deficient for inhibitor of NF-κB kinase 2 (IKK2) show a defect in IL-10 production in LPS-stimulated macrophages[50]. This κB site is exposed in IL-10-producing LPS-, CpG- or zymosan A-stimulated mouse macrophages and DCs but is absent in T cells[51]. This suggests that different molecular mechanisms might regulate the expression of *Il10* in the innate versus adaptive immune systems and that the production of IL-10 by innate immune cells might be subject to additional regulation, as it is the first checkpoint for the initiation of immune responses and determines the class of the resulting adaptive immune response. Of interest, however, is that ERK signalling is required for optimal IL-10 induction in macrophages, DCs and T_H cell subsets, which questions whether transcription factors that are required for IL-10 induction in DCs and macrophages may also have a role in inducing IL-10 in T cells. One of the transcription factors implicated in TLR-induced IL-10 expression in mouse macrophages and DCs, by the activation of ERK, is FOS, the expression of which is strongly induced by high levels of ERK activation[29,30,47].

Studies have suggested there is a role for JUN proteins in regulating *Il10* in mouse T_H2 cells, but not T_H1 cells, through binding to a regulatory element located ~6.45 kb downstream of the *Il10* start site[133,134] (FIG. 3). This finding supports our suggestion that alternative mechanisms probably operate to regulate *Il10* induction in T_H1 and T_H2 cells and possibly in other T_H cell subsets, although common factors, such as ERK and MAF, are required for *Il10* induction in various cell types.

Although there are no data so far to show direct binding of the various STATs to the *IL10* locus, an increasing amount of evidence suggests a role for the STAT proteins in regulating the induction of IL-10 expression in both primary macrophages and T cells. In mouse T cells, the induction of IL-10 by IL-27 seems to depend on both STAT1 (REFS 79,87) and STAT3 (REFS 79,85), and STAT3 is also involved in IL-6-induced IL-10 expression[79]. By contrast, a recent study on human monocytes describes an inhibitory role for IL-27 on IL-10 production through STAT1 (REF. 89). In addition, as STAT3 is required for T_H17 cell differentiation, this transcription factor may have an indirect role in IL-27-mediated induction of IL-10 by T_H17 cells by modulating T_H17 cell differentiation. Another study suggests that IL-10 induces its own expression by human monocyte-derived macrophages in an autocrine manner through the activation of STAT3 (REF. 67). In this study, activation of the *IL10* promoter depended on the integrity of the STAT3-binding site. Considering the data from human cell lines that show binding of STAT3 to the *IL10* promoter[124], it is possible that the STAT3-dependent effects on *IL10* activation in primary cells might be related to promoter transactivation. Finally, STAT4, which is important for the differentiation of IFNγ- and IL-10-producing mouse T_H1 cells[72], was also reported to have a role in inducing IL-10 expression by mouse NK cells[136]. The molecular mechanisms underlying the participation of other STAT molecules in the control of IL-10 expression require further clarification, particularly as several STAT molecules are required for the differentiation of T_H cell subsets and thus can modulate the induction of IL-10 in an indirect manner.

The list of transcription factors involved in the regulation of *IL10* expression is expanding (FIG. 4), which reflects the degree of precision and complexity that the expression of this cytokine demands. The exact contribution of many of the transcription factors discussed above remains elusive and may in some cases be cell specific, but may also depend on the type of stimulus that triggers *IL10* expression or may affect *IL10* expression indirectly. With the identification and characterization of distal regulatory regions in the *IL10* locus, a role for other transcription factors might also be revealed.

Silencing* IL10 *expression. Recent studies have provided evidence for the role of certain transcription factors in silencing *Il10* expression. For example, it has been suggested that the transcription factor ETS1 has a role in repressing the production of IL-10 by mouse T_H1 cells, as ETS1-deficient T_H1 cells show a marked increase in the production of this cytokine[137]. However, it is possible that the effect of ETS1 on IL-10 expression results from diminished T_H1 cell differentiation, as no interaction of ETS1 with the *Il10* locus has been shown. Similarly, an increase in IL-10 expression was observed in mice deficient for the T_H1 cell-specific transcription factor T-bet (also known as TBX21) that were infected with *M. tuberculosis*, suggesting that T-bet might have a role in the negative regulation of IL-10 expression by T_H1 cells[138]. However, as IFNγ expression is also lost in the absence of T-bet, this effect on IL-10 could reflect a blockade of T_H1 cell differentiation, with the increase in IL-10 expression resulting from other cells. This notion is strongly supported by the observation that IL-10 production by T_H1 cells is accompanied by the expression of high levels of IFNγ and T-bet[69,72].

