Biology and therapeutic potential of interleukin-10
Margarida Saraiva, Paulo Vieira, Anne O’garra

To cite this version:
Margarida Saraiva, Paulo Vieira, Anne O’garra. Biology and therapeutic potential of interleukin-10. Journal of Experimental Medicine, Rockefeller University Press, 2019, 216 (11), pp.jem.20190418. 10.1084/jem.20190418. pasteur-02365641

HAL Id: pasteur-02365641
https://hal-pasteur.archives-ouvertes.fr/pasteur-02365641
Submitted on 15 Nov 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - NonCommercial - ShareAlike 4.0 International License
REVIEW

Cytokines Focus

Biology and therapeutic potential of interleukin-10

Margarida Saraiva, Paulo Vieira, and Anne O’Garra

The cytokine IL-10 is a key anti-inflammatory mediator ensuring protection of a host from over-exuberant responses to pathogens and microbiota, while playing important roles in other settings as sterile wound healing, autoimmunity, cancer, and homeostasis. Here we discuss our current understanding of the regulation of IL-10 production and of the molecular pathways associated with IL-10 responses. In addition to IL-10’s classic inhibitory effects on myeloid cells, we also describe the nonclassic roles attributed to this pleiotropic cytokine, including how IL-10 regulates basic processes of neural and adipose cells and how it promotes CD8 T cell activation, as well as epithelial repair. We further discuss its therapeutic potential in the context of different diseases and the outstanding questions that may help develop an effective application of IL-10 in diverse clinical settings.

Introduction

The immune system evolved to fight infection while minimizing host damage. Several regulatory mechanisms are in place to ensure the delicate balance between an effective immune response and the appearance of tissue pathology. An anti-inflammatory response, of which IL-10 is the paradigm, is one such mechanism.

IL-10 (Fiorentino et al., 1989; Moore et al., 1990) is the founding member of a family of cytokines that also includes IL-19, IL-20, IL-22, IL-24, IL-28A, IL-28B, and IL-29 (reviewed in Ouyang and O’Garra, 2019). The involvement of IL-10 in many disease states has been demonstrated, both in animal models and in humans with mutations in the IL-10/IL-10R axis (reviewed in Engelhardt and Grimbacher, 2014; Shouval et al., 2014b). However, despite considerable progress in IL-10 biology, many outstanding questions still exist. In this review, we revisit the discovery of IL-10, highlight the latest developments toward understanding the metabolic regulation of IL-10 in various cell types, and discuss the molecular signals downstream of the IL-10R in responding cells. We present an overview of the biological functions of IL-10, including some surprising new findings on nonclassical roles for this cytokine. We finish by summarizing the progress made toward the therapeutic manipulation of IL-10.

The discovery of IL-10: A historic perspective

IL-10 was discovered 30 yr ago as a secreted cytokine synthesis inhibitory factor, produced by T helper (Th) 2 cell clones shown to inhibit cytokine production by Th1 cells (Fiorentino et al., 1989). The mouse and human IL-10-coding genes were subsequently cloned, and the predicted protein sequences were found to be highly homologous to an Epstein-Barr virus-encoded protein, BCRF1 (Moore et al., 1990; Vieira et al., 1991). This was the first suggestion that viruses may exploit the inhibitory properties of IL-10 as a mechanism of immune evasion. Indeed, recombinant BCRF1 protein was shown to mimic the activity of IL-10 (Hsu et al., 1990), namely inhibition of cytokine synthesis by activated human peripheral blood mononuclear cells and by a mouse Th1 cell clone (Vieira et al., 1991). Since then, a number of other viruses have also been shown to encode a homologue of the IIlO gene (Fleming et al., 1997; Kotenko et al., 2000; Jayawardane et al., 2008). Soon after the discovery of IL-10, its pleiotropic action was unveiled initially in the mouse, not only as a cytokine synthesis inhibitory factor, but additionally as a mast cell (Thompson-Snipes et al., 1991) and thymocyte (MacNeil et al., 1990) growth factor, and as an activator of B cells (Go et al., 1990; Roussel et al., 1992).

The mechanisms underlying the ability of IL-10 to inhibit cytokine production by Th1 cells were soon unveiled. IL-10-mediated inhibition of IFN-γ secretion by Th1 cells was demonstrated to occur via its action on the APC function of macrophages (Fiorentino et al., 1991b) and by its inhibition of cytokine production by activated macrophages and dendritic cells (DC; Bogdan et al., 1991; Fiorentino et al., 1991a; Macatonia et al., 1993). Additionally, IL-10 inhibited the killing of intracellular pathogens (Gazzinelli et al., 1992; Frei et al., 1993; Vouldoukas et al., 1997). In complementary studies, IL-10 was
shown to prevent antigen-specific proliferation of human T cells by inhibition of the antigen-presenting capacity of monocytes through the down-regulation of class II MHC (de Waal Malefyt et al., 1991b). Collectively, these initial studies placed IL-10 as a key mediator of the anti-inflammatory response.

Genetic ablation of Il10 showed its key role in controlling inflammation in vivo, as IL-10–deficient mice developed colitis (Kühn et al., 1993). These findings prompted many genetic association studies between deficiencies in the Il10 or Il10r genes and susceptibility to colitis, an association that is now well acknowledged (Glocker et al., 2009, 2010, 2011; Jostins et al., 2012; Moran et al., 2013; Engelhardt and Grimbacher, 2014; Ellinghaus et al., 2016). However, the role of IL-10 clearly exceeds the regulation of intestinal inflammation, as a function for this molecule has been also described in several other settings, from inflammatory or neurodegenerative diseases to infection or cancer (reviewed in Ouyang et al., 2011; Lobo-Silva et al., 2016; Ouyang and O’Garra, 2019; Wang et al., 2019).

In summary, IL-10 has emerged as a major suppressor of the immune response and a key player in human disease, and is thus an attractive therapeutic target. However, as discussed below, there is recent evidence that IL-10 may play a previously under-appreciated dual role, in some contexts stimulating the immune response instead of suppressing it. This depends on specific cell types and contexts, thus raising important issues related to its modulation in the clinic and calling for further research into the pleiotropic nature of this cytokine.

**Molecular mechanisms regulating IL-10 expression**

**IL-10–producing cells**

Although Th2 cells were the first identified cellular source of IL-10 (Florentino et al., 1989), the production of this cytokine by other CD4 and also CD8 T cells was soon demonstrated (Yssel et al., 1992; Tanchot et al., 1998). We now know that cells of both the myeloid and lymphoid lineages secrete IL-10 in response to different stimuli (reviewed in Saraiva and O’Garra, 2010; Ouyang and O’Garra, 2019). This includes macrophages, monocytes, DCs, neutrophils, mast cells, eosinophils, and natural killer cells, in addition to CD4 and CD8 T cells and B cells (reviewed in Moore et al., 2001; Gabryšová et al., 2014). More recently, the group of IL-10–producing cells was expanded to resident macrophages, such as microglia (reviewed in Lobo-Silva et al., 2016) and cardiac macrophages (Hulsmans et al., 2018). Notably, some nonhematopoietic cells, including epithelial cells, are also able to produce IL-10 (Jarry et al., 2008). Finally, tumor cells can also produce IL-10, which has been correlated with their ability to cause immunosuppression (Chen et al., 1994; Itakura et al., 2011).

**The regulation of IL-10 production: Global concepts**

The expression of IL-10 is tightly regulated to avoid diseases related to its excess or deficiency. Both common and cell-specific regulatory mechanisms including the triggering of specific signaling pathways, the expression and activation of particular transcription factors, and post-transcriptional and epigenetic regulation ensure the appropriate production of IL-10 (reviewed in Saraiva and O’Garra, 2010; Gabryšová et al., 2014; Ouyang and O’Garra, 2019). In myeloid cells, the expression of IL-10 accompanies that of pro-inflammatory cytokines, downstream of the activation of multiple pattern recognition receptors (PRRs; reviewed in Saraiva and O’Garra, 2010; Rutz and Ouyang, 2016; Gabryšová et al., 2018; Ouyang and O’Garra, 2019). MAPK are among the key pathways regulating IL-10. Activation of ERK1/2 downstream of the MAP3 kinase tumor progression locus 2 (Tpl2) regulates the expression of Il10 by TLR-activated macrophages and DCs, as shown by the reduction of IL-10 production observed inTpl2-deficient or upon chemical inhibition of ERK1/2 in macrophages (Yi et al., 2002; Banerjee et al., 2006; Liu et al., 2006; Kaiser et al., 2009). The MAPK p38 is also activated downstream of TLR signaling and shown to regulate IL-10 production by these cells (Yi et al., 2002; Chi et al., 2006; Kim et al., 2008). Several transcription factors known to regulate the transcription of the Il10 gene in macrophages and DCs are activated downstream of ERK1/2 or p38, as is the case for cAMP response element binding protein (CREB), cyclic AMP-dependent transcription factor 1 (ATF1), cFos, and Sp1 (reviewed in Saraiva and O’Garra, 2010; Rutz and Ouyang, 2016; Gabryšová et al., 2018; Ouyang and O’Garra, 2019). Cooperation between ERK and p38 activates the mitogen- and stress-activated protein kinase 1 (MSK1) and MSK2, which promote IL-10 production in TLR4–stimulated macrophages, through the transcription factors CREB and ATF1 (Ananieva et al., 2008). The regulation of IL-10 by MSK/CREB has been further demonstrated in the context of the activation of TLR2 in intestinal macrophages by a Helicobacter hepaticus–derived polysaccharide (Danne et al., 2017). NF-κB activation downstream of PRR also regulates IL-10 production in myeloid cells. Specifically, binding of several NF-κB family members to the Il10 locus promotes IL-10 production in TLR4–stimulated macrophages (Kanters et al., 2003; Saraiva et al., 2005; Chakrabarti et al., 2008). Crosstalk between the NF-κB p105 subunit and Tpl2 has also been reported (Banerjee et al., 2006). Finally, the phosphoinositide-3-kinase (PI3K)/serine/threonine protein kinase B (Akt) cascade also regulates the expression of Il10 in macrophages by blocking the kinase glycogen synthase kinase 3β, which inhibits IL-10 production (Ohtani et al., 2008; Nandan et al., 2012), and conversely potentiating IL-10 production through ERK1/2 (Martin et al., 2003) and mechanistic target of rapamycin (mTOR; Ohtani et al., 2008; Weichhart et al., 2008) activation. Thus, activation of ERK1/2 is at the crossroads of several other signaling cascades, demonstrating its importance as a central pathway for Il10 gene regulation in myeloid cells. In addition to direct PRR signaling, coreceptors and other cytokines regulate IL-10 production by macrophages and DCs. CD40, dectin-1, and DC-specific intercellular adhesion molecule-3–grabbing non-integrin enhance PRR-induced IL-10 (reviewed in Saraiva and O’Garra, 2010; Rutz and Ouyang, 2016; Gabryšová et al., 2018; Ouyang and O’Garra, 2019). Similarly, type I IFN has been shown to potentiate IL-10 production by LPS–stimulated (Chang et al., 2007a; Howes et al., 2016) and Mycobacterium tuberculosis–infected (McNab et al., 2014) bone marrow–derived macrophages. Mechanistically, type I IFN induces transcription of the Il10 gene and stabilizes the resulting mRNA, via STAT1 and ERK activation (Howes et al., 2016).

