The Interleukin-10 Signal Transduction Pathway and Regulation of Gene Expression in Mononuclear Phagocytes

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ABSTRACT

Interleukin-10 (IL-10) activates a diverse array of functional responses in mononuclear phagocytes. Functional IL-10 receptor (IL-10R) complexes are tetramers consisting of two IL-10R1 polypeptide chains and two IL-10R2 chains. Binding of IL-10 to the extracellular domain of IL-10R1 activates phosphorylation of the receptor-associated *Janus* tyrosine kinases, JAK1 and Tyk2. These kinases then phosphorylate specific tyrosine residues (Y446 and Y496) on the intracellular domain of the IL-10R1 chain. Once phosphorylated, these tyrosine residues (and their flanking peptide sequences) serve as temporary docking sites for the latent transcription factor, STAT3 (signal transducer and activator of transcription-3). STAT3 binds to these sites via its SH2 (*Src* homology 2) domain, and is, in turn, tyrosine-phosphorylated by the receptor-associated JAKs. It then homodimerizes and translocates to the nucleus where it binds with high affinity to STAT-binding elements (SBE) in the promoters of various IL-10-responsive genes. One of these genes, *SOCS-3* (<u>S</u>uppressor <u>o</u>f <u>C</u>ytokine <u>S</u>ignaling-3) is a member of a newly identified family of genes that inhibit JAK/STAT-dependent signaling. Moreover, the ability of IL-10 to induce *de novo* synthesis of SOCS-3 in monocytes correlates with its ability to inhibit expression of many genes in these cells, including endotoxin-inducible cytokines such as tumor necrosis factor-α (TNF-α) and IL-1. Thus, the ability of IL-10 to inhibit gene expression in monocytes is associated with its ability to rapidly induce synthesis of SOCS-3.

INTRODUCTION

THE CYTOKINE, INTERLEUKIN-10 (IL-10), was originally identified as a soluble factor produced by murine, CD4$^+$, Th2-type T cell clones that can inhibit production of cytokines such as IL-2 and interferon-γ (IFN-γ) by Th1-type clones.[1] Soon after, it was determined that the ability of IL-10 to inhibit cytokine production by T cells was primarily mediated via its effects on antigen-presenting cells (APC), including monocytes, macrophages, and dendritic cells.[2,3] Therefore, IL-10 acts directly on APCs to inhibit expression of costimulatory surface molecules such as major histocompatibility complex (MHC) class II and B7, as well as cytokines that are necessary for optimal T cell activation.[4,5] By inhibiting expression of these molecules by APCs, IL-10 indirectly suppresses activation of T cells and production of T cell-derived cytokines such as IFN-γ and IL-2. In addition to its effects on APCs, IL-10 can also

mediate direct effects on other hematopoietic cell types, including B and T lymphocytes (both CD4$^+$ and CD8$^+$), natural killer (NK) cells, mast cells, and eosinophils (reviewed in ref. 6). For example, IL-10 is a potent growth factor for activated B cells,[7] and can prevent activation-induced apoptosis in T cells.[8] Although IL-10 primarily inhibits T cell activation via its inhibitory effects on APCs, it can also act directly on T cells to inhibit production of certain cytokines, particularly IL-2.[9,10] How then does IL-10 transduce the signals that activate these diverse functional activities?

A single IL-10 gene encodes a polypeptide composed of 178 amino acids, including a hydrophobic, 18-amino acid, leader sequence.[11] Bioactive IL-10 is a homodimer composed of two noncovalently associated 18 kDa subunits. The native form of human IL-10 is nonglycosylated.[12] The cellular IL-10 gene is highly homologous to an open reading frame in the Epstein-Barr virus genome, termed BCRF1.[12,13] The viral IL-10 pro-

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tein (BCFR1) shares many of the functional properties that are associated with cellular IL-10. It has been suggested that the virus may have acquired this gene as a means of suppressing host antiviral immune responses. IL-10 is produced by a variety of cell types, including T cells (both CD4^{+} and CD8^{+}), B cells, monocytes, macrophages, and dendritic cells. IL-10 gene expression can be induced in monocytes by a variety of stimuli, including both Gram-positive and Gram-negative bacteria, purified endotoxin, bacterial exotoxins, and certain viruses. Expression of the IL-10 gene in lipopolysaccharid e (LPS)-stimulated monocytes is delayed relative to that of other cytokine genes such as tumor necrosis factor-α (TNF-α) and IL-1.[14] Furthermore, down-regulation of TNF-α and IL-1 gene expression coincides with the appearance of mRNA for IL-10.[15] Similarly, in activated T cells, IL-10 gene expression is delayed relative to the expression of other cytokine genes such as IL-2 and IL-4.[16] The delayed expression of IL-10 in both monocytes and T cells suggests that it functions to feedback inhibit continued cytokine production.

Numerous studies have shown that IL-10 treatment can decrease the severity of inflammatory processes *in vivo*. For example, IL-10 has been shown to reduce disease activity in animal models of microbial sepsis.[17,18] Conversely, treatment with neutralizing anti-IL-10 antibodies exacerbates disease activity and increases the risk of endotoxic shock in animals challenged with LPS or viable Gram-negative bacteria. Increased morbidity and mortality in these animal models is associated with increased production of proinflammatory cytokines, particularly TNF-α and IL-1.[19] Moreover, IL-10 gene knockout mice develop an inflammatory bowel disease that is associated with markedly elevated levels of certain cytokines, particularly TNF-α.[20] These findings have prompted clinical trials of recombinant human IL-10 as a possible treatment for chronic inflammatory bowel disease. IL-10 is also being evaluated as a potential therapeutic agent for other inflammatory diseases, including rheumatoid arthritis and multiple sclerosis.