Silencers of IL-10 expression have also been identified in cells of the innate immune response. MHC class II transactivator (CIITA) has been shown to negatively regulate the expression of IL-10 by mouse DCs and the activity of the *Il10* promoter in a mouse macrophage cell line[139]. As mentioned above, it is also possible that STAT1 negatively regulates IL-10 expression in human monocytes[89,140]. However, it is still unclear whether STAT1 directly or indirectly affects the *Il10* locus. In addition, isolated peritoneal macrophages from B cell lymphoma 3 (BCL-3)-deficient mice produce increased amounts of IL-10, suggesting that BCL-3 is an inhibitor of IL-10 expression in macrophages; although, again, it is questionable whether this effect is direct or indirect[141]. BCL-6 has been reported to have a role in inhibiting the production of T_H2 cell-specific cytokines including IL-10 (REF. 142), and in its absence T cells activated with strong co-stimulation show a large upregulation of *Il4*, *Il10* and *Il13* mRNA, whereas overexpression of BCL-6 in wild-type T cells strongly inhibits the production of IL-10 following activation[142]. However, it is unknown whether BCL-6 directly regulates the *Il10* locus. In recent reports, BCL-6 was required for T follicular helper cell differentiation, but it inhibited the differentiation of other T_H cell subsets, by direct interaction with T-bet, GATA3 and retinoic acid receptor-related orphan receptor-γt (RORγt)[143–145]. It is therefore possible that the previously reported BCL-6-mediated IL-10 suppression in T_H2 cells[142] is a consequence of the inhibition of the T_H2 cell differentiation pathways and therefore not due to a direct effect of BCL-6 on *Il10* transcription. In support of this, no BCL-6 consensus binding sites have as yet been found in the *Il10* promoter[142].

The complexity of *IL10* regulation by different cells of the immune system, having both positive and negative feedback loops, shows the tight control that is essential to achieve a balance between an effective immune response and immunopathology. This complex regulation ranges from common to distinct pathways of IL-10 induction in different cell types.

T follicular helper cell
(T_{FH} cell). A $CD4^+$ T cell that provides help to B cells in follicles and germinal centres. The T_{FH} cell signature includes the expression of CXCR5, ICOS, CD40 ligand and IL-21, factors that mediate T_{FH} cell homing to follicles and B cell help.

MicroRNAs
Single-stranded RNA molecules of approximately 21–23 nucleotides in length that regulate the expression of other genes.

Mechanisms of post-transcriptional regulation

Modulation of mRNA stability is an important component in the regulation of expression of several cytokines (reviewed in REF. 146) and most cytokine genes have a long 3′ untranslated region (UTR), containing class II adenosine–uridine-rich elements (ARE) that target mRNAs for rapid degradation. Multiple copies of potential mRNA destabilizing motifs are found in the 3′ UTR of *Il10* mRNA[147]. Various factors can alter the stability of *Il10* mRNA, including IL-10 itself, which triggers *Il10* mRNA degradation[148,149], and adenosine receptor activation, which acts by relieving the translational repressive effect of the *Il10* 3′ UTR thereby increasing the mRNA half-life and the amount of IL-10 produced[150].

More recently, *Il10* mRNA has been identified as a tristetraprolin (TTP) target in a wide genome screen[151]. TTP is a RNA-binding molecule that can induce rapid degradation of mRNA following binding to AREs in 3′ UTRs. Supporting this finding, macrophages from TTP-deficient mice showed a decrease in the rate of *Il10* mRNA decay and an increase in IL-10 secretion[151]. Moreover, activation of p38 has been reported to stabilize the *Il10* mRNA by inhibiting the action of TTP[152]. Interestingly, IL-10 induces TTP expression in a STAT3-dependent manner, contributing to the establishment of an anti-inflammatory programme[153].

Finally, a role for microRNAs in the regulation of IL-10 expression has been described[134]. A recent report shows that the human microRNA miR-106a, expressed in cells of both lymphoid and myeloid origin, binds the 3′ UTR of the *IL10* mRNA and induces its degradation[154].

This level of post-transcriptional regulation of IL-10 expression might explain why, despite the existence of common pathways for IL-10 induction, different cells ultimately secrete different amounts of IL-10. Thus, in addition to genetic regulation, post-transcriptional regulation contributes to the fine tuning of IL-10 expression.

Conclusion and outstanding questions

Owing to the key role of IL-10 in the immune response and the link between defective IL-10 production and certain autoimmune and inflammatory diseases, an understanding of the molecular mechanisms that regulate the expression of this cytokine is crucial.