IL-10 is produced by all T cell subsets. In CD4 Th cells, IL-10 production accompanies that of the hallmark cytokines IFN-γ,
IL-4/IL-5/IL-13, and IL-17 (reviewed in Saraiva and O’Garra, 2010; Rutz and Ouyang, 2016; Gabryšová et al., 2018; Ouyang and O’Garra, 2019). IL-10 production occurs downstream of TCR triggering, which activates Ras and subsequently MAPK ERK1/2 and API, which enhance Il10 transcription (Jones and Flavell, 2005; Wang et al., 2005; Saraiva et al., 2009). Transcription factors of the NFAT family also promote IL-10 production by T cells (Im et al., 2004; Lee et al., 2009). Additionally, STAT4, 6, and 3 activation during Th1, Th2, or Th17 CD4 T cell differentiation, respectively, have been suggested to contribute to Il10 gene expression (Saraiva et al., 2009), as have the basic leucine zipper ATF-like transcription factor and interferon regulatory factor (IRF) 4, which are critical for Th2 and Th17 differentiation (Ciofani et al., 2012; Glasmacher et al., 2012; Li et al., 2012b). Therefore, the mechanisms required for the differentiation and activation of Th cells seem to be required for regulation of IL-10 production, ensuring a feedback regulatory loop to limit immune pathology. In further support of this, the Th2 and Th17 cell lineage transcription factors GATA binding protein 3 (GATA3) and retinoid-related orphan receptor γt, respectively, have been described to modulate IL-10 production by CD4 T cells. GATA3 binds the Il10 promoter, inducing histone acetylation and remodeling the Il10 locus to facilitate Il10 gene expression (Shoemaker et al., 2006; Chang et al., 2007b), although it is not required for IL-10 production by differentiated Th2 cells (Zhu et al., 2004). In contrast, the Th17-associated transcription factor RORγt has been described to repress IL-10 production by Th17 cells (Ciofani et al., 2012). Several reports attest to the importance of PR domain zinc finger protein 1 (Blimp-1) in the regulation of IL-10 production by T cells (reviewed in Rutz and Ouyang, 2016; Ouyang and O’Garra, 2019). Blimp-1 is induced by STAT4 downstream of the IL-12R and shown to be absolutely required for IL-10 expression by IL-12–driven effector Th1 cells both in vitro and in vivo (Neumann et al., 2014). Furthermore, the induction of IL-10 by IL-27 in CD4 T cells requires Blimp-1 (Neumann et al., 2014), likely downstream of STAT1/STAT3 signaling (Stumhofer et al., 2007). IL-27 together with TCR triggering induced the differentiation of naive T cells into IL-10–producing type 1 regulatory T cells (Tr1; Pot et al., 2009). The induction of IL-10 in Tr1 cells is dependent on the cooperation between the transcription factors Eomes and Blimp-1 (Zhang et al., 2017). Of note, in vitro generation of Tr1 cells in the presence of TGF-β, in addition to IL-27, shifts the transcriptional requirements from Blimp-1 to cMaf and aryl hydrocarbon receptor (AhR; Apetoh et al., 2010; Neumann et al., 2014). Blimp-1 is also required for IL-10 expression by CD8 T cells (Sun et al., 2011).

Recent data place the leucine zipper transcription factor cMaf as a master regulator of IL-10 production, common to both myeloid and T cells (reviewed in Saraiva and O’Garra, 2010; Gabryšová et al., 2014; Rutz and Ouyang, 2016; Ouyang and O’Garra, 2019). cMaf was initially shown to bind to the Il10 promoter and enhance gene transcription in TLR4-stimulated macrophages (Cao et al., 2005). More recently, several studies support a role of cMaf in also regulating T cell–derived IL-10 in vitro (Xu et al., 2009; Apetoh et al., 2010; Ciofani et al., 2012; Gabryšová et al., 2018). IL-10 production in vivo by Th1, Th2, and Th17 cells in experimental disease models requires cMaf (Gabryšová et al., 2018). A link between cMaf and IL-10 expression by human Tr1 and Th17 cells has also been proposed (Gandhi et al., 2010; Aschenbrenner et al., 2018). cMaf cooperates with other transcription factors to regulate IL-10 expression. cMaf may require Blimp-1 to enhance IL-10 production by Th1 cells (Neumann et al., 2014). Although not absolutely required, the transcription factor AhR allows maximal IL-10 production by LPS-stimulated macrophages (Kimura et al., 2009) and T cells, where it directly binds to cMaf and synergistically transactivates the Il10 promoter (Apetoh et al., 2010). The transcription factor Bhlhe40 has been recently revealed as a repressor of IL-10 production in T cells. Mice deficient in Bhlhe40 are resistant to the development of experimental autoimmune encephalomyelitis, presumably by increasing the IL-10 production by Th1 and Th17 pathogenic cells (Lin et al., 2014). Targeted deletion of Bhlhe40 in either CD4 T cells or CD11c+ cells correlated with increased IL-10 and decreased IFN-γ production and resulted in increased bacterial burdens during infection with M. tuberculosis, rendering Bhlhe40-deficient mice more susceptible to infection (Huynh et al., 2018). Additionally, Bhlhe40 is a molecular switch for IL-10 production in Th1 cells and as a result, mice with conditional deletion of Bhlhe40 in T cells succumbed to Toxoplasma gondii infection (Yu et al., 2018). In both T cells and bone marrow–derived DCs, Bhlhe40 binds to a characterized enhancing region of the Il10 locus (Huynh et al., 2018), where the activating transcription factors API (Jones and Flavell, 2005) and IRF4 (Ahyi et al., 2009) have been shown to bind. The opposing effects of Bhlhe40 and cMaf on the regulation of IL-10 may result from the interaction between these two transcription factors. In the absence of cMaf, up-regulation of Bhlhe40 in T cells has been described (Gabryšová et al., 2018); conversely, deficiency of Bhlhe40 resulted in the up-regulation of Maf (Yu et al., 2018). Very recently, in macrophages, Bhlhe40 has been shown to bind to promoter elements of the Maf gene, repressing its transcription (Jarjour et al., 2019).

Metabolic regulation of IL-10 production
Recent findings show a metabolic reprogramming of immune cells in response to environmental cues (O’Neill and Pearce, 2016). In response to LPS and other stimuli, the metabolic profile of macrophages and DCs shifts toward glycolysis, with the accumulation of metabolites such as citrate and succinate that in turn regulate cytokine gene expression (O’Neill and Pearce, 2016). IL-10 is no exception, its production being regulated by alterations in the cell metabolic networks, as well as by specific metabolites (Fig. 1 A). The LPS-induced metabolic regulator pyruvate kinase isozyme M2 (PKM2) was shown to inhibit the cellular glycolytic reprogramming, succinate accumulation, and IL-1β production, while favoring IL-10 production by macrophages (Palsson-McDermott et al., 2018). Similarly, inhibition of the glycolytic shift promoted by M. tuberculosis infection of macrophages was shown to increase IL-10 production, while decreasing that of IL-1β (Glesson et al., 2016). In a different setting, macrophages activated by effectorcytosis during wound injury up-regulated their production of IL-10 via the SIRTUINI
IL-10 production is metabolically regulated. (A) Macrophages stimulated via TLR4 undergo a metabolic reprogramming toward glycolysis, which leads to the production of lactate and also to a break of the TCA cycle. As a result, succinate accumulates and activates HIF-1α, and the transcription of the Il1b gene is enhanced. Inhibition of this reprogramming, via the metabolic regulator PKM2 or during infection with M. tuberculosis, inhibits Il1b gene transcription, while promoting that of Il10. An increase of IL-10 production as a result of enhanced fatty acid metabolism has also been described, and is mediated by the transcription factor PBX1. In DCs, the maintenance of the pyruvate flux downstream of zymosan stimulation has been shown to reinforce the presence of acetylated histone 3 at the Il10 gene promoter, thus favoring IL-10 production. Other metabolic regulators, such as AMPK and mTOR, and metabolites, such as ATP, lactate, or butyrate, also contribute to IL-10 production by DCs, in different settings, but the molecular mechanisms in place remain largely unknown.

(B) Gut microbiota derived short chain fatty acids signal through GPR43 and activate STAT3 and mTOR in differentiated Th1 cells, up-regulating the expression of Blimp-1 and consequently that of IL-10. In IL-27-induced Tr1 cells, IL-27R signaling induces the oxysterol 25-hydroxycholesterol (25-OHC). 25-OHC activates liver X receptor (LXR) and presumably decreases the expression of the Il10 gene. In IL-27–differentiated Tr1 cells, extracellular ATP (eATP) has been shown to increase the amounts of HIF-1α and consequently reduce those of AhR. This stabilization of HIF-1α also occurs under hypoxic conditions. Given the role of AhR in enhancing IL-10 expression in Tr1 cells, HIF-1α stabilization ultimately decreases IL-10 production. The Th1 switching from IFN-γ single-producing cells, to IFN-γ/IL-10 double-producing ones, and eventually IL-10 single-producing cells, is coupled to the biosynthesis of cholesterol, via cMaf. In Th17 cells, a short chain fatty acid, pentanoate, has been suggested to inhibit AMPK activation, thus releasing the mTOR pathway, increasing the glycolytic flux, and leading to the production of IL-10. In parallel, through an epigenetic-mediated mechanism, pentanoate reduces Il17a expression.

Figure 1. IL-10 production is metabolically regulated. (A) Macrophages stimulated via TLR4 undergo a metabolic reprogramming toward glycolysis, which leads to the production of lactate and also to a break of the TCA cycle. As a result, succinate accumulates and activates HIF-1α, and the transcription of the Il1b gene is enhanced. Inhibition of this reprogramming, via the metabolic regulator PKM2 or during infection with M. tuberculosis, inhibits Il1b gene transcription, while promoting that of Il10. An increase of IL-10 production as a result of enhanced fatty acid metabolism has also been described, and is mediated by the transcription factor PBX1. In DCs, the maintenance of the pyruvate flux downstream of zymosan stimulation has been shown to reinforce the presence of acetylated histone 3 at the Il10 gene promoter, thus favoring IL-10 production. Other metabolic regulators, such as AMPK and mTOR, and metabolites, such as ATP, lactate, or butyrate, also contribute to IL-10 production by DCs, in different settings, but the molecular mechanisms in place remain largely unknown.

(B) Gut microbiota derived short chain fatty acids signal through GPR43 and activate STAT3 and mTOR in differentiated Th1 cells, up-regulating the expression of Blimp-1 and consequently that of IL-10. In IL-27–induced Tr1 cells, IL-27R signaling induces the oxysterol 25-hydroxycholesterol (25-OHC). 25-OHC activates liver X receptor (LXR) and presumably decreases the expression of the Il10 gene. In IL-27–differentiated Tr1 cells, extracellular ATP (eATP) has been shown to increase the amounts of HIF-1α and consequently reduce those of AhR. This stabilization of HIF-1α also occurs under hypoxic conditions. Given the role of AhR in enhancing IL-10 expression in Tr1 cells, HIF-1α stabilization ultimately decreases IL-10 production. The Th1 switching from IFN-γ single-producing cells, to IFN-γ/IL-10 double-producing ones, and eventually IL-10 single-producing cells, is coupled to the biosynthesis of cholesterol, via cMaf. In Th17 cells, a short chain fatty acid, pentanoate, has been suggested to inhibit AMPK activation, thus releasing the mTOR pathway, increasing the glycolytic flux, and leading to the production of IL-10. In parallel, through an epigenetic-mediated mechanism, pentanoate reduces Il17a expression.

signaling cascade and the transcription factor PBX1, driven by the respiratory chain (Zhang et al., 2019). In an experimental model of colitis, chemical inhibition of the nicotinamide phosphoribosyltransferase enzyme reduced mucosal nicotinamide adenine dinucleotide levels and the activities of nicotinamide adenine dinucleotide–dependent enzymes, while favoring the polarization of monocyte/macrophages toward IL-10 production (Gerner et al., 2018). IL-10 production by DCs is also modulated by metabolic pathways. Several metabolites, such as butyrate, adenosine, ATP, or lactic acid, have been proposed to favor IL-10 production by DCs (Li et al., 2008; Amiel et al., 2012; Boor et al., 2013; Hussaarts et al., 2013). Adiponectin also enhances IL-10 production by DCs through a pathway that depends on the cellular energy sensor AMP-activated protein kinase (AMPK; Tan et al., 2014). Moreover, a recent study showed that in DCs stimulated with zymosan, the production of IL-10 depended on the maintenance of pyruvate flux, as it reinforces the presence of acetylated histone 3 molecules bound to the Il10 gene promoter (Marquez et al., 2019).