IL-10 RECEPTORS

Homodimeric IL-10 binds to a single class of cell-surface receptors with a K_d in the range of 50–200 pM.[21] IL-10 receptors (IL-10R) are primarily expressed by hematopoietic cells such as B cells, T cells, NK cells, monocytes, and macrophages. IL-10Rs are not expressed by nonhematopoiet ic cells such as fibroblasts or endothelial cells. These observations are consistent with the results of functional studies that showed that IL-10 can inhibit cytokine production in monocytes but not in fibroblasts or endothelial cells.[22,23] The human IL-10R is species specific. In contrast, the murine IL-10R can bind both murine and human IL-10 with comparable affinity. Structural analysis of the IL-10 receptor-α chain (IL-10R1) has shown that it is a member of the class II cytokine receptor family, which includes the receptors for type-I and type-II IFNs, tissue factor, and the orphan receptor CRF2-4.[24,25] As will be discussed later in this review, CRF2-4 has recently been determined to be an essential component of functional IL-10R complexes.

IL-10R1: the ligand-binding chain

The gene for the first IL-10R chain, IL-10R1, was cloned by scientists at DNAX, Inc. in 1993.[24] The cDNA for the mouse IL-10R1 gene was isolated from cDNA libraries prepared from both a murine mast cell line (MC/9) and a murine macrophage cell line (J774). Expression of these cDNAs in COS-7 cells generated an IL-10R protein approximately 110 kDa in size. Most importantly, the expressed IL-10R1 chain bound radiolabeled IL-10 with an affinity comparable to that previously shown for native IL-10 receptors. Furthermore, when expressed in a mouse pre-B cell line, Ba/F3, the recombinant murine IL-10R1 chain endowed these cells with the ability to proliferate in the presence of IL-10.

The gene for the human homologue of the murine IL-10R1 chain was cloned from a cDNA library prepared from a human Burkitt lymphoma cell line, BJAB.[25] The human IL-10R1 gene encodes a 3.6-kb mRNA transcript, and similar to the murine IL-10R1 gene, generates an IL-10-binding protein approximately 90–110 kDa in size when expressed in COS-7 cells (Table 1). The full-length protein consists of 557 amino acids and is ≈60% identical to the murine homologue. Sequence analysis of both the murine and human IL-10R1 proteins revealed that the actual molecular mass of these molecules (≈60 kDa) was much less than that determined by chemical cross-linking and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). These findings indicated that a large portion of the total mass of the IL-10R1 molecule is due to carbohydrate. The human IL-10R1 chain exhibited a K_d for IL-10 in the range of 200–250 pM when expressed in Ba/F3 cells. Furthermore, expression of the human IL-10R1 chain in Ba/F3 cells enabled these cells to proliferate in response to stimulation by human IL-10.

Forced expression of the IL-10R1 chain is sufficient for many cell types to be able to respond functionally to IL-10, however, in some cell types this is not sufficient. These findings suggested that another receptor chain might be required to form a functional IL-10R complex. Both the mouse and human IL-10R1 proteins are structurally related to the IFN receptors, however, IL-10 does not compete with type-I or type-II IFNs for binding to IFN receptors, and IFNs do not compete with IL-10 for binding to IL-10 receptors. Therefore, it seemed unlikely that one or more of the known IFN receptor proteins are utilized by the IL-10 receptor. One candidate for this second IL-

TABLE 1. CHARACTERISTICS OF THE IL-10 RECEPTOR-α CHAIN (IL-10Rα, IL-10R1)

The ligand binding chain of the IL-10R complex
Recruits JAK1 to the receptor complex
Provides docking sites for STAT3 on its intracellular domain (Tyr[446], Tyr[496])
A member of the class II cytokine receptor family
A single gene encodes a 578-amino-acid protein that includes a 21-amino-acid signal sequence, a 215-amino-acid extracellular domain, a 25-amino-acid transmembrane domain, and a 317-amino-acid intracellular domain
Size of mRNA transcript: 3.6 kb
Expression is limited to hematopoietic cells and hematopoietic tissues (*e.g.*, spleen and thymus)

10R chain is a molecule known as CRF2-4 that is encoded by a gene (CRFB4) closely linked to that of the IFN-αR genes on chromosome 21.[26] The precise function of this molecule remained a mystery until 1997, when Kotenko et al. identified its role as the second chain of the IL-10R complex.[27]

IL-10R2: the accessory chain

Earlier studies had established that the Janus kinase Tyk2 is associated with the intracellular domain of CRF2-4.[28] Other studies showed that IL-10 mediates functional responses in IL-10R-positive cells by specifically activating JAK1 and Tyk2.[29,30] Furthermore, JAK1 was known to be associated with the IL-10R1 chain. Kotenko and colleagues deduced that Tyk2 must be associated with the putative second chain of the IL-10R complex, and considered the possible involvement of CRF2-4.[27] These investigators showed that expression of the IL-10R1 chain alone was not sufficient to transduce IL-10-mediated signals. However, coexpression of IL-10R1 together with CRF2-4 resulted in the formation of functional IL-10R complexes that were able to bind and respond to IL-10. Most importantly, the authors showed that IL-10R1 coimmunoprecipitated with CRF2-4 from cell lysates derived from IL-10-treated cells. Monoclonal antibodies to the CRF2-4 protein have recently been generated, and found to block IL-10R-mediated signaling (Kevin Moore, personal communication). Thus, CRF2-4 constitutes the essential second chain of the IL-10R complex, and is now referred to as IL-10R2. This molecule plays a critical role in IL-10-mediated signaling because, in the presence of IL-10, it recruits an additional JAK kinase, Tyk2, that catalyzes trans-phosphorylation of the IL-10R1 chain and JAK1, which is obligatory for IL-10 receptor-mediated signaling.