The fact that various cell types can express IL-10 makes the subject of *IL10* regulation challenging. It also highlights the complexity of this regulation. Several early studies on the molecular regulation of IL-10 reported apparent differences. The fact that cell lines and different conditions were used in most of these studies has certainly been a contributing factor to these discrepancies. Indeed, recent studies using primary T cells, macrophages and DCs show more consistent results between laboratories with respect to the regulation of *IL10* expression.

Several general conclusions from these studies can be made: first, many cells of the innate and adaptive immune response produce IL-10 regardless of the stimulus. Second, different stimuli, or the strength of the stimulus, give rise to different levels of IL-10 in the same cell type. Third, some of the molecular mechanisms for the regulation of IL-10 differ according to the cell

type, although common mechanisms also exist. And fourth, IL-10 is induced in many situations together with pro-inflammatory cytokines, although the pathways that induce IL-10 expression may actually negatively regulate the expression of these pro-inflammatory cytokines.

Several outstanding questions and future challenges remain. Which cells are induced to produce IL-10 during an immune response to specific pathogens and gut flora, and which IL-10-producing cells are required to prevent host damage or to conversely inhibit immune responses, thereby contributing to chronic infection? What signalling pathways and transcription factors can specifically induce IL-10 in different immune cells independently of the induction of pro-inflammatory cytokines? What signalling pathways are required in different cells, and what is the hierarchy of transcription factor binding to *IL10* regulatory elements? Is IL-10 production *in vivo* dictated by the environment and inflammatory stimuli, and what maintains the remodelling of the *IL10* locus? For example, can T_H1 cells detect signals and inflammatory molecules induced by microorganisms and their products in the microenvironment and turn on IL-10 production and thus reduce tissue damage? Many of these questions will be answered by comparing the kinetics and quantity of IL-10 expression and production in different immune cells stimulated with different stimuli and by elucidating the molecular signalling pathways leading to IL-10, by traditional biochemical methods, bioinformatics or high-throughput approaches, such as chromatin immunoprecipitation sequencing.

Outstanding questions might be answered by dissecting the mechanisms that regulate the expression of IL-10 in different cells, during different immune responses to microorganisms and in different anatomical locations (for example, comparing IL-10 expression in the lungs to the blood during *M. tuberculosis* infection[73]). An understanding of how IL-10 expression is regulated during such immune responses undoubtedly will be of use in developing therapeutic strategies to target IL-10 production in disease.

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DATABASES
UniProtKB: http://www.uniprot.org
CIITA | dectin 1 | DUSP1 | ETS1 | IL-10 | MAF | MYD88 | NOD2 | STAT1 | STAT3 | STAT4 | SYK | T-bet | TRAE3 | TRIF |

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About the authors

Margarida Saraiva received her Ph.D. from the University of Cambridge, UK, in 2002. She subsequently carried out her postdoctoral research at the Medical Research Council National Institute for Medical Research (NIMR) on the molecular mechanisms of interleukin-10 (IL-10) gene regulation. In 2007 she joined the Life and Health Sciences Research Institute (ICVS) in Portugal. Her research programme focuses on the molecular regulation of macrophage and dendritic cell (DC) responses to *Mycobacterium tuberculosis*.

Anne O'Garra trained at the NIMR. She was then recruited to the DNAX Research Institute, California, USA. Her findings identified the roles of several key cytokines in the activation or downregulation of immune responses, and with this knowledge she is now pursuing therapeutic strategies for intervention in infectious diseases. Her most important contributions relate to the discovery of the immunosuppressive functions of IL-10, the production of IL-12 by essential antigen-presenting cells (APCs), DCs and macrophages, and the roles of IL-18 and IL-12 in inducing $CD4^+$ T helper 1 (T_H1) cell responses. Despite several offers of distinguished posts in the USA, she returned to the UK in 2001 to a permanent position as the Head of a new Division of Immunoregulation at the NIMR, to be an interface between the Divisions of Immunology and Infectious Diseases.

Online summary

- Interleukin-10 (IL-10) is not a cell type-specific cytokine, but instead it is broadly expressed by many immune cells.
- Several layers of regulation regulate IL-10 production, including changes in the chromatin structure, enhancement or silencing of *IL10* transcription and post-transcriptional regulatory mechanisms.
- Many of the molecular events leading to *IL10* expression are similar and common to various IL-10-producing immune cells, but cell type-specific signals also exist.
- Induction of IL-10 often occurs together with pro-inflammatory cytokines, although pathways that induce IL-10 may actually negatively regulate these pro-inflammatory cytokines.
- Understanding the specific molecular events that regulate the expression of IL-10 will be important for the design of new strategies of immune intervention.

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