T cell immunity also relies on metabolic reprogramming (Buck et al., 2015), but how this affects IL-10 production is only now starting to be understood (Fig. 1 B). Initial evidence for this came from the finding that extracellular ATP or hypoxia inhibits the transcription factor HIF-1α in Tr1 cells, which in turn down-regulates AhR and IL-10 production (Apetoh et al., 2010; Mascanfroni et al., 2015). In addition, oxysterols, oxidized forms of cholesterol produced downstream of the IL-27R, negatively regulate IL-10 secretion in Tr1 cells via activation of the liver X receptor signaling and possibly through inhibition of the IL-10–inducing transcription factor Blimp-1 (Vigne et al., 2017). Furthermore, inhibition of cholesterol biosynthesis blocked the induction of cMaf and consequently IL-10 production by IFN-γ–producing Th1 cells (Perucha et al., 2019). Finally, the short-chain fatty acid pentanoate has been shown to induce IL-10 production in lymphocytes by reprogramming their metabolic–epigenetic crosstalk toward elevated glucose oxidation, while in parallel suppressing Th17 cells (Luu et al., 2019). Of note, in the same study, pentanoate has been shown to enhance Il10 gene
expression in regulatory B cells (Luu et al., 2019). Interestingly, gut microbiota–derived short-chain fatty acids have also been shown to induce Th1 cells to produce IL-10 to maintain intestinal homeostasis, through the activation of STAT3 and mTOR, upstream of Blimp-1 up-regulation (Sun et al., 2018). Despite these studies, the molecular mechanisms concerning IL-10 regulation by metabolic changes or metabolites across different subsets of T cells remain elusive. However, careful interpretation of experiments should be exercised. Indeed, findings from a recent study show that the widely used glucose analogue 2-deoxyglucose inhibited IL-10 production by Th1, Th2, and Th17 cells not through the inhibition of glycolysis, but instead through the modulation of glycosylation on cell surface proteins (Gabryšová et al., 2018).

The regulation of IL-10 in other cell types
In contrast to macrophages/DCs and T cells, the mechanisms regulating IL-10 in other cells are less explored and understood. IL-10 production by PRR-stimulated microglia follows the general basic mechanisms seen in peripheral myeloid cells (reviewed in Lobo-Silva et al., 2016). Molecules present in the microenvironment, such as adenosine, ATP, or glutamate, have been described to potentiate IL-10 production by microglia, but the precise mechanisms underlying these effects are mostly unknown (Seo et al., 2004, 2008; Werry et al., 2011; Koscsó et al., 2012; and reviewed in Lobo-Silva et al., 2016). Furthermore, other cytokines may impact IL-10 secretion by microglia. Although the effect of IFN-γ in regulating microglia-derived IL-10 is still controversial (Veroni et al., 2010; Ishii et al., 2013), type I IFN has been shown to up-regulate IL-10 in PRR-induced microglia (Lobo-Silva et al., 2017), similar to what has been described for bone marrow–derived macrophages (reviewed in Ouyang and O’Garra, 2019).

The regulation of IL-10 production by epithelial cells is also not fully understood. Engagement of CD1d on intestinal epithelial cells has been shown to induce IL-10 (Colgan et al., 1999), via a mechanism that involves the activation of STAT3 and STAT3-dependent transcription of IL-10 (Olszak et al., 2014). Crosstalk between TLR4-activated macrophages and intestinal epithelial cells has also been shown to sustain the expression of IL-10 in the latter, by the action of nuclear receptor peroxisome proliferator-activated receptor γ (Hyun et al., 2015). Furthermore, microbial and/or environmental oxazoles have been shown to induce tryptophan-derived metabolites and activate AhR in intestinal epithelial cells. The activated AhR in turn limits CD1d-restricted production of IL-10 by these cells (Iyer et al., 2018). Thus, metabolites in the intestine regulate IL-10 production. Given the importance of intestinal epithelial cells in maintaining the intestinal barrier and of IL-10 in controlling intestinal inflammation, more research in this area is needed.

The effects of IL-10 in responding cells
The IL-10R signaling cascade in brief
The cellular response to IL-10 depends on engagement of the IL-10R and intracellular signaling cascades (Fig. 2). IL-10, as the other family members, signals through a heterodimeric receptor (Pestka et al., 2004). In the case of IL-10, the receptor is composed two subunits: the high-affinity IL-10Ra, mainly expressed in leukocytes, and the ubiquitously expressed IL-10Rβ, which is shared by the receptor complex of other IL-10 family members (reviewed in Zdanov, 2010; Ouyang and O’Garra, 2019). The intracellular signaling cascades downstream of the IL-10R have been described in detail in previous reviews (Donnelly et al., 1999; Murray, 2006b; Ouyang et al., 2011). Building upon the extensive literature in this topic, an IL-10/IL-10R signaling map has been assembled (Verma et al., 2016).

Engagement of the IL-10Ra leads to its oligomerization with the IL-10Rβ subunit (Yoon et al., 2006). This in turn allows the phosphorylation of JAK1 associated with the IL-10Ra subunit and of tyrosine kinase 2 (TYK2) associated with the IL-10Rβ subunit (Rodig et al., 1998; Riley et al., 1999). These kinases further phosphorylate two functional tyrosine residues on the intracellular domain of the IL-10Ra, needed for the recruitment of STAT3 (Weber-Nordt et al., 1996). Activation of STAT3 by its phosphorylation allows its translocation to the nucleus, with the initiation of a specific transcriptional program that largely defines the IL-10–mediated anti-inflammatory response (Takeda et al., 1999; Lang et al., 2002; Williams et al., 2004a; reviewed in Murray, 2006b; Hutchins et al., 2013). In addition to the JAK/STAT3 pathway, the signaling cascade PI3K/Akt/GSK3β has also been shown to mediate IL-10–induced transcription in macrophages (Crawley et al., 1996; Antoniv and Ivashkov, 2011). Furthermore, IL-10 stimulation of primary monocytes results in PI3K-mediated mTORC1 activity (Crawley et al., 1996). Interestingly, IL-10 activation of the both STAT3 and PI3K/Akt/mTORC1 pathways in macrophages was shown to require AMPK (Zhu et al., 2015). Of note, activation of STAT1 and STAT5 downstream of the IL-10R has also been reported (Lehmann et al., 1994; Weiheger et al., 1996; Rahimi et al., 2005; Emmerich et al., 2012).

Interestingly, STAT3 activation has also been described for other cytokines in addition to IL-10, most notably IL-6 (Fig. 2). It is surprising that both pro- and anti-inflammatory cytokines may signal through the same molecule even in the same cell type, despite inducing profoundly different responses, and resulting in a different gene activation program (reviewed in Murray, 2006a, 2007; Hutchins et al., 2013). A likely explanation for this apparent paradox relies on the cooperation of STAT3 with selective cofactors to deliver a different gene expression program. One such cofactor is the suppressor of cytokine signaling 3 (SOCS3), which, despite being induced by both IL-10 and IL-6 in macrophages, only suppresses the activity of the IL-6R (gp130; Croker et al., 2003; Lang et al., 2003). Indeed, the IL-10R appears refractory to the effects of all members of the SOCS family (Williams et al., 2004b). Additionally, it is possible that in response to different stimuli, the epigenetic landscape of the STAT3-regulated genes changes, thus allowing for selective transcription of anti-inflammatory or inflammatory mediators (reviewed in Murray, 2006a, 2007; Hutchins et al., 2013).

Molecular basis for IL-10/STAT3 anti-inflammatory activities
As discussed above, a wealth of data positions the IL-10/STAT3 axis as a major transcriptional inhibitor of genes encoding cytokines, chemokines, cell-surface molecules, and other molecules required
for a full immune response (Murray, 2005; reviewed in Murray, 2006b). The requirement for STAT3 as a mediator of IL-10 responses is well-established (Takeda et al., 1999; Lang et al., 2002; Williams et al., 2004a). Macrophage/neutrophil-restricted deletion of STAT3 mimics IL-10 deficiency itself, resulting in the development of enterocolitis and high susceptibility to LPS-mediated shock and septic peritonitis (Takeda et al., 1999; Kobayashi et al., 2003; Matsukawa et al., 2003, 2005). How STAT3 activation results in the anti-inflammatory activity of IL-10 is not fully understood (Fig. 2).

Several independent reports performed over the years globally show that STAT3 acts indirectly by inducing the expression of a series of genes that execute the inhibitory effects associated with an anti-inflammatory response (reviewed in Hutchins et al., 2013). Among the STAT3-dependent anti-inflammatory mediators are several transcription factors, such as the E26 transformation–specific family transcriptional repressor, ETV3, and a helicase family corepressor, Strawberry notch homologue 2, that inhibit NF-κB–mediated transcription in LPS-stimulated macrophages (El Kasmi et al., 2007); and Nfil3, which negatively regulates the production of IL-12p40 by LPS-stimulated myeloid cells (Smith et al., 2011). Other molecular targets of IL-10–activated STAT3 have been described, including the up-regulation of the dual-specificity phosphatase 1, which impairs the inflammatory response of LPS-induced macrophages by down-regulating the activity of the MAPK p38 (Hammer et al., 2005). Also, in LPS-stimulated macrophages, a link between IL-10/STAT3 and the induction of the RNA destabilizing factor tristetraprolin has been proposed, and shown to be mediated by a delayed activation of p38 MAPK, resulting in diminished mRNA and protein levels of proinflammatory cytokines (Schaljo et al., 2009). Finally, in LPS-activated macrophages, IL-10 was shown to inhibit the transcription of the microRNA miR-155 in a STAT3-dependent manner, leading to increased expression of SH2 domain-containing inositol 5′-phosphatase 1 (SHIP1; McCoy et al., 2010), a negative regulator of TLR signaling (An et al., 2005). STAT3 is moreover activated downstream of receptors for other IL-10 family cytokines, IL-6, and a number of other cytokines and growth factors. How the cell distinguishes the anti- from the pro-inflammatory reprogramming downstream of STAT3 activation is not fully understood, but the IL-10–induced SOCS3 has been reported to play an important role by blocking STAT3 at least downstream of the IL-6R, but not of the IL-10R. DUSP, dual-specificity phosphatase; Gsk3β, glycogen synthase kinase 3β; Sbn2, protein strawberry notch 2; TTP, tristetraprolin.