The CRF2-4 protein is 325 amino acids long and is ≈69% identical to the murine homologue (Table 2).[31] When IL-10 is bound to its receptor, anti-CRF2-4 antibodies can coimmunoprecipitate IL-10R1 together with CRF2-4. The IL-10R complex formed by the ligand-induced bridging of two IL-10R1 molecules, and their paired association with two IL-10R2 molecules, initiates a cascade of signal transduction events that include tyrosine phosphorylation of JAK1 and Tyk2, trans-phosphorylation of the IL-10 receptor chains, and activation and nuclear translocation of the latent transcription factor, STAT3. It is noteworthy that although IL-10R1 and its associated JAK1 is absolutely required for IL-10-induced signaling, other Janus kinases may substitute for Tyk2 in IL-10R2. For example, using a chimeric receptor complex consisting of IL-10R1 and the IFN-γR2 chain, Kotenko et al. showed that JAK2 can replace the accessory function normally provided by Tyk2, and activate IL-10 signal transduction.[27] This may explain some of the known counterregulatory effects of IFN-γ and IL-10 that occur in monocytes and macrophages. Therefore, if cells express only the IL-10R1 chain, IL-10 signal transduction will not occur. However, in cells that coexpress IL-10R1 and IL-10R2 (CRF2-4), binding of dimeric IL-10 cross-links the IL-10R1 and IL-10R2 molecules which then activates the IL-10 signal transduction cascade.

The tetrameric configuration of the IL-10R complex is similar to that of the IFN-γ receptor, which also consists of two ligand-binding α-chains and two accessory β-chains that recruit an additional Janus tyrosine kinase to the receptor complex (reviewed in ref. 32). Also, like IFN-γ, bioactive IL-10 is a homodimer.[33] Two independent reports published in 1995 demonstrated that IL-10Rs utilize JAK1 and Tyk2 to transduce signals initiated by ligand binding to the extracellular domains of the IL-10R1 chain.[29,30] These kinases are physically associated with the membrane proximal intracytoplasmic portion of the receptor chains. JAK1 is associated with IL-10R1 and Tyk2 with IL-10R2.

IL-10R2 knockout mice (CRFB4−/− mice) have recently been generated by homologous recombination.[34] These animals develop normally and are fertile, however by ≈12 weeks of age, they develop chronic colitis and splenomegaly reminiscent of that which develops in IL-10 gene knockout mice.[20] Although cells from these animals respond normally to IFN stimulation, responses to IL-10 are severely diminished. For example, bone marrow-derived macrophages from these animals do not respond to IL-10 by down-regulating expression of TNF synthesis. Thus, the findings described in this report confirmed the essential role of CRF2-4 as a functional component of the IL-10R complex. The fact that these animals spontaneously develop chronic inflammatory bowel disease identical to that which develops in IL-10 knockout mice underscores the critical role of IL-10 as a negative regulator of inflammation. Unlike the IL-10R1 gene, which is primarily expressed by hematopoietic cells, the CRFB4 gene is expressed by most tissues.[31] These findings suggest that CRF2-4 might be utilized by other receptor complexes besides the IL-10R.

TABLE 2. CHARACTERISTICS OF THE IL-10 RECEPTOR-β CHAIN (IL-10Rβ, IL-10R2)

Serves an accessory role by recruiting Tyk2 to the IL-10R complex

A member of the class II cytokine receptor family

A single gene encodes a 325-amino-acid, membrane-spanning protein (~200 amino acids ECD; ~100 amino acids ICD)

Size of mRNA transcript: 2.0 kb

Broad tissue distribution

IL-10R2 gene knockout mice develop chronic inflammatory bowel disease similar to that which develops in IL-10 gene knockout mice

IL-10 ACTIVATES STAT1 AND STAT3

The first demonstration that IL-10 can activate STAT proteins (signal transducers and activators of transcription) was published in 1993.[35] In this report, it was demonstrated that IFN-γ activates DNA-binding proteins capable of binding to an oligonucleotide probe derived from the IFN-γ response region (GRR) of the proximal promoter of the human FcγRI (CD64) gene.[36] This probe contains a DNA sequence known as the IFN-γ activation sequence (GAS), which can bind a protein, referred to at that time simply as p91, that is specifically inducible by IFN-γ. When nuclear extracts from IL-10-treated monocytes were examined, it was found that they also contained a DNA binding activity similar to that induced by IFN-γ. This was the

