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Signal Transducer and Activator of Transcription 3 Is the Dominant Mediator of the Anti-Inflammatory Effects of IL-10 in Human Macrophages

Lynn Williams,* Laura Bradley, Laura Bradley,* Alexandra Smith,† and Brian Foxwell‡‡

The signaling mechanism by which the anti-inflammatory cytokine IL-10 mediates suppression of proinflammatory cytokine synthesis remains largely unknown. Macrophage-specific STAT3-null mice have demonstrated that STAT3 plays a critical role in the suppression of LPS-induced TNF-α release, although the mechanism by which STAT3 mediates this inhibition is still not clear. Using an adenoviral system, we have expressed a dominant negative (DN) STAT3 in human macrophages to broaden the investigation to determine the role of STAT3 in IL-10-mediated anti-inflammatory signaling and gene expression. Overexpression of STAT3 DN completely inhibited IL-10-induced suppressor of cytokine signaling 3, tissue inhibitor of MMP-1, TNF receptor expression, and the recently identified IL-10-inducible genes, T cell protein tyrosine phosphatase and signaling lymphocyte activation molecule. STAT3 DN also blocked IL-10-mediated inhibition of MHC class II and COX2 expression. In agreement with the studies in STAT3-null mice, overexpression of the STAT3 DN completely reversed the ability of IL-10 to inhibit LPS-mediated TNF-α and IL-6 production. However, real-time PCR analysis showed that STAT3 DN expression did not affect immediate suppression of TNF-α mRNA, but did reverse the suppression observed at later time points, suggesting a biphasic regulation of TNF-α mRNA levels by IL-10. In conclusion, although STAT3 does appear to be the dominant mediator of the majority of IL-10 functions, there are elements of its anti-inflammatory activity that are STAT3 independent. *The Journal of Immunology, 2004, 172: 567–576.

Interleukin-10 is a pleiotropic cytokine that has an important role in regulating the immune response (1). The cytokine potently inhibits macrophage activation, inhibiting the expression of inflammatory mediators such as cyclooxygenase 2 (COX2), proinflammatory cytokines (e.g., TNF-α and IL-6), and both CC and CXC chemokines, thus limiting the course of an inflammatory response by curtailing the activation and recruitment of a wide range of hemopoietic cells. IL-10 augments this activity by enhancing the release of soluble TNF receptors and IL-1R antagonist (2). 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 (3). Another key feature of IL-10 immunosuppressive capabilities is its effectiveness in disabling Ag presentation/T cell activation by inhibiting the expression of MHC class II, B7-1 and B7-2 on macrophages (4–6).

The potency of the anti-inflammatory effects of IL-10 has been demonstrated in animal models of inflammation such as sepsis (7), collagen-induced arthritis (8), insulitis (9), and in some models of EAE (10, 11). In a clinical setting, encouraging data have emerged from phase II trials of systemic administration of IL-10 in the treatment of psoriatic skin lesions (12), although similar data from Crohn’s disease and rheumatoid arthritis produced only a mild amelioration of disease activity (13, 14).

The intracellular mechanism by which IL-10 mediates its anti-inflammatory and other effects remains largely unknown. This subject, however, is of more than academic interest given the potential of IL-10 as a therapeutic agent. IL-10 mediates its diverse activities via a high affinity cell surface receptor (15–17) composed of two chains, IL-10R1 and CRF2–4/IL-10R2 (18, 19); both chains are members of the class IIFN receptor subgroup of cytokine receptors. IL-10 activates the Janus kinases, Jak-1 and Tyk-2 (20), and, as a consequence, the activation of STAT transcription factors, in particular STAT3, but the activation of STAT1 and STAT5 has also been reported (21–24). IL-10 also activates the phosphoinositol 3-kinase pathway (25, 26). Attempts to ascertain how IL-10 suppresses cytokine expression have been both controversial and contradictory; a variety of transcriptionally, post-transcriptionally, and translationally mediated mechanisms have been described (27–32). It is also unclear whether the effects of IL-10 on cytokine expression are direct or require de novo gene expression (33, 34), or whether IL-10 simply antagonizes the LPS-induced stability of mRNA as in the case of the chemokine KC (35).

