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REVIEW ARTICLE

Interleukin-10 suppression of myeloid cell activation — a continuing puzzle

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SUMMARY

Efforts to identify the signal transduction pathways used by interleukin-10 (IL-10) have resulted in limited success. The anti-inflammatory effects elicited by IL-10, and the mechanisms by which these are mediated, are still relatively unknown. Understanding the signalling mechanisms behind the suppression of cytokine expression by IL-10 could be of potential therapeutic interest. Although the consensus is that the Janus kinase, Jak1, as well as the signal transducer and activator of transcription STAT3 are central, much controversy exists about the participation and roles of many other signalling pathways targeted by IL-10. The mechanisms of cytokine suppression proposed by various groups have included transcriptional, post-transcriptional and post-translational regulation of IL-10 target genes; nevertheless no unifying model has emerged thus far. Here we would like to highlight novel findings and discuss their implications in the context of current understanding of IL-10 signalling.

Keywords signalling/signal transduction; cytokines/interleukins; inflammation/inflammatory mediators including eicosanoids

INTRODUCTION

The immune system's inflammatory response is essential to protect the host from infection, injury and neoplasia. The destruction of invading pathogens requires the production of powerful cytopathic factors; however, the immune response has to be of the appropriate amplitude and duration to prevent the unnecessary destruction of healthy tissue. Excessive production of these inflammatory factors can result in diseases such as rheumatoid arthritis, Crohn's disease and septic shock. The immune system has developed multiple anti-inflammatory mediators to prevent the inflammatory response from spiralling out of control. One of the most potent of these anti-inflammatory factors is interleukin-10 (IL-10). IL-10 is a pleiotropic cytokine that has an important role in regulating the immune response. It was originally described as a cytokine synthesis inhibitor factor produced by murine T helper 2 (Th2) cells.[1] However, its expression profile has now widened and it has been shown to be expressed in various subsets of T cells, macrophages, monocytes, dendritic cells, mast cells, B cells, eosinophils, keratinocytes, epithelial cells and various tumour cell lines.[2] Its main biological functions seem to be to limit and terminate the inflammatory responses, block pro-inflammatory cytokine secretion and regulate the differentiation and proliferation of several immune cells such as T cells, B cells, natural killer cells and mast cells.[3] IL-10 gene homologues have been found in numerous viral genomes and it is thought they act as virulence factors to manipulate the immune response.[4,5] In addition many tumours also acquire an IL-10-secreting phenotype that may permit malignant cells to evade cell-mediated immune defences.[6–8] The convergence of these evolutionary distinct mechanisms for immune evasion further underscores the importance of IL-10 as central immune modulator. More recently, five novel cytokines that display structural similarity to IL-10 have been identified in the human genome: IL-19, IL-20, IL-22, IL-24 and IL-26.[9–13] These, however, do not appear to display an anti-inflammatory function.

IL-10 potently inhibits macrophage activation. This results in reduced expression of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), IL-1, IL-12, IL-6 and granulocyte–macrophage colony-stimulating factor, inflammatory enzymes such as cyclo-oxygenase 2 and inducible nitric oxide synthase, chemokines such as regulated on activation, normal, T-cell expressed and

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secreted (RANTES), membrane inflammatory protein-1α (MIP1α), IL-8, and eotaxin, thus limiting the course of an inflammatory response by curtailing the activation and recruitment of a wide range of haematopoietic cells.[2]

IL-10 augments this inhibitory activity by enhancing the release of soluble TNF receptors and IL-1 receptor antagonist (IL-1RA).[14,15] Similarly, the potentially destructive activities of matrix metalloproteinases (MMP) are limited by IL-10, as it not only inhibits the production of MMP2 and MMP9 but also induces the production of tissue inhibitor of MMPs (TIMP), TIMP1.[16] Another key feature of IL-10's immunosuppressive capabilities is its effectiveness in disabling antigen presentation (AP)/T-cell activation by inhibiting expression of major histocompatibility complex (MHC) class II, CD80 (B7-1) and CD86 (B7-2) on macrophages and dendritic cells,[17,18] thus effectively blocking antigen presentation to T cells. Besides these indirect effects on T cells via inhibition of APC function, IL-10 does have some direct effects on T cells. It shows inhibitory activities towards $CD4^+$ T cells via suppressing IL-2, interferon-γ (IFN-γ), IL-4 and IL-5 production.[19–21] It has also been suggested that IL-10 plays a direct role in the differentiation of regulatory T cells.[22] In particular, the differentiation of Tr1 T cells might be controlled by tolerogenic dendritic cells that produce IL-10 and express tolerogenic costimulatory molecules.[23–25]