Two genome-wide studies were performed to reveal the universe of STAT3-regulated genes. The obtained data support a model of indirect STAT3 action in response to IL-10. The first study integrated STAT3 chromatin immunoprecipitation, RNA-sequencing, and computational approaches performed in

---

**Figure 2.** The anti-inflammatory response initiated downstream of the IL-10R. Binding of IL-10 to its receptor activates a major JAK1-TYK2-STAT3 signaling cascade, which culminates with the induction of the STAT3-mediated anti-inflammatory response. The STAT3 transcriptional reprogramming results in a number of molecules that include transcriptional repressors, chromatin modifiers, and post-transcriptional or post-translational regulators. Together, these molecules will limit the inflammatory response induced for example downstream of PRR activation. Recently, IL-10 has been shown to modulate the transcription of several metabolic regulators, including DDIT4, in a STAT3-dependent way. As DDIT4 is a regulator of mTORC, the IL-10R signaling limits the glycolytic flux downstream of TLR4 activation, which in turn controls the inflammatory response of the macrophage. In addition to the JAK–STAT3 pathway, STAT1, STAT5, and the PI3K-Akt cascade have also been reported to mediate IL-10 responses. The metabolic regulator AMPK has been reported to activate the PI3K-Akt-mTORC cascade, but also STAT3, in response to IL-30. STAT3 is moreover activated downstream of receptors for other IL-10 family cytokines, IL-6, and a number of other cytokines and growth factors. How the cell distinguishes the anti- from the pro-inflammatory reprogramming downstream of STAT3 activation is not fully understood, but the IL-10–induced SOCS3 has been reported to play an important role by blocking STAT3 at least downstream of the IL-6R, but not of the IL-10R. DUSP, dual-specificity phosphatase; Gsk3β, glycogen synthase kinase 3β; Sbn2, protein strawberry notch 2; TTP, tristetraprolin.
IL-10–treated resting or LPS-stimulated macrophages (Hutchins et al., 2012). The second study used chromatin immunoprecipitation sequencing to analyze the binding of STAT3 at the genomic level in DCs following histone deacetylases inhibition (Sun et al., 2017). Regardless of the critical role of STAT3 in mediating the response downstream of the IL-10R, it is possible that the STAT3 pathway may not be sufficient to mediate the full action of IL-10. Other anti-inflammatory mediators have been described downstream of the IL-10R, but their dependency on STAT3 is as yet unclear. Such is the case for Bcl3, which inhibits TNF production in LPS-treated macrophages via impairment of NF-κB (Kuwata et al., 2003) and A2O-binding inhibitor of NF-κB activation 3, which attenuates NF-κB signaling in human but not mouse macrophages (Weaver et al., 2007). IL-10–induced heme-oxygenase 1 has also been shown to contribute to the anti-inflammatory response triggered in macrophages (Lee and Chau, 2002).

Some lines of evidence suggest that chromatin accessibility might also be a mechanism of regulation of gene expression by the IL-10/STAT3 axis. For example, HDAC4, which acts as a repressor of gene expression by deacetylating histones, was identified as a STAT3 target (Hutchins et al., 2012). Furthermore, in adipose tissue, IL-10 has been shown to repress transcription of thermogenic genes by altering chromatin accessibility in a STAT3-dependent manner (Rajbhandari et al., 2018).

**Classic roles of IL-10**

In addition to being produced by many different cell types, IL-10 functionally targets diverse cells, resulting in a broad anti-inflammatory activity. As compared with most other cell types, macrophages express higher levels of the IL-10R (Moore et al., 2001). Compelling evidence provided by the study of cell-restricted IL-10R deficiency has pointed to macrophages being the main targets of the IL-10 inhibitory effects (Shouval et al., 2014a; Zigmond et al., 2014). Indeed, soon after its discovery, IL-10 was shown to trigger a robust immune suppressive response in macrophages and other APCs, mainly via the transcriptional inhibition of cytokines and chemokines, as well as of MHCIi, and costimulatory and adhesion molecules (Bogdan et al., 1991; de Waal Malefyt et al., 1991a,b; Fiorentino et al., 1991a,b; Ding and Shevach, 1992; D’Andrea et al., 1993; Willems et al., 1994; Creery et al., 1996). Overall, IL-10 both modulates the local cytokine micro-environment and limits antigen presentation, thus preventing the efficient development of T cell responses. Additionally, IL-10 also limits basic microbicidal mechanisms, namely the induction of nitric oxide synthase and the production of nitric oxide, as well as the release of reactive oxygen intermediates (Bogdan et al., 1991) by murine macrophages stimulated through PRR in the context of IFN-γ (Cunha et al., 1992).

The suppressive effect of IL-10 appears to be targeted to specific genes. This is well illustrated by a gene expression screen for IL-10–induced genes performed in LPS-stimulated macrophages, which identified a 15–20% inhibition of the LPS-induced genes (Lang et al., 2002). A more recent study further elucidated the mechanisms underlying the suppressive activity of IL-10 in LPS-stimulated macrophages, through genome-wide approaches. In line with previous reports, inhibition of transcription was found to be the primary mechanism for IL-10–mediated suppression (Conaway et al., 2017). Novel targets of IL-10 in macrophages have been recently revealed, including the modulation of the macrophage metabolic reprogramming downstream of TLR4 activation (Ip et al., 2017). Specifically, IL-10 inhibited LPS-induced glucose uptake and glycolysis, promoting a shift toward oxidative phosphorylation (Ip et al., 2017).

Inhibition of cytokine and chemokine production by IL-10 is also documented for neutrophils (Cassatella et al., 1993; Kasama et al., 1994) and for resident macrophages, such as microglia (Balasingam and Yong, 1996; and reviewed in Lobo-Silva et al., 2016). However, and most interestingly, the comparison of the anti-inflammatory response induced by IL-10 across different LPS-stimulated myeloid cells showed a divergent pattern. Specifically, in macrophages, IL-10 mostly inhibited NF-κB target genes, whereas in DCs and mast cells, an indirect disruption of IRF was proposed, and in neutrophils, IL-10 appeared to induce both IRF disruption and indirect NF-κB inhibition (Hutchins et al., 2015).

Classically, IL-10 is known to inhibit T cell responses via APCs (Macatonia et al., 1993). However, IL-10 can also limit T cell responses by acting directly on CD4 T cells to induce nonresponsiveness or anergy (Groux et al., 1996). More recently, IL-10 has been shown to act directly on memory/effector T cells (Kamanaka et al., 2011) in experimental colitis, in Th17 cells during intestinal inflammation (Huber et al., 2011), and in Th2 cells in a murine model of house dust mite allergy (Coomes et al., 2017). Surprisingly, IL-10–stimulatory effects on T cells have also been described. IL-10 enhances CD8 T cell proliferation, cytotoxic activity, and IFN-γ production (Groux et al., 1998; Mumm et al., 2011; Naing et al., 2018). IL-10 promotes the survival and action of Foxp3 regulatory T cells (Murai et al., 2009; Chaudhry et al., 2011). Stimulatory activities of IL-10 on mast cells and B cells have also been well documented (Thompson-Snipes et al., 1991; Rouset et al., 1992; Itoh and Hirohata, 1995).

**Nonclassic roles of IL-10**

Although the effects of IL-10 have been classically studied in immune cells, the range of IL-10–responding cells is expanding (Fig. 3). It is now evident that IL-10 also acts in nonhematopoietic cells, where it plays important homeostatic roles. An example of this comes from central nervous system (CNS) and peripheral nervous system cells, which respond to IL-10 and other IL-10 family members (reviewed in Lobo-Silva et al., 2016; Burmeister and Marriott, 2018). In this context, IL-10 plays important roles in limiting neuronal damage during infection or other inflammatory processes, as well as in regulating homeostatic processes. IL-10 has been associated with increased neuronal survival (Zhou et al., 2009a,b) and the regulation of adult neurogenesis (Perez-Asensio et al., 2013; Perreira et al., 2015). Administration of IL-10 in different settings proved beneficial for axon regeneration, through mechanisms that implicate STAT3 and the regulation of NF-κB signaling (reviewed in Vidal et al., 2013). Finally, IL-10 was shown to reduce vulnerability of neurons to CNS ischemia and trauma (Knoblah and Faden, 1998; Grilli et al., 2002).
Importantly, whether the effects of IL-10 on neurons and oligodendrocytes are direct or indirect is still controversial. To support a direct effect on cells of the CNS, resting astrocytes express the IL-10R and respond to IL-10 (Molina-Holgado et al., 2001; Ledeboer et al., 2002; Xin et al., 2011; Perriard et al., 2015). IL-10 limits reactive astrogliosis in response to pathogens and promotes the expression of TGF-β by astrocytes (Balasingam and Yong, 1996; Norden et al., 2014; and reviewed in Burmeister and Marriott, 2018). At the organismal level, variations in IL-10 expression have been associated with altered depressive-like behavior. IL-10–deficient female mice displayed increased depressive-like behavior, whereas IL-10–overexpressing mice displayed a decreased depressive-like behavior (Mesquita et al., 2008). More research is clearly needed to fully elucidate how IL-10 regulates the CNS in health and disease.

Another nonclassic function ascribed to IL-10 relates to its regulation of adipose tissue (Fig. 3). Epidemiological studies link a low production of IL-10 with metabolic syndrome and type 2 diabetes (van Exel et al., 2002), but in animal models, ablation of IL-10 did not support an anti-obesity function for this cytokine (den Boer et al., 2006; Miller et al., 2011; Mauer et al., 2014). However, previous studies proposed that IL-10 might promote the activity of M2 macrophages in adipose tissue (Lumeng et al., 2007; Hong et al., 2009; Gao et al., 2013; Xie et al., 2014), or act directly on adipocytes to decrease their inflammatory response (Lira et al., 2012). Very recently, and contrary to the previously established idea that IL-10 might stimulate brown tissue activity by limiting inflammation, direct IL-10 signaling in adipocytes was strikingly shown to limit thermogenesis and energy expenditure (Rajbhandari et al., 2018). Mechanistically this effect involved remodeling the chromatin landscape of adipocytes and specific transcriptional down-regulation of thermogenic genes (Rajbhandari et al., 2018). As a result, mice lacking IL-10 had increased energy expenditure and were protected against diet-induced obesity (Rajbhandari et al., 2018). It will be interesting in future to evaluate the therapeutic value of IL-10 manipulation in obesity, taking all these findings into consideration.
The last “nonclassic” role of IL-10 that we will discuss relates to its function as a promoter of epithelial wound repair (Fig. 3). Several studies highlight the IL-10-mediated pro-repair activities, which have been classically attributed to its anti-inflammatory activities (reviewed in Sziksz et al., 2015). However, this concept is changing, and an effect of IL-10 as a regulator of the extracellular matrix and fibroblast function is emerging (Balaji et al., 2017; and reviewed in King et al., 2014). Similarly, the critical role of IL-10 in gut homeostasis is well documented, with the majority of the studies focusing on its anti-inflammatory activities (reviewed in Kole and Maloy, 2014). Recent evidence reveals an additional role for IL-10 as an orchestrator of epithelial wound repair (Loren et al., 2015); however, the molecular bases for this are only starting to be unveiled. Macrophage-derived IL-10 was shown to activate CREB signaling in intestinal epithelial cells, leading to the secretion of the WNT1-inducible signaling protein 1, which in turn induced epithelial cell proliferation and wound closure (Quiros et al., 2017). Furthermore, up-regulation of the IL-10R in intestinal epithelial cells has been described (Kominsky et al., 2014), and recent studies not only confirm the importance of the IL-10R in wound healing but also implicate tryptophan metabolites and AhR in its up-regulation (Lanis et al., 2017).

**Therapeutic manipulation of IL-10**

Given the wide spectrum of the anti-inflammatory properties attributed to IL-10, therapeutic manipulation of this cytokine has attracted a great deal of interest (reviewed in O’Garra et al., 2008; Ouyang and O’Garra, 2019; Wang et al., 2019). We next discuss the therapeutic opportunities for IL-10 in various pathologies, and the strategies used to manipulate IL-10 levels in the organism (Fig. 4).