Studies focused on linking specific signaling events with anti-inflammatory processes are also very limited, mainly using cells from knockout mice. In murine macrophages deficient in STAT3 expression, IL-10 was unable to suppress TNF-α and IL-6 production (36, 37). However, in studies using a truncated form of STAT3 (C-terminal deletion after Lys685), a dominant negative (DN) did not support a role for STAT3 in IL-10 suppression of cytokine production (38). No other studies have been performed investigating the mechanisms of additional anti-inflammatory effects of IL-10.
Overall, therefore, there is still much to learn about the mechanisms of IL-10’s anti-inflammatory effects, and previous studies have often produced contradictory data. In an attempt to begin to address these questions we have investigated the role of STAT3 in IL-10 suppression of macrophage function. This study has been performed in primary human cells, because we consider that this is more relevant to human physiology. In addition, the study has not been confined to solely investigating the suppression of cytokine production, but has also examined multiple aspects of IL-10 anti-inflammatory activity. The data show that whereas STAT3 is a common route for many of IL-10’s anti-inflammatory effects, there is an exception in certain aspects of the mechanisms involved in blocking TNF-α production. These studies therefore highlight the complexity of IL-10’s anti-inflammatory activity.

**Materials and Methods**

**Cells**

Single-donor plateletpheresis residues were purchased from the North London Blood Transfusion Service (Tooting, U.K.). Mononuclear cells were isolated by Ficoll-Hyphaque centrifugation (specific density, 1.077 g/ml) proceeding T cell/monocyte separation in a Beckman JE6 elutriator (Fullerton, CA). T cell purity was assessed by flow cytometry using directly conjugated anti-CD3 (BD Biosciences, Oxford, U.K.), and monocyte purity, assessed using anti-CD45 and anti-CD14 Abs (Leucogate; BD Biosciences, Oxford, U.K.), and monocytes were rejected if the endotoxin concentration exceeded 0.1 U/ml. Macrophages were derived from elutriated monocytes by culturing the cells with M-CSF at 100 ng/ml (Wyeth, Boston, MA) in 10% heat-inactivated FCS RPMI 1640 for 3 days (39).

**Reagents**

IL-10 was a gift from Schering-Plough (Kennilworth, NJ), and *Salmonella typhimurium* LPS was purchased from Sigma-Aldrich (Dorset, U.K.). Anti-IL-10 and anti-IL-1Rα were purchased from R&D Systems (Oxon, U.K.), isotype control was provided by D. Mason (University of Oxford, Oxford, U.K.), anti-HLA DR-PE and Ig-conjugated PE were purchased from BD Biosciences. TNF-α and IL-6 ELISA reagents were purchased from BD Biosciences, the TIMP-1 ELISA kit was purchased from Amersham Pharmacia Biotech (Little Chalfont, U.K.), and soluble TNF-R reagents were purchased from BioSource International (Nivelles, Belgium).

**Adenoviral (Ad) vectors and viral infections**

A recombinant, replication-deficient, Ad vector encoding the human STAT3 Tyr705→Phe (AdSTAT3 DN) (40) was provided by Y. Fasjio (University of Osaka, Osaka, Japan). An identical construct lacking the insert (Ad0) was provided by A. Byrnes and M. Wood (University of Oxford, Oxford, U.K.). The recombinant viruses were purified and concentrated as described previously (39). Macrophages were routinely infected with virus at the stated multiplicity of infection (m.o.i.) for 1 h in serum-free medium. Cells were then washed and recultured in growth medium with 5% (v/v) FCS for 24 h.

**Western blot analysis**

The following Abs were used: anti-Tyr705 phospho-STAT3 and anti-STAT3 (NEB, Hitchin, U.K.), anti-STAT1 (BD Biosciences), anti-β-actin (Sigma-Aldrich), anti-COX 2 (Alexis, Oxford, U.K.), and anti-suppressor of cytokine signaling 3 (SOCS3) (Santa Cruz Biotechnology, Santa Cruz, CA). Cell extracts were resolved by SDS-PAGE and transferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA), which were blocked for 1 h with blocking buffer (5% (w/v) fat-free milk and 0.1% (v/v) Tween 20 in PBS), followed by 1-h incubation with the Abs, diluted 1/1000 in blocking buffer. HRP-conjugated anti-mouse IgG or anti-rabbit IgG (Amersham Pharmacia Biotech) were used as secondary Abs at a dilution of 1/2000. Bound Ab was detected using the ECL kit (Amersham Pharmacia Biotech) and was visualized using Hyperfilm MP (Amersham Pharmacia Biotech).