first demonstration that a cytokine other than IFN-γ could activate DNA-binding proteins capable of binding to these elements. It was further demonstrated in this study that anti-p91 antibodies could supershift the IL-10-inducible DNA:protein complexes detectable in gel-shift assays. This molecule, p91, had previously been shown to be a transcription factor involved in IFN-induced transcription. [37] These findings suggested a potential relationship between the IL-10 and IFN-γ signal transduction pathways. This hypothesis was further supported by the finding that IL-10 could induce expression of FcγRI (CD64), a gene that is also activated by IFN-γ.[38] Related studies by others subsequently confirmed that IL-10 activates p91 in macrophages. [39] The p91 protein was renamed STAT1α in 1993 when it became apparent that p91 was one member of a family of structurally related proteins that mediate cytokine-induced *trans*-activation of multiple genes. This family now contains seven members: STAT1α, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6. STAT1β is an alternatively spliced variant of STAT1α that lacks the nuclear transactivation domain that is present in STAT1α. In most IL-10R-positive cell types, IL-10 activates both STAT1α and STAT3.[29] Activation of these STATs can result in the formation of three distinct STAT complexes: STAT1 homodimers, STAT3 homodimers, and STAT1:STAT3 heterodimers.[40,41] The full significance of this differential assembly of STAT complexes is not clear, but it may facilitate induction of distinct patterns of gene expression in different cell types.

Weber-Nordt *et al*. identified two, membrane-distal, tyrosine residues (Y427 and Y477) on the intracellular domain (ICD) of the murine IL-10R1 chain that are required for binding and activation of STAT3 by IL-10.[41] Similarly, the intracellular domain of the human IL-10R1 protein also contains two conserved tyrosine residues (Y446 and Y496) that correspond to the two essential tyrosine residues present in the murine IL-10R1 protein. STAT3 but not STAT1 is recruited to the ligand-activated IL-10R1 chain by binding to these phosphotyrosine residues. It is noteworthy that the sequences that flank the tyrosine residues on the ICD are highly conserved in both the mouse and human proteins. Weber-Nordt *et al*. noted that very similar flanking peptide sequences are also present adjacent to the ICD tyrosine residues of the gp130 protein. Similar to the IL-10R complex, receptors such as the IL-6 and IL-11 receptors, which utilize gp130 for signaling, also activate STAT3.[42] The consensus sequence for the conserved peptide sequence noted by Weber-Nordt *et al*. is GYLKQHy, where Hy is an amino acid with a terminal hydrophilic group.

The notion that the IL-10 receptor may utilize the same signaling pathway as that used by IL-6-type cytokines was strengthened by the studies of Lai *et al*., who showed that the intracellular domain of the murine IL-10R1 chain can efficiently recruit STAT3 to its ICD when transfected into the hepatocyte cell line, HepG2.[43] Forced expression of the murine IL-10R1 molecule by transfection of the IL-10R1 gene specifically recruited STAT3 to the membrane-distal tyrosine residues (Y427 and Y477) that constitute the so-called Box 3 motif. Cotransfection of the human IL-10R1 chain together with IL-6-responsive reporter gene constructs into IL-10R-negative HepG2 cells endowed these cells with the ability to *trans*-activate these reporter genes when the cells were subsequently stimulated with IL-10. Therefore, although hepatocytes do not normally express

IL-10R, IL-10 can efficiently activate STAT3 in these cells if the IL-10R1 gene is transfected into them. It is noteworthy that reconstitution of a functional IL-10R complex in HepG2 cells required transfection of only the IL-10R1 chain, not both IL-10R1 and IL-10R2. It is likely, therefore, that these cells already express IL-10R2, and that IL-10R1 heterodimerized with the endogenous IL-10R2 protein to generate a functional IL-10R complex.

IL-10 INHIBITS EXPRESSION OF LPS-INDUCIBLE GENES

IL-10 inhibits expression of a number of LPS-inducible genes in monocytes, including many cytokines. A list of some of the LPS-inducible genes that are known to be inhibited by IL-10 is shown in Table 3. These include TNF-α IL-1, IL-6, IL-8, IL-10, IL-12, granulocyte colony-stimulating factor (G-CSF), and granulocyte macrophage (GM)-CSF. In addition, expression of other LPS-inducible genes, including tissue factor and cyclooxygenase-2 (Cox-2), is also inhibited by IL-10. The molecular mechanism by which IL-10 inhibits LPS-inducible gene expression has not been clearly defined. One report suggested that IL-10 inhibits activation of nuclear factor-kappa B (NF-κB) in monocytes,[44] however other studies have failed to confirm this.[45,46] Thus, no clear consensus has been reached concerning the effects of IL-10 on NF-κB activity in LPS-stimulated monocytes.

Similar studies aimed at determining whether IL-10 inhibits activation of mitogen-activated protein kinases (MAPK) have also yielded conflicting results. An initial report by Geng *et al*. indicated that IL-10 inhibits LPS-induced MAPK activity in monocytes. [47] However, other investigators have found that IL-10 does not inhibit activation of MAPK in LPS-stimulated macrophages. [48,49] We examined the effects of IL-10 on activation of p38 MAPK in purified human monocytes. In these