**Enhancing IL-10 in inflammatory bowel disease (IBD)**

IBD is perhaps the best studied disease in the context of IL-10, owing to the initial findings that IL-10-deficient mice develop colitis (Kühn et al., 1993), together with the profound genetic association of IBD with deficient IL-10 responses (Glocker et al., 2009, 2010, 2011; Jostins et al., 2012; Moran et al., 2013; Engelhardt and Grimbacher, 2014; Ellinghaus et al., 2016). Administration of IL-10 or IL-10 overexpression in different animal models of colitis has proved consistently beneficial (Hagenbaugh et al., 1997; Steidler et al., 2000; Cardoso et al., 2018). In humans, systemic (intravenous or subcutaneous) administration of recombinant IL-10 has been tested to treat IBD in different trials, but in general it did not significantly improve the clinical outcome of the patients (van Deventer et al., 1997; Fedorak et al., 2000; Schreiber et al., 2000; Colombel et al., 2001). A likely explanation for this relies on the possible low concentration of IL-10 achieved locally. Thus, efforts have been placed in designing strategies to specifically deliver IL-10 to the intestine. An interesting approach has been the development of bacteria engineered for intestinal IL-10 delivery. Specifically, the dairy microbe Lactococcus lactis has been modified to express the il10 gene and intragastrically administered to mice (Steidler et al., 2000). This strategy has proved efficient in dextran sulfate sodium−induced and spontaneous (IL-10−deficient) colitis models (Steidler et al., 2000). Subsequent improvements of the engineered L. lactis have been devised, all with positive effects in ameliorating experimental colitis (reviewed in Shigemori and Shimosato, 2017; Wang et al., 2019). Genetic modification of Bifidobacterium bifidum for IL-10 expression has also been described (Mauras et al., 2018), thus expanding the species of bacteria amenable for IL-10 intestinal delivery. Although only tested in a small cohort of Crohn’s disease patients, therapeutic L. lactis expressing human IL-10 ameliorated the clinical score of disease (Braat et al., 2006), which, together with the promising findings obtained in the mouse model, suggests that further improving these approaches may render them applicable for human therapy.

**IL-10 as an immunotherapy for cancer**

The immunosuppressive role of IL-10 has led to the general view that its presence during cancer would facilitate tumor immune escape (Dunn et al., 2004). This hypothesis is validated in several animal models. Overexpression of IL-10 resulted in failure to control an immunogenic tumor (Hagenbaugh et al., 1997), and blockade of IL-10R increased the therapeutic benefit of chemotherapy in a breast cancer model (Ruffell et al., 2014) and to other transplantable tumors in the context of CpG immunostimulatory oligonucleotide by reversal of tumor-induced DC paralysis (Vicari et al., 2002). Likewise, IL-10 deficiency conferred resistance to UV-induced skin carcinogenesis (Loser et al., 2007). In patients, the presence of IL-10 has been described in the tumor-microenvironment of different cancers and overall correlated with poor prognosis (Nemunaitis et al., 2001; Mannino et al., 2015; reviewed in O’Garra et al., 2008). However, early lines of evidence suggest an unanticipated protective role for IL-10 in cancer. Both IL-10 overexpression and IL-10 administration have been reported to associate with tumor shrinkage and rejection (Berman et al., 1996; Zheng et al., 1996; Groux et al., 1999). More recently, administration of pegylated IL-10 to mouse models of breast cancer or squamous cell carcinoma provided compelling preclinical evidence on the efficacy of IL-10 as an immunotherapy for cancer (Mumm et al., 2011). Mechanistically, IL-10 increased CD8 T cell infiltration in tissue, induced IFN-γ production, and favored effective T cell memory responses (Mumm et al., 2011; Emmerich et al., 2012). Prompted by these studies, a pegylated human IL-10 was developed (Naing et al., 2016) and shown to induce hallmarks of CD8 T cell immunity in patients with solid tumors (Naing et al., 2018). Thus, IL-10 may in some types of cancer be useful as an immunotherapy by potentiating the activity of antitumor CD8 T cells (reviewed in Autio and Oft, 2019; Ouyang and O’Garra, 2019; Wang et al., 2019). However, a recent study has shown that tumor-infiltrating regulatory T cells through their expression of IL-10 and IL-35 may contribute to the exhaustion of intratumoral CD8 T cells, via the up-regulation of the transcription factor Blimp-1 (Sawant et al., 2019). Several factors may explain the discrepancies between these different studies, including the cell targeted by IL-10 in the different tumors (for example, myeloid versus T cell), or differences in the T cell response to IL-10 at different effector stages. Thus, it is critical to determine the
context whereby IL-10 may provide a protective or detrimental role in the treatment of tumors, in order to appropriately tailor cancer therapy.

The potential of IL-10 to treat other diseases

In addition to IBD and cancer, the therapeutic manipulation of IL-10 has been envisaged in the context of several other pathologies (reviewed in O’Garra et al., 2008; Ouyang and O’Garra, 2019; Wang et al., 2019). In both rheumatoid arthritis and psoriasis, IL-10 administration has yielded some promising results at the preclinical and clinical levels (McInnes et al., 2001; Trachsel et al., 2007; Galeazzi et al., 2014). In allergic asthma, where pathological responses to inhaled allergens develop due to a failure of immune tolerance, successful therapeutic strategies are linked to an increase of IL-10. This is the case for the glucocorticoid dexamethasone, which favors IL-10 production by human CD4 and CD8 T cells (Richards et al., 2000; Barrat et al., 2002). Furthermore, severe steroid-resistant asthma associates with failure of patient cells to enhance IL-10 in response to dexamethasone (Xystrakis et al., 2006; Gupta et al., 2014). A recent study showed that the dexamethasone-driven IL-10 production by human memory CD4 T cells is accompanied by that of IL-17A, IL-17F, and IL-22. Interestingly, administration of IL-10 to a small cohort of chronic hepatitis C patients who had not previously responded to IFN-based therapies did not impact antiviral activity and resulted in improved liver histology and function, as well as in reduced liver fibrosis in a large proportion of patients receiving treatment (Nelson et al., 2000). In studies using experimental models of Listeria monocytogenes (Dai et al., 1997), lymphocytic choriomeningitis virus (Brooks et al., 2006), Leishmania major (Belkaid et al., 2001), Leishmania donovani (Murray et al., 2002), and M. tuberculosis (Redford et al., 2010), IL-10 deficiency resulted in

Figure 4. The potential of enhancing IL-10 to treat disease. Several IL-10 enhancing strategies have been tried in a number of diseases and models of disease. The best understood effects of IL-10 at the mechanistic level have been described in the case of IBD and cancer. Side effects of IL-10 administration have been reported in both patients and healthy volunteers, but the underlying mechanisms remain unknown. The human figure represents clinical data, and the mouse figure preclinical findings. HCV, hepatitis C virus.

Saraiva et al. Journal of Experimental Medicine
New insights on the anti-inflammatory cytokine IL-10

Burmeister and Marriott, 2018). These included administration of recombinant IL-10, enhancement of IL-10 production through agonists, or delivery of IL-10 through viral vectors (reviewed in Kwilasz et al., 2015). However, the preclinical success of IL-10 in this setting has been conflicting, and targeting IL-10 to treat neuroimmune disorders has not been tested in the clinic. An opposing strategy consists of IL-10 blockade, in pathologies where its excess is detrimental. This is the case of systemic lupus erythematos. A small clinical trial performed in systemic lupus erythematos patients showed an improvement of symptoms following IL-10 blockade (Llorente et al., 2000).

Given the anti-inflammatory action of IL-10, its therapeutic manipulation in infection is appealing. However, the effects of IL-10 in infection significantly depend on the infecting agent (reviewed in Couper et al., 2008; Redford et al., 2011). In a number of experimental models of infection including T. gondii (Gazzinelli et al., 1996), malaria (Li et al., 1999), and Trypanosoma cruzi (Hunter et al., 1997), ablation of IL-10 led to minimal pathogen clearance, while allowing fatal tissue damage. Interestingly, administration of IL-10 to a small cohort of chronic hepatitis C patients who had not previously responded to IFN-based therapies did not impact antiviral activity and resulted in improved liver histology and function, as well as in reduced liver fibrosis in a large proportion of patients receiving treatment (Nelson et al., 2000). In studies using experimental models of Listeria monocytogenes (Dai et al., 1997), lymphocytic choriomeningitis virus (Brooks et al., 2006), Leishmania major (Belkaid et al., 2001), Leishmania donovani (Murray et al., 2002), and M. tuberculosis (Redford et al., 2010), IL-10 deficiency resulted in
increased pathogen clearance, beneficial to the host. In the case of tuberculosis, both human and mouse studies highlight a role for IL-10 in limiting the host protective immune response (reviewed in Redford et al., 2011). Interestingly, a recent study has shown that the delivery of aerosolized peptide inhibitors targeting the IL-10/STAT3 pathway to mice chronically infected with M. tuberculosis led to a reduction of bacterial burden accompanied by the up-regulation of antimicrobial effector molecules and of apoptotic/autophagy mediators (Upadhyay et al., 2018).

Despite the large number of disease settings where IL-10 modulation has been tried, of which we only discussed a few, the overall data are conflicting, and a clear argument in favor of IL-10 as a therapy is still nonexistent. Several variables need to be fine-tuned, including the anatomical location where IL-10 is needed for treatment and the context of diseases where IL-10 has a stimulatory or anti-inflammatory effect.

Secondary effects associated with increased doses of IL-10

Most data assessing the side effects of IL-10 administration have been obtained in the context of systemic administration of recombinant IL-10 to Crohn’s disease patients. The overall results show that systemic administration of recombinant IL-10 is well-tolerated, particularly at low doses (van Deventer et al., 1997). However, when higher doses of IL-10 were administered, some unexpected side effects have been observed (Fig. 4), including fatigue and headache, accompanied by a reduction of red blood cells and thrombocytes (Buruiana et al., 2010). Additionally, the induction of the pro-inflammatory cytokine IFN-γ and decreased hemoglobin and platelet counts have been reported (Tilg et al., 2002b). Similar side effects have been described during the administration of recombinant IL-10 to treat psoriatic arthritis (Mclnnes et al., 2001). Importantly, these effects seemed to be reversible (Tilg et al., 2002a) and did not appear to be disease-related, as even in healthy volunteers, an up-regulation of IFN-γ in the serum has been observed (Lauw et al., 2000). The mechanisms underlying this immune-stimulatory role of IL-10 remain unknown. It has been suggested that the observed anemia might be caused by an IL-10-driven deregulation of iron metabolism (Tilg et al., 2002a). Whether the up-regulation of IFN-γ and granzyme B result from the activation of CD8 T cells, as shown in the case of pegylated IL-10, or from alternative mechanisms remains unknown. More research is needed to elucidate the aforementioned effects of IL-10, as they may provide key insights on combinatory therapies to increase the efficacy of IL-10 in inflammatory conditions.

Concluding remarks

The wide range of IL-10–producing and IL-10–responding cells offers the immune system the possibility of regulating the immune response in different situations and anatomical sites. However, its opposing context-specific anti-inflammatory and stimulatory effects make the therapeutic manipulation of IL-10 challenging. Progress in this area will require a deeper understanding of IL-10 biology, specifically of the molecular mechanisms that govern IL-10 production and action, as well as of the contrasting roles this cytokine may play in different contexts. Also important will be obtaining information on nonclassic functions of IL-10, which are only now being unveiled. Given the history of IL-10 research, exciting new findings on the biology of this cytokine are expected in the future.

Acknowledgments

M. Saraiva is financed by Fundo Europeu de Desenvolvimento Regional funds through the COMPETE 2020 Operational Program for Competitiveness and Internationalisation, Portugal 2020, and by Portuguese funds through Fundação para a Ciência e Tecnologia in the framework of the project “Institute for Research and Innovation in Health Sciences” (POCI-01-0145-FEDER-007274), and by Fundação para a Ciência e Tecnologia through Estímulo Individual ao Emprego Científico. P. Vieira is funded by Agence National de la Recherche, through the project MYELOTEN (ANR-13-ISV1-0003-01), and by the Institut Pasteur, France. A. O’Garra is funded by the Francis Crick Institute, which receives its core funding from Cancer Research UK (FC001126), the UK Medical Research Council (FC001126), and the Wellcome Trust (FC001126).