The effects of IL-10 on B cells are more stimulatory. IL-10 enhances B-cell survival, and is a potent cofactor for proliferation of human B-cell precursors and mature B cells activated by *Staphylococcus aureus* Cowan 1 (SAC), anti-immunoglobulin M (IgM) or CD40 ligation.[26,27] IL-10 can affect B-cell differentiation and isotype switching.[28] Long-term culture of B cells with anti-CD40 and IL-10 can result in the development of plasma cells.[28] The effects of IL-10 on B cells could suggest that antagonism of IL-10 function could be useful in the treatment of antibody mediated autoimmune disease such as systemic lupus erythematosus (SLE). In support of this view, the spontaneous *in vitro* production of IgM, IgG, and IgA by peripheral blood mononuclear cells (PBMC) from SLE patients is strongly increased by IL-10. Conversely, anti-IL-10 antibodies strongly inhibited the production of autoantibodies in severe combined immunodeficiency mice injected with PBMC from SLE patients.[29] Furthermore, anti-IL-10 treatment of SLE patients ameliorated disease in a limited trial.[30]

IL-10 deficient mice have highly polarized Th1 responses and develop a severe colitis which supports the essential role of IL-10 in balancing the cytokine network. Generally considered as a Th2 cytokine, IL-10 inhibits Th1-dominated responses. The potency of the anti-inflammatory effects of IL-10 has been demonstrated in animal models of inflammation such as sepsis,[31] collagen-induced arthritis,[32] inflammatory bowel disease,[33] insulitis,[34] and in some models of experimental autoimmune encephalomyelitis (EAE).[35,36] In a clinical setting, encouraging data has emerged from phase II trials of systemic administration of IL-10 in the treatment of psoriatic skin lesions[37] although similar data from Crohn's disease and rheumatoid arthritis produced only a mild amelioration of disease activity.[38,39]

The potent anti-inflammatory activity of IL-10 could be harnessed therapeutically, from a more comprehensive understanding of the signalling pathways involved. We will review the current literature regarding the signalling mechanisms employed by IL-10 focusing particularly upon its cytokine suppressing activities.

THE IL-10R: STRUCTURE AND FUNCTION

The IL-10R is a heterotetramer comprising of two molecules of the IL-10R1 and two molecules of the IL-10R2 (also known as CRF 2–4) chain. Janus kinase-1 (Jak1) and Tyk2 are permanently associated with the IL-10R1 and IL-10R2 chain, respectively.[40–42] Knockout models of IL-10R2[42] and signal transducer and activator of transcription-3 (STAT3)[43] have confirmed that these components of the IL-10 signalling machinery mediate IL-10 activity, as the phenotype of spontaneous enterocolitis in all these ablations is analogous to that found in IL-10-deficient mice. In contrast, the absence of Tyk2 did not have an effect on IL-10 activity nor did it result in a related phenotype.[44] The functional relevance of several segments within the amino acid sequences of the human (578 residues)[40] and murine (576 residues)[45] IL-10R1 has been investigated through various deletion and substitution mutations of the cytoplasmic or signalling part of the receptor. This strategy has led to the identification of several active domains and residues. The tyrosine residues present in a cytokine receptor box 3 motif (YXXQ) at positions 446 and 496 in the human IL-10R1 chain were found to be critical for IL-10 function and highly conserved between murine and human sequences.[46,47] This motif has been described as being common to all STAT3-activating cytokine receptors including IL-10R1 and gp130[48] and more recently the IL-20R1 and IL-22R1.[49,50] In addition, a stretch of 30 C-terminal amino acids, which was thought to contain at least one critical serine residue, was also implicated in mediating the anti-inflammatory activity of IL-10. It was this segment of 30 C-terminal amino acids that was said to be the unique receptor domain that conferred the specificity for the anti-inflammatory activity of IL-10.[47] This finding has gained new relevance since the cloning and characterization of the receptors for the IL-10 homologues IL-22 and IL-26. The receptors for these IL-10 family cytokines have distinct receptor chains (IL-22R1 for IL-22 and IL-20R1 for IL-26) and the same accessory chain as the IL-10 receptor complex the IL-10R2/CRF2-4 chain and are thought to form multimeric receptors,[50–54] as shown in Fig. 1. Despite these basic structural similarities, IL-22 has been shown to activate not only the Jak/STAT pathway, but all three mitogen-activated protein (MAP) kinase pathways (JNK, extracellular signal-related kinase and p38) in a rat hepatoma cell line and IL-26 activated STAT1 in human colon carcinoma cells.[54–56] There is also data to suggest that IL-22 is capable of binding the IL-10R2 chain, which is unlike IL-10 and like IL-26, possibly activating additional pathways.[49,51,54] More significantly though is the finding that both IL-22 and IL-26 are strong inducers of STAT3 phosphorylation in target cells expressing the