TABLE 3. GENES THAT ARE DOWN-REGULATED BY IL-10 IN MONOCYTES

LPS-inducible genes
    Tumor necrosis factor-α
    Interleukin-1
    Interleukin-6
    Interleukin-8
    Interleukin-10
    Interleukin-12
    Granulocyte colony-stimulating factor
    Granulocyte-macrophage colony-stimulating factor
    Cyclooxygenase-2 (Cox-2)
Interferon-γ-inducible genes
    MHC class II molecules (HLA-D)
    B7.1, B7.2 (CD80, CD86)
    ICAM-1 (CD54)
    IFN-γ-inducible protein (IP-10)
    Inducible nitric oxide synthetase (iNOS)
Interleukin-4-inducible genes
    MHC class II molecules (HLA-D)
    FcεRIIb (CD23)
    IL-1 receptor type-I
    IL-1 receptor type-II

experiments, monocytes were preincubated with medium alone (control) or IL-10 for 1 h at 37°C. At the end of this initial incubation period, LPS was added, and the cells were incubated for 0–60 min longer. As shown in Fig. 1, LPS stimulation induced a rapid increase in the levels of activated (phosphorylated) p38 MAPK. Pretreatment with IL-10 did not decrease the levels of phosphorylated p38 MAPK induced by LPS stimulation. IL-10 also did not inhibit activation of other MAP kinases, including ERK-1, ERK-2, and JNK/SAPK in LPS-stimulated monocytes (unpublished observations). Therefore, our results support the conclusions of Clarke et al.[48] and Foey et al.[49] that IL-10 does not inhibit activation of MAPKs in monocytes.

The ability of IL-10 to inhibit LPS-induced gene expression requires JAK1 because IL-10 does not inhibit LPS-induced cytokine (TNF) production by macrophages from JAK1-deficient mice.[50] These findings indicate that JAK1 is essential for mediating IL-10-induced inhibitory activity. In contrast, although IL-10 has been shown to activate STAT1 in certain cell types, STAT1 is not required for IL-10 to inhibit LPS-induced cytokine production because IL-10 inhibits cytokine production in macrophages from STAT1 knockout mice.[51] A critical role for STAT3 in mediating IL-10-induced inhibition has recently been demonstrated by targeted deletion of the STAT3 gene in macrophages and neutrophils.[52]

Two other points regarding the ability of IL-10 to inhibit LPS-induced gene expression have been confirmed in multiple laboratories: first, inhibition of cytokine gene expression results largely from decreased transcription,[15,53] and, second, inhibition of cytokine gene expression by IL-10 requires new protein synthesis because suppression can be blocked by cycloheximide.[53,54] These findings indicate that IL-10 rapidly induces de novo expression of one or more inhibitory gene(s) that in turn mediate suppression of cytokine synthesis.

Although IL-10 is best known for its ability to inhibit cytokine gene expression, it also activates expression of certain genes in monocytes (Table 4). These genes include FcγRI (CD64), the high-affinity Fc receptor for immunoglobulin G (IgG),[35] tissue inhibitor of metalloproteinases-1 (TIMP-1),[55] monocyte chemoattractant protein-1 (MCP-1),[56] and certain

TABLE 4. GENES THAT ARE UP-REGULATED BY IL-10 IN MONOCYTES

Gene	References
FcγRI (CD64)	(35, 38)
Tissue inhibitor of metalloproteinases-1 (TIMP-1)	(55)
Monocyte chemoattractant protein-1 (MCP-1)	(56)
CCR5	(57)
IL-1 receptor antagonist (IL-1ra)	(58)
TNF-R2 (p75, CD120b)	(59)
Suppressor of cytokine signaling-3 (SOCS-3)	(60)

CC chemokine receptors, particularly CCR5.[57] In addition, IL-10 can potentiate expression of the IL-1 receptor antagonist and the type-2 (p75) TNF receptor by LPS-stimulated monocytes.[58,59] Most recently, we have shown that IL-10 induces expression of a novel gene known as SOCS-3 (Suppressor of Cytokine Signaling-3) in monocytes.[60] SOCS-3 is a member of a newly identified family of highly conserved genes that mediate feedback inhibition of cytokine-induced responses.[61–63] IL-10 rapidly up-regulates transcription of SOCS-3 in monocytes. An increase in SOCS-3 mRNA levels is apparent within 15 min after addition of IL-10.[60] Peak mRNA levels for this gene occur approximately 30–60 min post stimulation. Forced expression of the SOCS-3 gene in myeloid cell lines markedly inhibits cytokine-induced activation of the JAK/STAT signaling pathway.[61–63] These findings indicate that IL-10 may inhibit production of cytokines in monocytes by rapidly inducing expression of SOCS-3.

IL-10 INHIBITS EXPRESSION OF IFN-INDUCIBLE GENES

In addition to its effects on LPS-inducible gene expression, IL-10 has also been shown to inhibit gene expression induced by specific cytokines. For example, IL-10 inhibits expression of several IFN-γ-inducible genes, including MHC class II molecules,[4] B7,[5] intercellular adhesion molecule-1 (ICAM-1),[64,65] IP-10,[60] and inducible nitric oxide synthase (iNOS).[66] Induction of these genes by IFN-γ is mediated in large part through the activation and nuclear translocation of STAT1α. Binding of IFN-γ to the IFN-γR complex on monocytes activates tyrosine phosphorylation of JAK1 and JAK2, which, in turn, mediate trans-phosphorylation of the receptor chains on specific tyrosine residues (reviewed in ref. 32). Phosphorylation of these tyrosine residues generates docking sites for cytosolic STAT1α on the ICD of the IFN-γR1 chain. STAT1α then binds via its SH2 domain to these phosphorylated tyrosine residues, and is itself tyrosine-phosphorylated by the receptor-associated JAKs. Activated STAT1 then dissociates from the receptor, and homodimerizes with other tyrosine-phosphorylated STAT1 molecules. STAT1 dimers then translocate to the nucleus where they bind with high affinity to GAS elements in the promoters of various IFN-γ-inducible genes.