The authors declare no competing financial interests.

Submitted: 10 June 2019
Revised: 5 August 2019
Accepted: 11 September 2019

References

Ahyi, A.N., H.C. Chang, A.L. Dent, S.L. Nutt, and M.H. Kaplan. 2009. IFN regulatory factor 4 regulates the expression of a subset of Th2 cytokines. J. Immunol. 183:1598–1606. https://doi.org/10.4049/jimmunol.0803302
Amiel, E., B. Everts, T.C. Freitas, L.L. King, J.D. Curtis, E.L. Pearce, and E.J. Pearce. 2012. Inhibition of mechanistic target of rapamycin promotes dendritic cell activation and enhances therapeutic autologous vaccination in mice. J. Immunol. 189:2151–2158. https://doi.org/10.4049/jimmunol.1103741
An, H., H. Xu, M. Zhang, J. Zhou, T. Feng, C. Qian, R. Qi, and X. Cao. 2005. Src homology 2 domain-containing inositol-5-phosphatase 1 (SHIP1) negatively regulates TLR4-mediated LPS response primarily through a phosphatase activity- and PI-3K-independent mechanism. Blood. 105:4685–4692. https://doi.org/10.1182/blood-2005-01-0191
Ananieva, O., J. Darragh, C. Johansen, J.M. Carr, J. McIhrath, J.M. Park, A. Wingate, C.E. Monk, R. Toth, S.G. Santos, et al. 2008. The kinases MSK1 and MSK2 act as negative regulators of Toll-like receptor signaling. Nat. Immunol. 9:1028–1036. https://doi.org/10.1038/ni.1644
Antoniv, T.T., and L.B. Ivashkiv. 2011. Interleukin-10-induced gene expression and suppressive function are selectively modulated by the PI3K-Akt-GSK3 pathway. Immunology. 132:567–577. https://doi.org/10.1111/j.1365-2567.2010.03402.x
Apetoh, L., F.J. Quintana, C. Pot, N. Joller, S. Xiao, D. Kumar, E.J. Burns, D.H. Sherr, H.L. Weiner, and V.K. Kuchroo. 2010. The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. Nat. Immunol. 11:854–861. https://doi.org/10.1038/ni.1912
Achenbrenner, D., M. Fogliarini, D. Jarrossay, D. Hu, H.L. Weiner, V.K. Kuchroo, A. Lanzavecchia, S. Notarbartolo, and F. Sallusto. 2018. An immunoregulatory and tissue-residency program modulated by c-MAF in human Th17 cells. Nat. Immunol. 19:1126–1136. https://doi.org/10.1038/s41590-018-0200-5
Auito, K., and M. Oft. 2019. Pegylated Interleukin-10: Clinical Development of an Immunoregulatory Cytokine for Use in Cancer Therapeutics. Curr. Oncol. Rep. 21:19. https://doi.org/10.1007/s11912-019-0760-z
Balaji, S., X. Wang, A. King, L.D. Le, S.S. Bhattacharya, C.M. Molen, M.J. Butte, V.A. de Jesus Perez, K.W. Liesch, T.N. Wight, et al. 2017. Interleukin-10-mediated...
regenerative postnatal tissue repair is dependent on regulation of hyaluronan metabolism via fibroblast-specific STAT3 signaling. J Exp. Biol. 201:161–171. https://doi.org/10.1242/jeb.008568

Balasingam, V., and V.W. Yong. 1996. Attenuation of astroglial reactivity by interleukin-10. J. Neurosci. 16:2945–2955. https://doi.org/10.1523/JNEUROSCI.16-09-02945.1996

Banerjee, A., R. Gugasyan, M. McMahon, and S. Gerondakis. 2006. Diverse Toll-like receptors utilizes TLR2 to activate extracellular signal-regulated kinase (ERK) in hemopoietic cells. Proc. Natl. Acad. Sci. USA. 103: 3274–3279. https://doi.org/10.1073/pnas.0511330103

Barrat, F.J., D.J. Cua, A. Boonstra, D.F. Richards, C. Crain, H.F. Savelkoul, R. de Buck, M.D., D. O’Neill, A.R. Burmeister, A.R., and I. Marriott. 2018. The Interleukin-10 Family of Cytokines. J Immunol. 178: 6705–6709. https://doi.org/10.4049/jimmunol.178.11.6705

Barrat, F.J., F.J. Cua, D.F. Richards, C. Crain, H.F. Savelkoul, R. de Buck, M.D., D. O’Neill, A.R. Burmeister, A.R., and I. Marriott. 2018. The Interleukin-10 Family of Cytokines. J Immunol. 178: 6705–6709. https://doi.org/10.4049/jimmunol.178.11.6705

Buck, M.D., D. O’Neill, A.R. Burmeister, A.R., and I. Marriott. 2018. The Interleukin-10 Family of Cytokines. J Immunol. 178: 6705–6709. https://doi.org/10.4049/jimmunol.178.11.6705

Chi, H., S.P. Barry, R.J. Roth, J.J. Wu, E.A. Jones, A.M. Bennett, and R.A. Flavell. 2006. Dynamic regulation of pro- and anti-inflammatory cytokines by IL-10. J. Exp. Med. 203: 2337–2346. https://doi.org/10.1084/jem.20061629

Chen, Q., V. Daniel, D.W. Maher, and P. Hersey. 1994. Production of IL-10 by melanoma cells: examination of its role in immunosuppression mediated by melanoma. Int. J. Cancer. 59:755–760. https://doi.org/10.1002/ijc.29150

Chen, Q., V. Daniel, D.W. Maher, and P. Hersey. 1994. Production of IL-10 by melanoma cells: examination of its role in immunosuppression mediated by melanoma. Int. J. Cancer. 59:755–760. https://doi.org/10.1002/ijc.29150

Colombel, J.F., P. Rutgeerts, H. Macchial, M. Jacyna, O.H. Nielsen, J. Rask-Madsen, S. Van Deventer, A. Ferguson, P. Desreumaux, A. Forbes, et al. 2001. Interleukin 10 (Tenonovil) in the prevention of postoperative recurrence of Crohn’s disease. Gut. 49:42–46. https://doi.org/10.1038/gut.49.144

Conaway, E.A., D.C. de Oliveira, C.M. McNisni, S.B. Snapper, and B.H. Horiwitz. 2017. Inhibition of Inflammatory Gene Transcription by IL-10 Is Associated with Rapid Suppression of Lipopolysaccharide-Induced Enhancer Activation. J. Immunol. 198:2906–2915. https://doi.org/10.4049/jimmunol.1601781

Coomes, S.M., Y. Kannan, V.S. Pelly, J.L. Entwistle, R. Guidi, J. Perez-Lloret, N. Nikolov, W. Müller, and M.S. Wilson. 2017. CD4+ Th2 cells are directly regulated by IL-10 during allergic airway inflammation. Mucosal Immunol. 10:150–161. https://doi.org/10.1080/17518300.2016.460717

Couper, K.N., D.G. Blount, and E.M. Riley. 2008. IL-10: the master regulator of immunity to infection. J. Immunol. 180:5771–5777. https://doi.org/10.4049/jimmunol.180.9.5771

Crawley, J.B., I. Fugier, J.G. Zhang, S. Wormald, T.A. Willson, E.G. Stanley, L. Croker, B.A., D.L. Krebs, J.G. Zhang, S. Wormald, T.A. Willson, E.G. Stanley, L. Oldstone. 2006. Interleukin-10 determines viral clearance or persistence of immunity to infection. J. Exp. Med. 203: 2337–2346. https://doi.org/10.1084/jem.20061629

Cardoso, A., A. Gil Castro, A.C. Martins, G.M. Carriche, V. Murineux, I. Castro, A. Cunaso, P. Vieira, and M. Baravia. 2018. The Dynamics of Interleukin-10–Afforded Protection during Dextran Sulfate Sodium-Induced Colitis. Front. Immunol. 9:400. https://doi.org/10.3389/fimmu.2018.00400

Cassatella, M.A., L. Meda, S. Bonora, M. Ceska, and G. Constantin. 1993. Interleukin 10 (IL-10) inhibits the release of proinflammatory cytokines from human polymorphonuclear leukocytes. Evidence for an autocrine role of tumor necrosis factor and IL-1 beta in mediating the production of reactive oxygen species by lipopolysaccharide. J. Exp. Med. 178:2207–2211. https://doi.org/10.1083/jem.178.6.2207

Chakrabarti, A., A.J. Sadler, N. Kar, H.A. Young, R.H. Silverman, and B.R. Williams. 2008. Protein kinase R-dependent regulation of interleukin-10 in response to double-stranded RNA. J. Biol. Chem. 283:25132–25139. https://doi.org/10.1074/jbc.M804770200

Chen, C.S., A. Ming-Lung, G.B. Golds, S.J. Lee, R.J. Anderson, and A.L. Mui. 2012. Interleukin-10 inhibits lipopolysaccharide-induced tumor necrosis factor-α translation through a SHIP1–dependent pathway. J. Biol. Chem. 287:38020–38027. https://doi.org/10.1074/jbc.M112.348599

Cheng, E.C. 2007a. Cutting edge: involvement of the type I IFN production and signaling pathway in lipopolysaccharide-induced IL-10 production. J. Immunol. 178: 6705–6709. https://doi.org/10.4049/jimmunol.178.11.6705
Defective IL-10 expression and in vitro steroid-induced IL-17A in pae- diatric severe therapy-resistant asthma. Thorax. 69:508–515. https://doi.org/10.1136/thoraxjnl-2013-203342

Hagenbaugh, A., S. Sharma, S.M. Dunibett, S.H. Wei, R. Aranda, H. Cher- outre, D.J. Fowell, S. Binder, B. Tsao, R.M. Locksley, et al. 1997. Altered immune responses in interleukin 10 transgenic mice. J. Exp. Med. 185: 2101–2110. https://doi.org/10.1083/jem.185.12.2101

Hammer, M., J. Mages, H. Dietrich, F. Schmitz, F. Striebel, P.J. Murray, H. Wagner, and R. Lang. 2005. Control of dual-specificity phosphatase-1 expression inactivated macrophages by IL-27. Eur. J. Immunol. 35: 2991–3001. https://doi.org/10.1002/eji.200526192

Hong, E.G., H.J. Ko, Y.R. Cho, H.J. Kim, Z. Ma, R.H. Friedline, E. Kurt- Jones, R. Finberg, M.A. Fischer, et al. 2009. Interleukin-10 prevents diet-induced insulin resistance by attenuating macrophage and cyto- kine response in skeletal muscle. Diabetes. 58:2525–2535. https://doi.org/10.2337/db09-0179

Howes, A., C. Taubert, S. Blankley, N. Spink, X. Wu, C.M. Graham, J. Zhao, M. Saraula, P. Ricciardi-Castagnoli, G.J. Bancroft, and A. O’Garra. 2016. Differential Production of Type I IFN Determines the Reciprocal Levels of IL-10 and Proinflammatory Cytokines Produced by C57BL/6 and BALB/c Macrophages. J. Immunol. 197:2838–2853. https://doi.org/10.4049/jimmunol.1501923

Hsu, D.H., R. de Waal Malefyt, D.F. Florentino, M.N. Daog, P. Vieira, J. de Vries, H. Spits, T.R. Moorsman, and K.W. Moore. 1990. Expression of interleukin-10 activity by Epstein-Barr virus protein BCRF1. Science. 250:830–832. https://doi.org/10.1126/science.2173142

Huber, S., N. Gagliani, E. Espugues, W. O’Connor Jr., F.J. Huber, A. Chaudhry, M. Kamanaka, Y. Kobayashi, C.J. Booth, A.Y. Rudensky, et al. 2011. Th17 cells express interleukin-10 receptor and are controlled by Foxp3 and Foxp3 regulatory CD4+ T cells in an interleukin-10-dependent man- ner. Immunity. 34:545–565. https://doi.org/10.1016/j.immuni.2011.01 .020

Hulsman, M., H.B. Sager, J.D. Roh, M. Valero-Munoz, N.E. Houlist, Y. Iwamoto, Y. Sun, R.M. Wilson, G. Wojtkiewicz, B. Tricot, et al. 2018. Cardiac macrophages promote diastolic dysfunction. J. Exp. Med. 215: 492–510. https://doi.org/10.1084/jem.20172274

Hunter, C.A., L.A. Ellis-Neyes, T. Slifer, S. Kanaly, G. Grünig, M. Fort, D. Rennick, and F.G. Araújo. 1997. IL-10 is required to prevent immune hyperactivity during infection with Trypanosoma cruzi. J. Immunol. 158: 3311–3316.