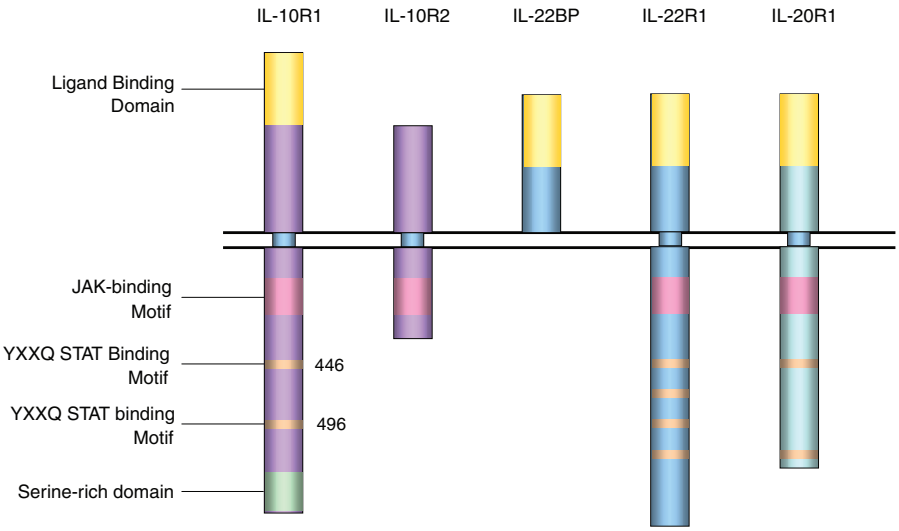

Figure 1. Domains and binding motifs of the IL-10 receptor family which utilize the IL-10R2 chain. Shown in this figure are the receptor complexes involved in IL-10, IL-22 and IL-26 signalling which all utilize the IL-10R2 chain as part of the active receptor complex. IL-22 binding protein (BP) is believed to act as an inhibitor of IL-22 signalling by competition for ligand binding. The human IL-10R1 and IL-20R1 chains both contain two YXXQ STAT binding motifs while IL-22R1 has four.

relevant receptor complexes.[54,57–60] The expression of the ligand-binding chains of the IL-22R1 and IL-20R1 of the complexes were predominantly present in non-haematopoietic cells including epithelial cells of the colon, as well as hepatocytes, stromal cells and fibroblasts of various other tissues.[9,57–60] Despite the ability to induce STAT3 in target cells, IL-22 and IL-26 have been shown to have pro-inflammatory activities.[56,57,61] This suggests that the presence of a box 3 YXXQ motif and the activation of STAT3[49] do not solely confer anti-inflammatory activity to a receptor complex as seen in the case of the IL-10R complex and other motifs within the IL-10R complex may be uniquely responsible for mediating the anti-inflammatory activity of IL-10. However, in the case of IL-22 more recent work has indicated that in a model of T-cell mediated murine viral hepatitis, IL-22 may have a protective role indicating that IL-22 may harbour some anti-inflammatory properties,[60] thus warranting further structural comparisons of the IL-22R1 and the IL-10R1 chains in the context of inflammatory responses.

The association of Jak1 to the IL-10R1 is dependent on a membrane-proximal part of the receptor (amino acids 269–274 in the human IL-10R1) containing a region designated a box 2B motif. This is characterized by a core of four hydrophobic residues flanked by a serine and charged residues.[62] Mutation of the serine and the phenylalanine in the SVLLFKK sequence led to the loss of Jak1 binding to a human GST-IL-10R1 fusion protein. In a related study further mapping of the IL-10R1 and Jak1 interaction domains revealed significant binding of amino acids 300–598 of the human IL-10R1 to Jak1.[63]

Another investigation has characterized three major domains within the murine IL-10R1. Using stably transfected pro-B-cell lines, the activity of the three domains was assayed by the proliferative response (amino acids 459–559 and amino acids 401–432) and changes in the expression of cell surface antigens (amino acids 459–559), a mechanism that was related to differentiation of the cells. One region also had a potential negative repressor function (amino acids 282–389), as cells lacking this region showed enhanced sensitivity to IL-10.[64] A polymorphism (glycine to arginine) in the human IL-10R1 region analogous to the mouse IL-10R1 region (amino acids 282–389) appears to confer altered sensitivity to IL-10 *in vitro* in cells from polymorphic carriers.[65]

Taken together, molecular events involving IL-10RI structures have only been partly elucidated. The current understanding of downstream steps following receptor activation will be discussed in more detail in the following section.

STAT PROTEINS IN IL-10 SIGNALLING

Binding of IL-10 to its receptor causes the activation of the receptor-associated Janus tyrosine kinases, Jak1 and Tyk2.[66] These kinases are responsible for the phosphorylation of tyrosine residues within the intracellular domain of IL-10R1 which serve as docking sites for STAT molecules.[64] IL-10 was initially shown to activate STAT1 and STAT3.[67,68] These molecules can form either STAT1 or STAT3 homodimers, or a heterodimer comprising of STAT1 and STAT3 and the authors suggest that the three different STAT complexes may induce different sets of genes according to the particular cell type involved.