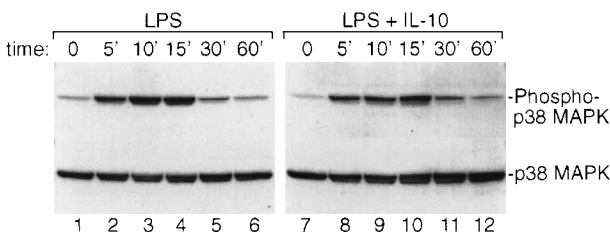The promoters of IFN-γ-inducible genes such as B7, ICAM-1, and IP-10, which are known to be inhibited by IL-10, con-

FIG. 1. Effect of pretreatment with IL-10 on LPS-induced activation of p38 MAPK in purified human monocytes. Monocytes (5×10^6 cells/ml) were preincubated at 37°C with medium alone or medium containing rhIL-10 (10 ng/ml) for 1 h. LPS (100 ng/ml) was then added, and the cultures were incubated for 0–60 min longer. At the designated timepoints, cell lysates were prepared, and analyzed by immunoblotting with anti-phospho-p38 MAPK antibody (New England Biolabs). The blots were then stripped, and reprobed with an anti-p38 MAPK antibody to confirm equivalent levels of p38 protein in each sample.

tain one or more GAS elements. Activation and nuclear translocation of STAT1α is essential for *trans*-activating these genes.[51] Recently, we showed that IL-10 can inhibit IFN-γ-induced tyrosine phosphorylation and nuclear translocation of STAT1α in monocytes.[60] This inhibitory effect of IL-10 is both dose- and time-dependent, and correlates with suppression of several IFN-inducible genes, including IP-10, ISG54, and ICAM-1. Furthermore, we showed that the ability of IL-10 to inhibit IFN-γ-induced gene expression correlates temporally with induction of SOCS-3 mRNA.[60] Recent studies have shown that forced expression of the *SOCS-3* gene in either hematopoietic or nonhematopoietic cell lines markedly inhibits IFN-mediated functional responses, including activation and nuclear translocation of STAT1α.[67,68] Therefore, IL-10 appears to inhibit expression of IFN-inducible genes, at least in part, by inducing rapid expression of *SOCS-3*. Like other SOCS proteins, the SOCS-3 molecule contains a central SH2 (*Src* homology-2) domain that enables it to bind physically to phosphorylated tyrosine residues in the tyrosine kinase domain (JH1 domain) of JAK kinases. Once bound, SOCS-3 blocks the activity of these kinases resulting in diminished IFN-induced STAT1 activity.

IL-10 INHIBITS EXPRESSION OF IL-4-INDUCIBLE GENES

In addition to its inhibitory effects on LPS-induced and IFN-induced responses, IL-10 can also suppress gene expression induced by the Th2 cytokines, IL-4 and IL-13, in monocytes. These genes include MHC class II (HLA-D) Ags,[4] CD23b,[69] and the type-I and type-II IL-1 receptors.[70] Most of these genes are distinct from those activated by IFN-γ. IL-4 and IL-13 activate gene expression by inducing tyrosine phosphorylation and nuclear translocation of STAT6.[71] STAT6-responsive elements are distinct from classical GAS elements because they are characterized by the presence of an intervening 4-base spacer (N4 sites) instead of the more common 3-base spacer (N3 sites) typically present in many GAS elements.[72] This difference (*i.e.*, the presence of a 4-base spacer instead of a 3-base spacer) makes these sites highly selective for STAT6. Examples of this type of STAT-binding element (SBE) can be found in the promoters of several IL-4-inducible genes, including IL-1ra, IL-4Rα, and IgE (Iε).[73–75] In addition, induction of certain other genes that contain N3-type GAS elements in their promoters are also inhibited by IL-10. These include IL-1RI and CD23 (FcεRIIb).[70,75]

The mechanism by which IL-10 inhibits expression of these genes in monocytes appears to be similar to that by which IL-10 inhibits expression of IFN-inducible genes. As shown in Fig. 2A, pretreatment of monocytes with IFN-γ or IL-10 inhibits IL-4-induced tyrosine phosphorylation of STAT6. The ability of IL-10 to inhibit IL-4-induced activation of STAT6 is dose- and time-dependent, and requires that cells be preincubated with IL-10 for at least 1 h prior to stimulation with IL-4. In addition, the ability of IL-10 to inhibit activation of STAT6 can be blocked by actinomycin D indicating a requirement for *de novo* transcription. Furthermore, both IFN-γ and IL-10 rapidly induce *SOCS-3* gene expression in monocytes (Fig. 2B). It is possible, therefore, that IFN-γ and IL-10 inhibit activation of IL-

4-inducible genes by inducing *de novo* expression of SOCS-3. Because activation of STAT6 is essential for expression of IL-4-responsive genes, the ability of IL-10 to inhibit activation of STAT6 provides an explanation for how IL-10 can suppress IL-4-inducible gene expression.