Hussaarts, L., H.H. Smits, G. Schramm, A.J. van der Ham, G.C. van der Zon, H. Korman, S. O’Shea, S. Monticelli, K.H. Kang, and A. Rao. 2015. Chromatin- mediated epithelial-macrophage crosstalk. J. Exp. Med. 210:1657–1675. http://doi.org/10.1084/jem.2015175347

Itakura, E., R.R. Huynh, J.P., C.C. Lin, J.M. Kimmey, N.N. Jarjour, E.A. Schwarzkopf, T.R. Bradstreet, I. Shchukina, C.C. Lin, S.C. Huynh, C.W. Lai, M.E. Cook, T. Taneja, T.S. Steppenbeck, et al. 2019. Blh40e mediates tissue-specific control of macrophage proliferation in homeostasis and type 2 immunity. Nat. Immunol. 20:687–700. https://doi.org/10.1038/s41555-019-0382-5

Jarry, A., C. Bossard, C. Bou-Hanna, D. Masson, E. Espazie, M.G. Denis, and C.L. Laboisse. 2008. Mucosal IL-10 and TGF-beta play crucial roles in preventing LPS-driven, IFN-gamma-mediated epithelial damage in human colon explants. J. Clin. Invest. 118:132–142.

Jayawardane, G.C., G.C. Russell, J. Thomson, D. Deane, H. Cox, D. Gatherer, M. Ackermann, D.M. Haig, and J.P. Stewart. 2008. A captured viral in- terleukin 10 gene with cellular exon structure. J. Gen. Virol. 89: 2477–2485. https://doi.org/10.1099/jgv.0.017743-0

Jones, E.A., and R.A. Flavell. 2005. Distal enhancer elements transcribe in- tergenic RNA in the IL-10 family gene cluster. J. Immunol. 175: 7427–7446. https://doi.org/10.4049/jimmunol.175.11.7427

Jostins, L., S. Riple, R.K. Weersma, R.H. Duerr, D.P. McGovern, K.Y. Hui, J.C. Lee, L.P. Schumm, Y. Sharma, C.A. Anderson, et al. International IBD Genetics Consortium (IIBDGC). 2012. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 491:119–124. https://doi.org/10.1038/nature11582

Kaiser, C., D. Cook, S. Papoutsopoulou, R. Rajasham, Y. Wu, H.T. Yang, S. Grant, P. Ricciardi-Castagnoli, P.N. Tsichlis, S.C. Ley, and A. O’Garra. 2009. TPL-2 negatively regulates interferon-beta production in macrophages and myeloid dendritic cells. J. Exp. Med. 206:1863–1871. https://doi.org/10.1084/jem.20091059

Kamanaka, M., S. Huber, L.A. Nenewicz, N. Gagliani, C. Rathinam, W. O’Connor Jr., Y.Y. Fan, S. Nakas, Y. Iwakura, L. Hao, and R.A. Flavell. 2011. Memory/effector (CD45RB(lo)) CD4 T cells are controlled directly by IL-10 and cause the IL-22-dependent intestinal pathology. J. Exp. Med. 208:1027–1040. https://doi.org/10.1084/jem.20102149

Kanters, E., M. Pasparakis, M.J. Gijbels, M.N. Vergouwe, I. Portens-Hendriks, R.J. Fijneman, B.E. Clausen, I. Förster, M.M. Koekx, K. Rajewsky, et al. 2003. Inhibition of NF-kappaB activation in macrophages in creases atherosclerosis in LDL receptor-deficient mice. J. Clin. Invest. 112:1176–1185. https://doi.org/10.1172/JCI200318850

Kasama, T., R.M. Strieeter, N.W. Lukacs, M.D. Burdick, and S.L.unkel. 1994. Regulation of neutrophil-derived chemokine expression by IL-10. J. Immunol. 152:3559–3569.

Kim, C., Y. Sano, K. Todorova, B.A. Carlson, L. Arpa, A. Celada, T. Lawrence, K. Otsu, J.L. Brissette, J.S. Arthur, and J.M. Park. 2008. The kinase p38 alpha serves cell type-specific inflammatory functions in skin injury and coordinates pro- and anti-inflammatory gene expression. Nat. Immunol. 9:1019–1027. https://doi.org/10.1038/ni.1640

Kimura, A., T. Nakahama, I. Chinen, K. Masuda, K. Nohara, Y. Fujii-Kuriyama, and T. Kishimoto. 2009. Aryl hydrocarbon receptor in combination with Stat1 regulates LPS-induced inflammatory responses. J. Gen. Virol. 90: 775–786. https://doi.org/10.1128/JCVI.00719-08

King, A., S. Balaji, L.D. Le, T.M. Crombleholme, and S.G. Keswani. 2014. Re- generative Wound Healing: The Role of Interleukin-10. Adv. Wound Care (New Rochelle). 3:315–323. https://doi.org/10.1089/wound.2013.0461

Knoblauch, S.M., and A.I. Faden. 1998. Interleukin-10 improves outcome and alters proinflammatory cytokine expression after experimental traumatic brain injury. Exp. Neuro 153:143–151. https://doi.org/10.1006/exnr.1998.6877

Kobayashi, M., M.N. Kweon, H. Kuwata, D.R. Schreiber, H. Kiyono, K. Takeda, and S. Akira. 2003. Toll-like receptor-dependent production of IL-12p40 causes chronic enterocolitis in myeloid cell-specific Stat3-deficient mice. J. Clin. Invest. 111:2973–3001. https://doi.org/10.1172/JCI17035

Kole, A., and K.J. Maloy. 2014. Control of intestinal inflammation by interleukin-10. Curr. Top. Microbiol. Immunol. 389:19–38.
Lee, T.S., and L.Y. Chau. 2002. Heme oxygenase-1 mediates the anti-inflammatory cytokine IL-10 https://doi.org/10.1084/jem.20190418

Saraiva et al. Journal of Experimental Medicine https://doi.org/10.1084/jem.20190418
induces the transcription factor c-Maf, cytokine IL-21, and the co-stimulatory receptor ICOS that coordinately act to promote differentiation of IL-10-producing Treg cells. J. Immunol. 183:1041–1046. https://doi.org/10.4049/jimmunol.175.2.1041

Saravia, M.J., R.C. Christensen, A.V. Tsytsinskaya, A.E. Goldfeld, S.C. Ley, D. Kloussis, and A. O’Garra. 2005. Identification of a macrophage-specific chromatin signature in the IL-10 locus. J. Immunol. 175:1041–1046. https://doi.org/10.4049/jimmunol.175.2.1041

Sawant, D.V., H. Yano, M. Chikina, Q. Zhang, M. Liao, C. Liu, D.J. Callahan, Z. Sun, T. Sun, T. Tabib, et al. 2019. Adaptive plasticity of IL-10 and IL-35+ Treg cells cooperatively promotes tumor T cell exhaustion. Nat. Immunol. 20:724–735. https://doi.org/10.1038/s41590-019-0346-9

Schalj, B., F. Kratochvill, N. Graz, I. Sedacz, I. Sauer, M. Hammer, C. Vogl, B. Strobl, M. Müller, P.J. Blackshear, et al. 2009. Tristetraprolin is required for full anti-inflammatory response of murine macrophages to IL-10. J. Immunol. 183:1197–1206. https://doi.org/10.4049/jimmunol.0803883

Schreiber, S.R., N. Fedorak, O.H. Nielsen, G. Wild, C.N. Williams, S. Nikolaus, M. Jacyna, B.A. Lashner, A. Gang, P. Rutgeerts, et al. 2000. Safety and efficacy of recombinant human interleukin 10 in chronic active Crohn’s disease. Crohn’s Disease IL-10 Cooperative Study Group. Gastroenterology. 119:1461–1472. https://doi.org/10.1053/gast.2000.19196

Lee, D.R., K.Y. Kim, and Y.B. Lee. 2004. Interleukin-10 expression in lipopolysaccharide-activated microglia is mediated by extracellular ATP in an autocrine fashion. Neuroreport. 15:1157–1161. https://doi.org/10.1097/00001756-200405190-00015

Lee, D.R., W.Y. Kim, K.Y. Kim, H.G. Lee, J.H. Moon, S.S. Lee, U.S. Kim, and Y.B. Lee. 2008. Cross talk between P2 purinergic receptors modulates extracellular ATP-mediated interleukin-10 production in rat microglial cells. Exp. Mol. Med. 40:19–26. https://doi.org/10.3858/emmm.2008.40.119

Shigemori, S., and T. Shimosato. 2017. Application of Genetically Modified Immunobiotics with High Immunoregeneratory Capacity for Treatment of Inflammatory Bowel Diseases. Front. Immunol. 8. https://doi.org/10.3389/fimmu.2017.01005

Shoemaker, J., M. Saraiva, and A. O’Garra. 2006. GATA-3 directly remodels the IL-10 locus independently of IL-4 in CD4+ T cells. J. Immunol. 176:3470–3479. https://doi.org/10.4049/jimmunol.176.6.3470

Shouval, D.S., A. Biswas, J.A. Goettel, K. McCann, E. Conaway, N.S. Redhu, I.D. Mascalfroni, Z. Al Adham, S. Lavoie, M. Bourk, et al. 2014a. Interleukin-10 receptor signaling in innate immune cells regulates mucosal immune tolerance and anti-inflammatory macrophage function. Immunity. 40:706–719. https://doi.org/10.1016/j.immuni.2014.03.001

Shouval, D.S., J. Ouahed, A. Biswas, J.A. Goettel, B.H. Horwitz, C. Klein, A.M. Muise, and S.B. Snapcer. 2014b. Interleukin 10 receptor signaling: master regulator of intestinal mucosal homeostasis in mice and humans. Adv. Immunol. 122:177–210. https://doi.org/10.1016/B978-0-12-338957-5.00005-5

Singh, N., A. Guruv, S. Sivaprakasam, E. Brady, R. Padia, H. Shi, M. Thangaraju, P.D. Prasad, S. Manicassamy, D.H. Munn, et al. 2014. Activation of Gpr109a receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. Immunity. 40:128–138. https://doi.org/10.1016/j.immuni.2013.12.007

Smith, A.M., J.E. Quilis, K. Brien, L. Baloutian, P.F. Johnson, S. Schultz-Cherry, S.T. Smale, and P.J. Murray. 2011. A distal enhancer in IIfib2 is the target of transcriptional repression by the STAT3 pathway and requires the basic leucine zipper (B-ZIP) protein NFIL3. J. Biol. Chem. 286:23582. https://doi.org/10.1074/jbc.M111.49233