The role STAT1 plays in IL-10 signalling still remains unclear. We and others observed a low level or even absent STAT1 phosphorylation in human macrophages in

response to IL-10. Herrero *et al*. have shown that IL-10 induced STAT1 phosphorylation was only observed after a high dose of IFN-γ priming and the consequence of this alteration in STAT phosphorylation resulted in IL-10 no longer being able to suppress cytokine synthesis or down regulate MHC class II expression.[69] Furthermore, macrophages isolated from STAT1 deficient mice show normal responses to IL-10 suggesting that STAT1 plays only a minor role in IL-10 signalling.[70]

The role STAT3 plays in IL-10 signalling has been studied to a much greater degree. IL-10 rapidly activates STAT3 and it remains phosphorylated over a sustained period. This is in contrast to IL-6 mediated STAT3 activation which is transient.[71,72] This observation may be explained by the receptor coupling of suppressor of cytokine signalling-3 (SOCS3) to the IL-6 receptor. SOCS3 is rapidly induced by both IL-6 and IL-10, in a STAT3-dependent manner, and has recently been shown to specifically inhibit IL-6 but not IL-10 signalling. The SOCS family of proteins are thought to regulate the responses of immune cells to cytokines. They are rapidly induced after cytokine receptor engagement and can bind directly to either Jaks or cytokine receptors. Through the recruitment of ubiquitin transfer system, these proteins then mediate the degradation of proteins associating through the N-terminal region of the SOCS proteins. Neither of the IL-10 receptor chains contains a SOCS consensus motif (SOCS box), whereas the IL-6 gp130 signalling chain contains the necessary tyrosine motif which targets these receptor complexes for degradation. Pretreatment of macrophages with SOCS3-inducing cytokines prevents the activation of STAT3 in response to IL-6, but has no effect upon IL-10-induced STAT3 activation. Consistent with these findings are the recent papers describing the generation of SOCS3-deficient macrophages.[73–75] In these cells, IL-6-induced STAT3 is sustained for many hours, the consequence of this is that it becomes strongly anti-inflammatory, whereas IL-10 signalling was not affected. This would suggest that even though IL-10 strongly induces SOCS3 it plays no role in the negative regulation of IL-10 signalling, although what role it does play remains to be resolved. The IL-10 receptor system may have simply evolved to escape this level of regulation which may account for ability to maintain a high level of STAT3 activation that may be necessary for it be an effective immunosuppressive cytokine.

MOLECULAR MECHANISMS OF CYTOKINE INHIBITION BY IL-10

The mechanism by which IL-10 mediates the suppression of proinflammatory cytokine synthesis remains an area of contention and contradiction. Most research focuses upon lipopolysaccharide (LPS), the outer membrane component of Gram-negative bacteria, as an inducer of proinflammatory cytokine/chemokine release in macrophages. LPS induces a number of signalling cascades, many of which have been shown to play a critical role in the production of pro-inflammatory cytokine release. TNF-α is perhaps the most extensively studied cytokine in this context. LPS rapidly induces TNF-α, with mRNA levels detectable within 15 min of stimulation, and protein levels evident after 60 min of LPS stimulation.[72] In human macrophages, IL-10 can inhibit TNF-α mRNA as soon as the mRNA is detected; however, the levels of suppression of mRNA are more profound at later time points (2 hr). In the murine system, IL-10 can also inhibit TNF-α mRNA accumulation but not to the same extent as in the human system.[76] IL-10 potently inhibits TNF-α protein production, even when given up to 2 hr post LPS stimulation.[77–79] However, *in vivo* IL-10 only effectively suppresses cytokine production when given before LPS, but not when given after LPS in experimental endotoxaemia.[80]

At what point in the LPS signalling cascade does IL-10 intervene? Fig. 2 shows the signalling cascades activated by LPS which have been shown to be critical to cytokine release and highlights the reports of IL-10 intervention. We will review this literature in terms of the LPS signalling cascade and what signalling mechanisms IL-10 employs to target these pathways.

Nuclear factor (NF)-κB

A body of work exists in support of this transcriptional control of TNF-α by IL-10 and focuses on the inhibition of the NF-κB pathway. NF-κB is a transcription factor utilized by different cytokines and LPS for the induction of a large array of pro-inflammatory cytokines.[81,82] The NF-κB complex is maintained in the cytoplasm in an inactive state through association with the inhibitor of NF-κB (IκB).[83] Following stimulation, IκB kinases (IKK) phosphorylate IκB, targeting it for ubiquitination and subsequent degradation. The NF-κB protein complex is then free to translocate to the nucleus and bind to target DNA sequences.[84] Cytokines such as TNF-α have numerous κB consensus sites within the promoter region which are engaged upon LPS stimulation with a heterodimer comprised predominantly of p65 and p50 subunits of NF-κB.[85–87] Over-expression of the inhibitor IκB markedly suppresses the expression of cytokines such as TNF-α, IL-6 and vascular endothelial growth factor in human macrophages, and along with the generation of knockout mice deficient in components of the NF-κB pathway has identified this pathway, as a key effector of cytokine production in response to LPS.[88,89]

Numerous reports have shown an effect of IL-10 upon the NF-κB pathway. The first report by Wang *et al*. proposed that IL-10 acted through the regulation of transcription. In peripheral blood mononuclear cells (PBMC) IL-10 had no effect on cytokine mRNA accumulation during the first hour of IL-10 stimulation but after 2·5 hr stimulation substantial inhibition was observed and this was then reflected in the levels of TNF-α protein produced.[90] This work was extended to propose that this control was exerted through the inhibition of LPS induced NF-κB binding to its motif and thereby inhibiting cytokine production in PBMCs. Two further papers also supported this observation.[91,92] Shames *et al*. demonstrated that