Many atopic disorders are characterized by overproduction of IL-4 and IL-13. The ability of IL-10 to antagonize IL-4- and IL-13-inducible gene expression suggests a potential role for IL-10 in the treatment of allergic diseases.[76] Consistent with this concept, recent studies have shown that IL-10 knockout mice are much more susceptible to bronchial inflammation than wild-type mice.[77] Moreover, STAT6 knock-out mice exhibit markedly diminished bronchial inflammation when challenged with allergens that induce IL-4 and IL-13 activity[78,79] Together, these findings demonstrate that the ability of IL-10 to inhibit activation of STAT6 by IL-4 and/or IL-13 may be of therapeutic value in the treatment of allergic respiratory diseases.
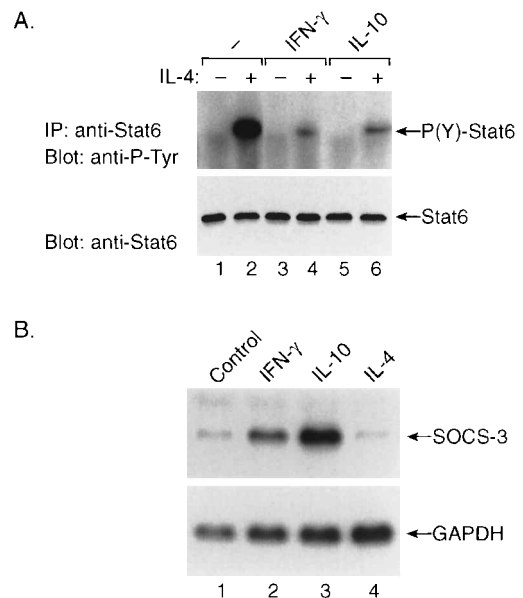

FIG. 2. **(A)** Effect of IL-10 on induction of tyrosine phosphorylation of STAT6 by IL-4 in purified monocytes. Monocytes (5×10^6 cells/ml) were preincubated with medium (control), IFN-γ (10 ng/ml), or IL-10 (10 ng/ml) for 1 h at 37°C, after which the cells were treated with (+) or without (−) IL-4 (1 ng/ml) for 30 min at 37°C. At the end of the second incubation period, cell lysates were prepared, and STAT6 was immunoprecipitated using rabbit anti-STAT6 antibody. The levels of tyrosine-phosphorylated STAT6 were then measured by western blotting with anti-phospho(Y) antibody. The blot was subsequently stripped, and reprobed with an anti-STAT6 antibody (Santa Cruz Biotechnology, Inc.) to confirm equivalent levels of STAT6 protein in each sample. **(B)** Effect of IL-10 on induction of SOCS-3 gene expression in monocytes. Elutriated monocytes (5×10^6 cells/ml) were treated with medium alone (Control), IFN-γ (10 ng/ml), IL-10 (10 ng/ml), or IL-4 (10 ng/ml) for 1 h at 37°C. At the end of this incubation period, RNA was extracted, and the levels of SOCS-3 mRNA were measured by northern blotting.

CONCLUDING REMARKS

Since its initial description in 1989,[1] much has been learned about the biochemical and biological properties of IL-10. Interest in this cytokine increased significantly when it was found that IL-10 inhibits production of endotoxin-inducible cytokines such as TNF-α, IL-1, and IL-6 in mononuclear phagocytes.[2–4] LPS stimulation induces rapid expression of the TNF-α and IL-1 genes in monocytes. Downregulation of TNF-α gene expression in LPS-stimulated monocytes coincides with latent IL-10 gene expression.[14,15] Furthermore, neutralization of IL-10 activity with anti-IL-10 antibodies prolongs TNF-α gene expression and increases net TNF-α production by LPS-stimulated monocytes.[14,15] These findings suggest a critical role for IL-10 in preventing overexpression of TNF-α by monocytes. This hypothesis is supported by the fact that IL-10 gene knockout mice develop a chronic inflammatory bowel disease-like syndrome that is associated with markedly elevated levels of TNF-α.[20]

The overall structure of the IL-10 receptor complex as well as the sequence of signal transduction events initiated by ligand binding are very similar to the IFN-γR signaling pathway.[32] Figure 3 provides a current model of the IL-10 signaling pathway. Functional IL-10R complexes are tetramers consisting of
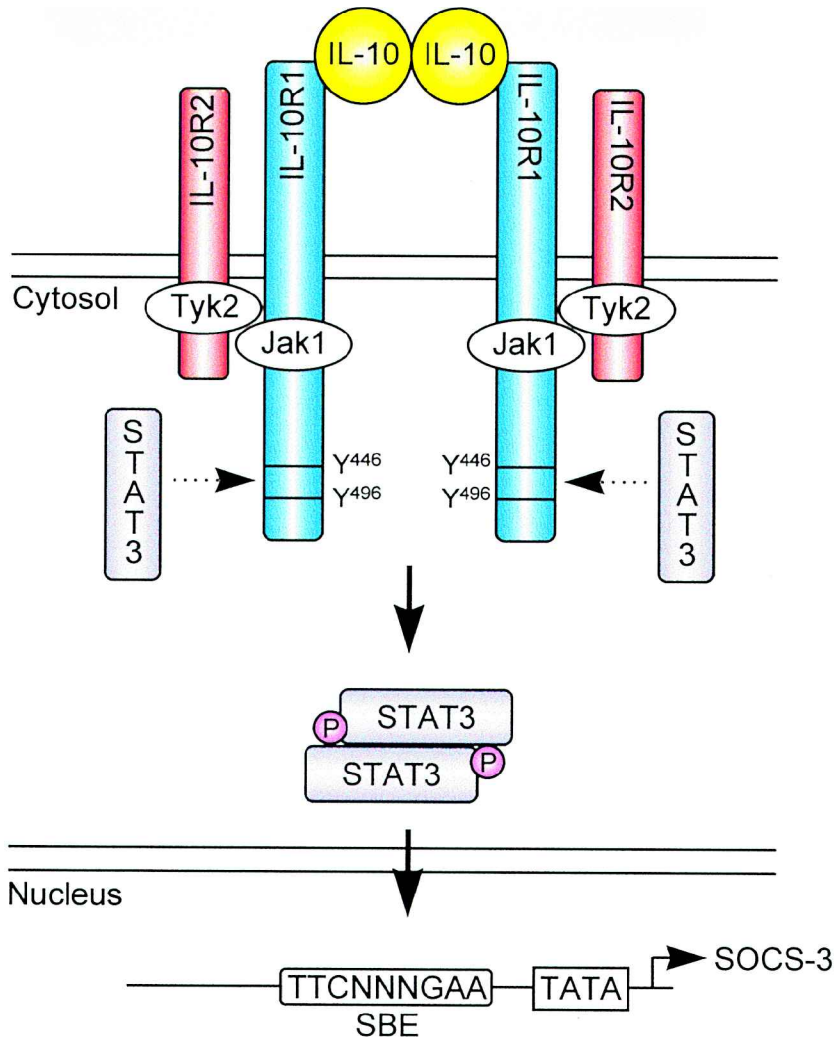