Steidler, L., W. Hans, L. Schotte, S. Neirynck, F. Obermeier, W. Falk, W. Fiers, and E. Remaut. 2000. Treatment of murine colitis by Lactobacoccus lactis secreting interleukin-10. Science. 289:1352–1355. https://doi.org/10.1126/science.289.5483.1352

Stumhofer, J.S., J.S. Silver, A. Laurence, P.M. Porrett, T.H. Harris, L.A. Turka, M. Ernst, C.J. Saris, J.J. Sheaea, and C.A. Hunter. 2007. Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin-10. Nat. Immunol. 8:1336–1341. https://doi.org/10.1038/ni1357

Sun, J., H. Dodd, E.K. Mozer, R. Sharma, and T.J. Braciale. 2011. CD4+ T cell help in innate-derived IL-21 induces Bmp1- and p210-dependent IL-10 production by antiviral CTLs. Nat. Cell Biol. 13:327–334. https://doi.org/10.1038/ni.1996

Sun, M., W. Wu, L. Chen, W. Yang, H. Huang, C. Ma, F. Chen, Y. Xiao, Y. Zhao, C. Ma, et al. 2018. Microbiota-derived short-chain fatty acids promote Th1 cell IL-10 production to maintain intestinal homeostasis. Nat. Commun. 9:3355. https://doi.org/10.1038/s41467-018-05900-2

Sun, Y., X. Ji, M. McEachin, M. Zhao, Y.M. Wu, X. Cao, M. Oravec-Wilson, C. Zajac, N. Mathewson, S.J. Wu, et al. 2017. Genome-Wide STAT3 Binding Analysis after Histone Deacetylase Inhibition Reveals Novel Target Genes in Dendritic Cells. J. Immunol. 9:126–144. https://doi.org/10.4049/jimmunol.11500450681

Sziksz, E., D. Pap, R. Lippai, N.J. Béres, A. Fekete, A.J. Szabó, and A. Vannay. 2015. Fibrosis Related Inflammatory Mediators: Role of the IL-10 Cytokine Family. Mediators Inflamm. 2015:76441. https://doi.org/10.1155/2015/76441

Takeda, K., B.E. Clausen, T. Kaisho, T. Tsujimura, N. Terada, I. Forster, and S. Akira. 1999. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. Immunity. 10:39–49. https://doi.org/10.1016/S1074-7613(00)80005-9

Tan, P.H., H.E. Tyrrell, L. Gao, D. Xu, J. Quan, D. Gill, L. Rai, Y. Ding, G. Plant, Y. Chen, et al. 2014. Adaptive receptor signaling on dendritic cells blunts antimicrobial immunity. Cancer Res. 74:5711–5722. https://doi.org/10.1158/0008-5472.CAN-13-1397

Tanchot, C., S. Guillaume, J. Delen, C. Bourgeois, A. Franzek, A. Sarukhan, A. Trautmann, and B. Rocha. 1998. Modifications of CD8+ T cell function during in vivo memory or tolerance induction. Immunity. 8:581–590. https://doi.org/10.1016/S1074-7613(00)0568-3
Weaver, B.K., E. Bohn, B.A. Judd, M.P. Gil, and R.D. Schreiber. 2007. ABIN-3: tyrosine-phosphorylated docking sites in the interleukin-10 receptor intracellular domain. J. Biol. Chem. 272:27954–27961. https://doi.org/10.1074/jbc.M701310200

Wehinger, J., F. Gouilleux, B. Groner, J. Flinke, R. Mertelsmann, and R.M. Weber-Nordt. 1996. IL-10 induces DNA binding activity of three STAT proteins (Stat1, Stat3, and Stat5) and their distinct combinatorial assembly in the promoters of selected genes. FEBS Lett. 394:365–370. https://doi.org/10.1016/S0014-5793(96)00990-8

Welchhart, T., G. Costantino, M. Foglitsch, M. Rosner, M. Zeyda, K.M. Stunzinger, T. Kolbe, T.M. Stunz, W.H. Hiel, M. Hengstschläger, et al. 2008. The TSC-mTOR signaling pathway regulates the innate inflammatory response. Immunity. 29:565–577. https://doi.org/10.1016/j.immuni.2008.08.012

Werry, E.L., G.J. Liu, M.D. Lovelace, R. Nagarajah, I.B. Hickie, and M.R. Bennett. 2011. Lipopolysaccharide-stimulated interleukin-10 release from neonatal spinal cord microglia is potentiated by glutamate. Neuropsychos. 175:93–103. https://doi.org/10.1016/j.neuroscience.2010.10.080

Willems, F., A. Marchant, J.P. Delville, C. Gérard, A. Delvaux, T. Velu, M. De Boer, and M. Goldman. 1994. Interleukin-10 inhibits B7 and intercellular adhesion molecule-1 expression on human monocytes. Eur. J. Immunol. 24:1007–1009. https://doi.org/10.1002/eji.1802404343

Williams, L., L. Bradley, A. Smith, and B. Foxwell. 2004a. Signal transducer and activator of transcription 3 is the dominant mediator of the anti-inflammatory effects of IL-10 in human macrophages. J. Immunol. 172:567–576. https://doi.org/10.4049/jimmunol.172.1.567

Williams, L.M., G. Ricchetti, U. Sarma, T. Smallie, and B.M. Foxwell. 2004b. Interleukin-10 suppression of myeloid cell activation—a continuing puzzle. Immunology. 113:281–292. https://doi.org/10.1111/j.1365-2657.2004.01988.x

Xie, L., Q. Fu, T.M. Ortega, L. Zhou, D. Rasmussen, J. O’Keefe, K.K. Zhang, and S.K. Chaples. 2014. Overexpression of IL-10 in CD2 macrophages promotes a macrophage phenotypic switch in adipose tissue environments. PLoS One. 9:e86541. https://doi.org/10.1371/journal.pone.0086541

Xin, J., D.A. Wainwright, N.A. Mesnard, C.J. Serpe, V.M. Sanders, and K.J. Jones. 2011. IL-10 within the CNS is necessary for CD4+ T cells to mediate neuroprotection. Brain Behav. Immun. 25:820–829. https://doi.org/10.1016/j.bbi.2010.08.004

Xu, J., Y. Yang, G. Qiu, G. Lal, Z. Wu, D.E. Levy, J.C. Ochando, J.S. Bromberg, and Y. Ding. 2009. c-Maf regulates IL-10 expression during Th17 polarization. J. Immunol. 182:6226–6236. https://doi.org/10.4049/jimmunol.0900123

Xystrakis, E., S. Kusumakar, S. Boswell, E. Peek, Z. Urry, D.F. Richards, and S.K. Krieg. 2002. Role of mitogen-activated protein kinases in CpG DNA-mediated IL-10 regulation of IL-10 gene expression in CD4+ T cell clones and peripheral blood T cells. J. Immunol. 168:4711–4720. https://doi.org/10.4049/jimmunol.168.9.471

Yoon, S.I., N.L. Logsdon, F. Sheikh, R.P. Donnelly, and M.R. Walter. 2006. Conformational changes mediate interleukin-10 receptor 2 (IL-10R2) binding to IL-10 and assembly of the signaling complex. J. Biol. Chem. 281:35088–35096. https://doi.org/10.1074/jbc.M606912000

Yssel, H., R. De Waal-Malefyt, M.N. Dang, K.E. Johnson, R. Kastelein, D.F. Florentino, J.E. de Vries, M.G. Roncarolo, T.R. Mosmann, and K.W. Moore. 1991. Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: homology to Epstein-Barr virus open reading frame BCRFI. Proc. Natl. Acad. Sci. USA. 88:1172–1176. https://doi.org/10.1073/pnas.88.4.1172

Vigne, S., F. Chalmin, D. Duc, A.S. Clotou, L. Apetou, J.A. Lebacarco, I. Christen, J. Zhang, and C. Pot. 2017. IL-27 induced Type I Regulatory T-Cells Produce Oxysterols that Constrain IL-10 Production. Front. Immunol. 8:1814. https://doi.org/10.3389/fimmu.2017.01814

Vouloudioukis, I., P.A. Béchere, V. Riveros-Moreno, M. Aroc, O. da Silva, P. Debré, D. Mazerier, and M.D. Massaly. 1997. Interleukin-10 and interleukin-4 inhibit intracellular killing of Leishmania infantum and Leishmania major by human macrophages by decreasing nitric oxide generation. Eur. J. Immunol. 27:860–865. https://doi.org/10.1002/eji.18027020409

Wang, X., K. Wong, W. Ouyang, and S. Rutz. 2019. Targeting IL-10 Family Cytokines for the Treatment of Human Diseases. Cold Spring Harb. Perspect. Biol. 11:028548. https://doi.org/10.1101/cshperspect.a028548

Wang, Z.Y., H. Sato, S. Kusam, S. Sehra, L.M. Toney, and A.L. Dent. 2005. Regulation of IL-10 gene expression in TH2 cells by Jun proteins. J. Immunol. 174:2098–2105. https://doi.org/10.4049/jimmunol.174.4.2098

Weaver, B.K., E. Bohn, B.A. Judd, M.P. Gil, and R.D. Schreiber. 2007. ABIN-3: a molecular basis for species divergence in interleukin-10-induced anti-inflammatory actions. Mol. Cell. Biol. 27:4605–4616. https://doi.org/10.1128/MCB.00223-07

Weber-Nordt, R.M., J.K. Riley, A.C. Greenland, K.W. Moore, J.E. Darnell, and R.D. Schreiber. 1996. Stat3 recruitment by two distinct ligand-induced,...
Efferocytosis Fuels Requirements of Fatty Acid Oxidation and the Electron Transport Chain to Polarize Macrophages for Tissue Repair.

Zheng, L.M., D.M. Ojcius, F. Garaud, C. Roth, E. Maxwell, Z. Li, H. Rong, J. Chen, X.Y. Wang, J.J. Catino, and I. King. 1996. Interleukin-10 inhibits tumor metastasis through an NK cell-dependent mechanism. J. Exp. Med. 184:579–584. https://doi.org/10.1084/jem.184.2.579

Zhou, Z., X. Peng, R. Insolera, D.J. Fink, and M. Mata. 2009a. IL-10 promotes neuronal survival following spinal cord injury. Exp. Neurol. 220: 183–190. https://doi.org/10.1016/j.expneurol.2009.08.018

Zhou, Z., X. Peng, R. Insolera, D.J. Fink, and M. Mata. 2009b. Interleukin-10 provides direct trophic support to neurons. J. Neurochem. 110:1617–1627. https://doi.org/10.1111/j.1471-4159.2009.06263.x

Zhu, J., B. Min, J. Hu-Li, C.J. Watson, A. Grinberg, Q. Wang, N. Killeen, J.F. Urban Jr., L. Guo, and W.E. Paul. 2004. Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. Nat. Immunol. 5: 1157–1165. https://doi.org/10.1038/ni128

Zhu, Y.P., J.R. Brown, D. Sag, L. Zhang, and J. Suttles. 2015. Adenosine 5’- monophosphate-activated protein kinase regulates IL-10-mediated anti-inflammatory signaling pathways in macrophages. J. Immunol. 194: 584–594. https://doi.org/10.4049/jimmunol.1401024

Zigmond, E., B. Bernshtein, G. Friedlander, C.R. Walker, S. Yona, K.W. Kim, O. Brenner, R. Krauthgamer, C. Varol, W. Müller, and S. Jung. 2014. Macrophage-restricted interleukin-10 receptor deficiency, but not IL-10 deficiency, causes severe spontaneous colitis. Immunity. 40:720–733. https://doi.org/10.1016/j.immuni.2014.03.012

---

Saraiva et al.
New insights on the anti-inflammatory cytokine IL-10

Journal of Experimental Medicine
https://doi.org/10.1084/jem.20190418