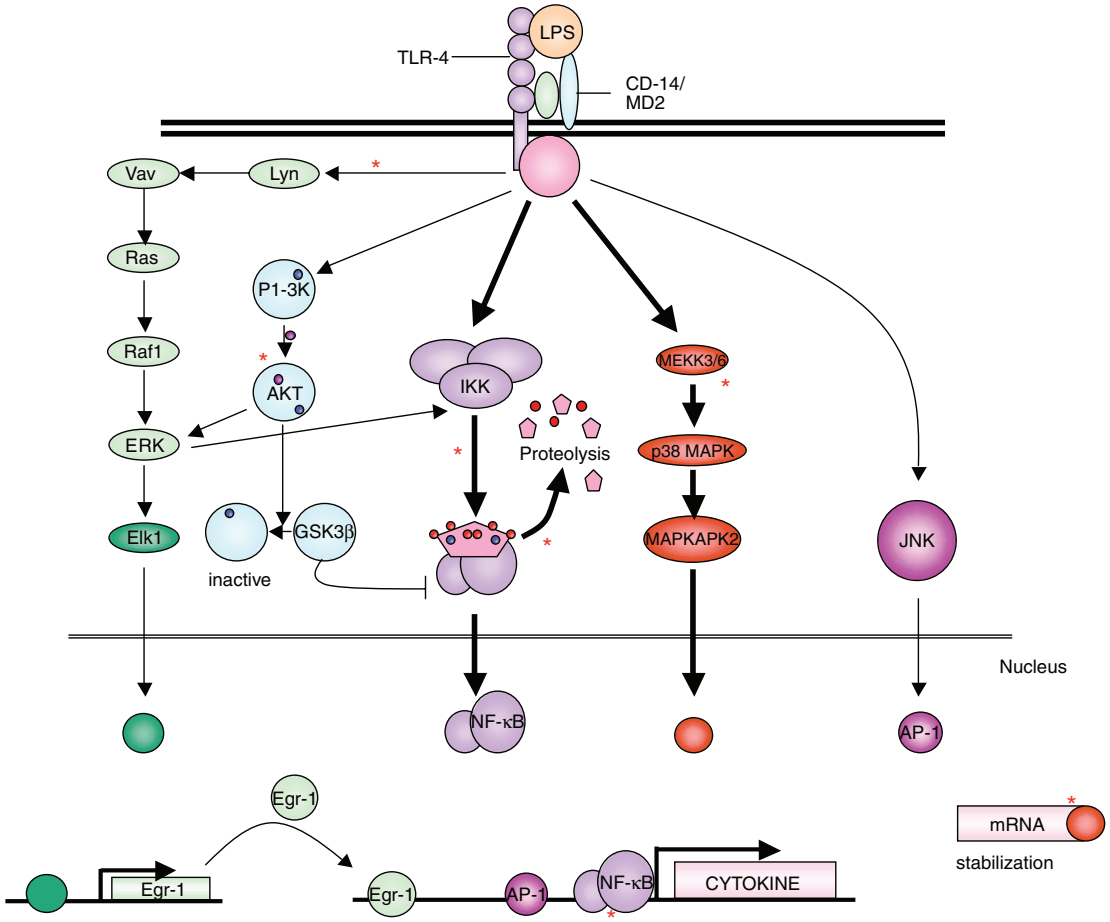

Figure 2. Signalling cascades of the LPS pathway and reported sites of IL-10 intervention. LPS activates numerous signalling cascades that are critical for the production of cytokines by macrophages. The two dominant pathways of cytokine production, the NF-κB and the p38 MAPK pathway have both been cited as being inhibited by IL-10. The NF-κB pathway has been suggested to be regulated at several levels, with IL-10 inhibiting the activation of IKK and thereby the degradation of IκB by the proteosome and also preventing the binding of the NF-κB subunits to its binding motif. Other reports have suggested IL-10 prevents translation in murine systems by inhibiting p38-MAPK. In addition 3′ UTR of the TNF-α gene was found to be critical to IL-10 activity. With the exception of JNK, the other pathways activated by LPS have also demonstrated sensitivity to the effects of IL-10. *, Suggested site of IL-10 inhibition.

pretreatment with IL-10 prevented the degradation of IκBα and thereby the translocation of NF-κB to the nucleus and its activation of cytokine production. Schottelius *et al.* demonstrated substantial inhibition by IL-10 of NF-κB activity using a reporter construct with three tandem MHC class I NF-κB binding motifs, this inhibition by IL-10 was proposed to act by blocking the activity of the IKK molecule, thereby preventing the degradation of IκB. This inhibition by IL-10 resulted in a 60% inhibition of NF-κB inducible genes. However, this blockage of IκB degradation was a transient effect of IL-10, IKK activity was inhibited at 5–10 min post IL-10 stimulation, and therefore IL-10 inhibition of NF-κB binding at 1 hr post stimulation was independent of IκB activity. and finally, using the same MHC class I NF-κB binding motifs, a recent study has shown that with pretreatment of immature dendritic cells with IL-10 results in a reduction of LPS induced NF-κB activation characterized by reduced degradation of IκBα and inhibition of serine phosphorylation of p65.[93] However, in primary human macrophages the effects of IL-10 upon the NF-κB pathway have been shown to be negligible at concentrations where the effects of IL-10 are saturating.[79] Supporting this observation, Zhou *et al.* have shown that rather than IL-10 affecting the recruitment of NF-κB to the IL-12p40 gene, IL-10 abolished the recruitment of RNA polymerase II to the promoter and the authors suggested that several mechanisms were employed by IL-10; including, reduced C/EBPβ binding to the promoter and reduced nucleosome remodelling.[94] Moreover, Clarke *et al.* only see an inhibitory effect of IL-10 on NF-κB activity, in the murine macrophage cell line, RAW 264.7, at concentrations much higher than those required to suppress cytokine production.[95] Clearly, this field remains contentious and requires clarification. The heterogeneity of