FIG. 3. A unified model of the IL-10 signal transduction pathway in human mononuclear phagocytes. IL-10 signal transduction in monocytes is initiated by binding of homodimeric IL-10 to the extracellular domains of two adjoining IL-10R1 molecules. Ligation of IL-10R1 by IL-10 activates the receptor-associated *Janus* tyrosine kinases, JAK1 and Tyk2, which then *trans*-phosphorylate the intracellular domains of the IL-10R1 chains on specific tyrosine residues. These phosphorylated tyrosine residues and their flanking peptide sequences serve as temporary docking sites for the latent, cytosolic, transcription factor, STAT3. STAT3 transiently docks on the IL-10R1 chain via its SH2 domain, and is in turn tyrosine phosphorylated by the receptor-associated JAKs. Once activated, it dissociates from the receptor, homodimerizes with other STAT1 molecules, and translocates to the nucleus where it binds with high affinity to SBEs in the promoters of IL-10-inducible genes. One of these genes, *SOCS-3*, is a member of a newly identified family of JAK/STAT inhibitory genes. The ability of IL-10 to rapidly induce expression of *SOCS-3* may explain, at least in part, how IL-10 inhibits induction of many genes in monocytes.

two IL-10R1 polypeptide chains and two IL-10R2 chains. Binding of homodimeric IL-10 to the extracellular domains of two adjoining IL-10R1 molecules activates phosphorylation of the receptor-associated *Janus* tyrosine kinases, JAK1 and Tyk2.[29,30] These kinases then phosphorylate specific tyrosine residues (Y446 and Y496) on the intracellular domain of the IL-10R1 chain.[30,41,43] Once phosphorylated, these tyrosine residues (and their flanking peptide sequences) serve as temporary docking sites for the latent transcription factor, STAT3. STAT3 binds to these sites via its SH2 domain, and is, in turn, tyrosine-phosphorylated by the receptor-associated JAKs. It then homodimerizes and translocates to the nucleus where it binds with high affinity to SBEs in the promoters of various IL-10-responsive genes. One of these genes, *SOCS-3*, is a member of a newly identified family of genes that are now known to inhibit JAK/STAT-dependent signaling. Moreover, the ability of IL-10 to induce *de novo* synthesis of SOCS-3 in monocytes correlates with its ability to inhibit expression of many genes in these cells, including endotoxin-inducible cytokines such as TNF-α and IL-1. Thus, the ability of IL-10 to inhibit gene expression in monocytes is associated with its ability to rapidly induce synthesis of SOCS-3.

The ability of IL-10 to inhibit LPS-inducible gene expression in monoctyes is transcriptionally mediated, and can be blocked by the protein synthesis inhibitor, cycloheximide.[15,53,54] These findings indicate that the inhibitory effects of IL-10 are mediated via the induction of an intermediate gene, possibly *SOCS-3*. Definitive evidence that SOCS-3 can directly inhibit LPS-inducible gene expression has not yet been provided. If forced expression of *SOCS-3* in a macrophage cell line is found to inhibit LPS-induced cytokine production, it will be interesting to determine exactly how SOCS-3 inhibits the LPS-induced signaling pathway.

Based on the results of preclinical studies in various animal models, clinical trials of recombinant human IL-10 (rhIL-10) have begun to be carried out to explore the possible utility of IL-10 as a treatment for inflammatory diseases such as rheumatoid arthritis and Crohn's disease.[80] In addition, analysis of proinflammatory cytokine production by monocytes from individuals treated with rhIL-10 as part of a baseline safety and pharmacokinetic study in normal volunteers confirmed that IL-10 can inhibit activation of monocytes *in vivo*.[81,82] Inhibition of proinflammatory cytokine production by IL-10 requires the continued presence of IL-10. However, like many other cytokines, IL-10 has a relatively short half-life *in vivo*.[82] This problem might be overcome by physically modifying the protein to prolong its serum half-life. It might also be possible to deliver a continued supply of IL-10 through the use of gene therapy approaches. Preliminary studies in animal models support this approach. It is likely, therefore, that rhIL-10 will eventually join other recombinant cytokines such as IFN-β (which is already approved for clinical use in the treatment of multiple sclerosis) as a treatment for certain chronic inflammatory diseases. Consequently, interest in this cytokine is likely to continue for years to come.

ACKNOWLEDGMENTS

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