results may be a consequence of different systems used by different laboratories, in particular the different NF-κB consensus sequences used may be a key factor in the apparent conflicting results obtained.

P42/44 MAP kinase (MAPK)

LPS also rapidly induces numerous tyrosine kinases including the *src* family tyrosine kinases. Small molecular weight inhibitors of tyrosine kinases such as genistein, herbimycin A and PP2 have shown that tyrosine kinases play a key role in the regulation of cytokine production from LPS stimulated monocytes,[96,97] as have inhibitors of p42/p44 MAPK activation.[98] The *src* family member *lyn*, associates with the guanine exchange factor Vav. IL-10 has been shown to inhibit LPS-induced activation of *lyn* and subsequent activation of downstream events in this pathway such as Ras activation and p42/44 MAPK kinase activation.[99] However this early study has never been repeated and other researchers have even shown that IL-10 actually induces p42/44 MAPK activation.[71]

P38 MAPK

p38 MAPK is a stress response kinase rapidly activated by LPS. The generation of inhibitors of p38 MAP kinase such as the pyridyl imidozole compound SB203580 have shown that this kinase plays a pivotal role in the control of TNF-α and many other pro-inflammatory cytokines.[100] p38 MAPK is thought to fulfil an important role in the stabilization of cytokine mRNA by targeting adenosine-uracil (AU) rich elements (ARE) in the 3′ untranslated regions (UTR) of these genes.[101] In the case of TNF-α, removal of the 3′ UTR results in an elevated level of expression of this cytokine. In transgenic mice engineered to express the human TNF-α transgene, an excessive production of TNF-α is observed which leads to the development of an inflammatory arthritis.[102] More specifically, the removal of a 69 bp ARE from the murine TNF-α-3′ UTR also leads to an overproduction of TNF-α.[103] Interestingly, in the TNF-α-3′ UTR modified transgenic mice, IL-10 is no longer capable of inhibiting TNF-α production, implying this region of gene as a critical target. Further support for such a conclusion has been provided by the generation of TNF-α luciferase constructs by Denys *et al.*[79] In these experiments, when IL-10 was given simultaneously with LPS, IL-10 was only capable of inhibiting constructs containing the 3′ UTR, as constructs carrying the TNF promoter alone were only modified by IL-10 when the cells were pretreated for 24 hr prior to LPS stimulation. Given that p38 MAP kinase plays such an important role in cytokine mRNA stability, it has been an attractive candidate target for the action of IL-10 intervention. Kontoyannis *et al.* have proposed that the main effect of IL-10 signalling is the inhibition of the p38 MAPK pathway and have demonstrated in murine macrophage cells a 90% inhibition of p38 MAPK phosphorylation and suggest that the inhibition of MAP KAP K2 (a downstream target of p38 MAPK) prevents the polysomal coupling of cytokine mRNA without affecting mRNA stability but rather disrupting mRNA–peptide translation. However, we and others have failed to show any affect of IL-10 on p38 MAPK activity in human mononuclear cells and would suggest that the effects of IL-10 are independent of p38 MAPK and are at the level of post-transcriptional control.[79,104] It is difficult to reconcile such opposing views, however, work done by Biswas *et al.*[105] support the post-transcriptional control hypothesis. Although IL-10 has no direct effect on mRNA decay it was demonstrated, using *in vitro* systems, that the effects of IL-10 on the chemokine KC mRNA stability were to antagonize the stabilizing effects of LPS. Kishore *et al.* also support this hypothesis as IL-10-mediated inhibition of a KC reporter construct required the AU-rich elements in the 3′ UTR, a characteristic of post-transcriptional control.[106]

STAT3

The literature sites only two main signalling cascades that are initiated by IL-10: the Jak/STAT pathway; and the phosphatidylinositol-3 (PI-3) kinase pathway, as summarized in Fig. 3. Of these, only the Jak/STAT pathway has been shown to have an anti-inflammatory role in monocytes/macrophages. Studies using the Jak1 and macrophage conditional STAT3 knockout mice have shown that these two molecules are absolutely required for IL-10 mediated cytokine suppression.[47,107,108] More recently, it has been shown that it is the STAT3α isoform, rather than the β isoform that specifically mediates IL-10 inhibition of TNF-α and IL-6 production in murine macrophages.[109] Further studies in murine cell lines have deleted the STAT3 binding sites within the IL-10 receptor and shown an ablation of IL-10's ability to suppress TNF-α production in response to LPS.[46,47] Finally, the use of a STAT3 dominant-negative (Y705F) suggests that STAT3 is the dominant effector molecule mediating the majority of IL-10 s anti-inflammatory effects in human macrophages such as inhibition of cytokine production, induction of soluble TNF-receptor production and TIMP-1 production and down regulation of MHC class II expression. However, expression of the dominant negative was unable to inhibit early phases of IL-10 signalling suggesting STAT3-independent signalling.[72]

PI-3 kinase

IL-10 activates PI-3 kinase, and its downstream effectors, AKT and p70 S6 kinase.[110–112] LPS also induces PI 3 kinase and AKT,[113] and it has been suggested that PI-3 kinase limits LPS signalling pathways *in vitro*[114] and *in vivo*,[115] potentially via AKT mediated regulation of NF-κB. However in human monocytes, the inhibition of IL-10 induced PI-3 kinase pathway had no effect on anti-inflammatory activity of IL-10 and the authors suggest that activation of this pathway promotes cell viability/cell proliferation.[110] This is supported by Zhou *et al.* who have shown that in myeloid precursors, IL-10 promotes cell survival via the activation of the PI-3 kinase pathway. PI-3 kinase is activated by IL-10 via its association with the adaptor molecule IRS-2, and proceeds to then activate AKT.[111]

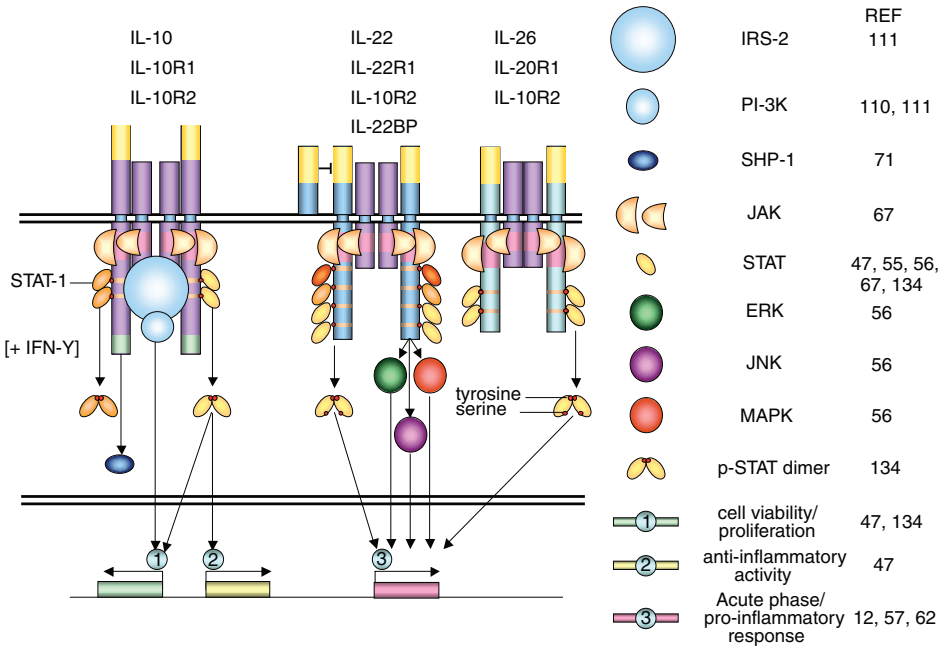

Figure 3. IL-10, IL-22 and IL-22 receptor signalling. Binding of IL-10, IL-22 and IL-26 to their respective receptor complex results in the initiation of various signalling cascades. In all cases the JAK/STAT pathway is the primary pathway known to be activated by ligand binding. IL-10 also activates the PI-3K pathway via the binding of the IRS-2 adaptor molecule to the IL-10R complex. STAT-1 and SHP-1 have also been shown to be activated by IL-10, however, the former is only induced with IFN-γ; preincubation. IL-22 and IL-26, like IL-10, initiate the dimerization of STAT-3, however, IL-22 also activates the JNK, ERK and MAPK pathways as well as STAT1 and -5. IL-22 and -26 also activate the acute phase/pro-inflammatory responses in cells rather than de-activation of the inflammatory response.

Conversely, Bhattacharya *et al.* have suggested that *pre*treatment of bone-marrow-derived dendritic cells with IL-10 resulted in the inhibition of LPS induced PI-3 kinase pathway, which in turn prevented the activation of IKK and the subsequent degradation of IκB.[93] Once again, contradiction plagues this field. The key factor in this case may be the requirement for pretreatment, clearly suggesting that IL-10 is inducing a gene whose actions are central to the suppression of LPS signalling pathways leading to inhibition of cytokine synthesis.

IL-10 INDUCIBLE GENES

A number of studies have suggested that protein synthesis is required for IL-10 to mediate its cytokine suppressive effects, as many groups report a requirement for pretreatment of IL-10 to inhibit many aspects of LPS signalling.[72,93,116,117] The identity of the gene(s) mediating these effects still remains unclear. Until the advent of expression profiling using microarrays there was only a limited number of genes known to be regulated by IL-10 in mononuclear cells: these included FcR1 (CD64),[67] TIMP-1,[16] monocyte chemoattractant protein-1 (MCP-1),[118] CCR5,[119] CD163,[120] IL-1ra,[15] TNF-R2[121] and SOCS3.[122] In the last 2 years a number of profiling studies have greatly expanded our knowledge of IL-10 inducible genes in both human and murine mononuclear cells.[69,123–127] In the most extensive study of Jung *et al.* up to 500 genes were shown to be induced by IL-10 over multiple time points reflecting coherent IL-10 responses in human PBMCs. However, when the data generated from the human studies are compared to the murine studies only a limited number of genes are common to both (Table 1). It seems surprising that only 21 genes are common to both species. This may be a reflection of the human and murine Affymatrix chips used containing a differential set of genes or it could be due to the fact that the murine studies were all performed in mature macrophages where as most of the human studies were performed in monocytes. We found that as human monocytes matured into macrophages, many genes become insensitive to IL-10 regulation.[123]

One notable group of IL-10-inducible genes absent from Table 1 are the leucocyte immunoglobulin-like receptor (LIR) family. This family comprises 10 family members, related to the natural killer cell immunoglobulin receptors for human leucocyte antigen class I molecules.[128] Distinct LIR family members are differentially expressed in lymphocytes, monocytes, macrophages, DC cells and granulocytes. These receptors can either be activating or inhibitory, the cytoplasmic domain of the later contain immunoreceptor tyrosine-based inhibitory receptor motifs which recruit SH2 containing protein tyrosine phosphatase-1 (SHP-1) to the receptor complex. At the RNA level, IL-10 has been shown to induce LIR-1, -2, -3, -4, -6a, and -7.[125] At the protein level LIR-3 and -4 (both inhibitory) are up-regulated by IL-10 in dendritic cells.[129] These two LIR have

Table 1. IL-10 inducible genes observed in both human and murine mononuclear cells

Name	Function
B-ATF	Transcriptional regulator
GADD45β	Signal transduction
SOCS3	
Vav-1	
Tyrosine phosphatase	
IL-10	Cytokine/chemokines
MIP-1β	
MCP2	
MCP5	
IL-1ra	Cytokine antagonist
IL-4Rα	Cytokine/chemokine receptors
CCR1	
CCR5	
FcRII	Immunoglobulin receptor
CD163	Scavenger receptor
Arginase II	Enzymes
Glycerol kinase	
Chondroitin sulphate	Miscellaneous
Proteoglycan-2 (versican)	
Pentraxin	
Latent TGF-β binding protein	
Metallothionein-l	

B-ATF, B-activating transcription factor; GADD, growth arrest and DNA damage; TGF-β, transforming growth factor-β.

been shown to play a crucial role in the tolerization of dendritic cells.[130] Up-regulation of these two LIR by IL-10 may represent an additional mechanism by which IL-10 treatment of dendritic cells renders these cells tolerogenic.

Bcl-3, a member of the IκB protein family, was shown to be strongly up-regulated by IL-10 in murine macrophages in two profiling studies[124,126] but absent from any of the human studies. Kuwata *et al*. were able to demonstrate that IL-10 also up-regulated Bcl-3 at the protein level in a STAT3-dependent manner. Using a combination of approaches they show convincing data for a role of Bcl-3 in the suppression of LPS-induced TNF-α, but not IL-6, production via antagonism of the NF-κB signalling pathway. Bcl-3 preferentially associates with the p50 subunit of NF-κB and the authors suggest that Bcl-3 acts as a negative regulator of NF-κB and enhances p50-mediated inhibition of TNF-α promoter activity by antagonizing the formation of a functional NF-κB p50/p65 heterodimer

Another recently described IL-10 inducible gene is heme oxygenase 1. This protein has been shown to be regulated at the protein level in both human and murine macrophages.[131,132] In the murine system this protein has been shown to be critical to the anti-inflammatory activities of IL-10 but it does not appear to have the same role in human systems.[125,133]

CONCLUSION

The IL-10 field is still centred round the Jak1/STAT3 pathway as a central mediator of IL-10 anti-inflammatory effects. However, it is still unclear how STAT3 mediates these effects, given that many other cytokines also activate this factor. Possibly other signalling moieties may be involved or alternatively, the duration of the STAT3 response induced by IL-10 may be the critical factor, as unlike other cytokines, IL-10 induction of STAT3 has evolved to escape the control mechanism such as SOCS3 to limit STAT3 activation. What is also unclear is whether STAT3 is acting solely as a transcription factor or whether it fulfils another role in IL-10 signalling. If STAT3 is acting as a transcription factor, the identity of the key gene it induces to suppress cytokine synthesis still remains elusive. Although candidate genes such as Bcl-3 exist, no one mechanism has emerged that encompasses all the data published regarding both the NF-κB transcriptional control and the control of mRNA stability via the 3′ UTR. It may simply be that IL-10 employs multiple mechanisms within different target cells and that this puzzle still remains to be solved.

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