**STAT3 EMSAs**

After stimulation, cells were scraped into ice-cold PBS, then lysed in hypotonic lysis buffer (0.0125% Nonidet P-40, 5 mM HEPES (pH 7.9), 10 mM KCl, and 1.5 mM MgCl₂) and nuclei were harvested by centrifugation.
It has previously been shown that SOCS3 mRNA is up-regulated by IL-10 in human macrophages (42) and in studies of murine macrophage cell lines, SOCS3 has been suggested to play a role in the anti-inflammatory effects of this cytokine (43). We therefore investigated whether there was a corresponding regulation of SOCS3 protein by IL-10 and whether this required STAT3. Fig. 2a presents an IL-10 time course of SOCS3 expression in human macrophages, showing that it is induced within 20 min. Maximal expression occurs between 1–4 h; however, SOCS3 protein expression is still detected 24 h after IL-10 stimulation (Fig. 2a). IL-10 induces a sustained level of phosphorylation of STAT3 as well as SOCS3 expression, in contrast to IL-6-induced STAT3 and SOCS3 expression, which was more transient in nature. Infection of cells with Ad-STAT3-DN completely abrogated IL-10-induced SOCS3 expression, whereas there was no effect of the Ad0 vector control (Fig. 2b). Ad STAT3 DN also inhibited IL-6-induced SOCS3 expression. These data indicate that even though SOCS3 is very rapidly induced by IL-10 and IL-6, there is still a requirement for STAT3 activity for its expression.

Overexpression of STAT3 DN inhibits IL-10-induced TIMP-1 and p75 TNF-R release and IL-10-induced SLAM and TCPTP mRNA accumulation

IL-10 has a potent and unique effect on macrophages by enhancing TIMP-1 secretion while decreasing metalloproteinase biosynthesis (3). Similarly, while down-regulating TNF-α production, IL-10 increases the production of a natural inhibitor of TNF-α, soluble p75 (sp75) TNF-R (2). Using the Ad STAT3 DN, we wanted to determine whether STAT3 plays a role in the production of these soluble anti-inflammatory mediators. As shown in Fig. 3, macrophages spontaneously produce both sp75 TNF-R (Fig. 3a) and TIMP-1 (Fig. 3b); however, stimulation with IL-10 increases production up to 3-fold higher than background levels (this varies from donor to donor). Unlike infection with Ad0, the expression of Ad STAT3 DN inhibited IL-10 and IL-6-induced TIMP-1 and p75 TNF-R release. Additionally, the expression of Ad STAT3 DN inhibited IL-10-induced SOCS3 expression, whereas there was no effect of the Ad0 vector control (Fig. 2b). Ad STAT3 DN also inhibited IL-6-induced SOCS3 expression. These data indicate that even though SOCS3 is very rapidly induced by IL-10 and IL-6, there is still a requirement for STAT3 activity for its expression.
Ad STAT3 DN effectively inhibited IL-10-induced TIMP-1 and sp75 TNF-R production. As a result of IL-10 expression profiling in monocytes, we recently reported a number of newly identified, IL-10-inducible genes (41). Of the eight novel genes we focused upon in monocytes, only two were still regulated by IL-10 in macrophages (data not shown). As the regulation of these two genes, TCPTP and SLAM, remains unknown, we examined whether STAT3 was involved in their regulation. As shown in Fig. 3 (c and d), real-time PCR analysis of TCPTP and SLAM mRNA levels shows that their expression is absolutely dependent on STAT3 activity.

Overexpression of Ad STAT3 DN inhibits down-regulation of MHC class II and induction of CD64 expression by IL-10

One mechanism of IL-10-mediated macrophage deactivation is the down-regulation of MHC class II expression by a mechanism involving the inhibition of transport of mature, peptide-loaded MHC class II molecules to the plasma membrane (44). Blocking STAT3 function completely reversed IL-10-mediated inhibition of MHC class II expression (Fig. 4c), whereas infection with Ad0 had no effect (Fig. 4b). IL-10 also enhances the expression of CD64 (FcγR) on monocytes/macrophages (45). Up-regulation of CD64 correlates with enhanced Ab-dependent cell-mediated cytotoxicity and enhanced capacity of monocytes/macrophages to phagocytose opsonized particles and bacteria (46). Unlike MHC class II, IL-10 positively regulates CD64; however, once again, the expression of Ad STAT3 completely inhibits this aspect of IL-10 function (Fig. 4, d–f).

Overexpression of Ad STAT3 DN inhibits IL-10 suppression of LPS-induced TNF-α and IL-6 production

To date the data had indicated that STAT3 was important in aspects of IL-10 anti-inflammatory mechanisms that had not been previously investigated. The study was now extended to examine the IL-10 suppression of TNF-α production that has been investigated in the murine system with conflicting results (36–38). Infection of human macrophages with Ad STAT3 DN reversed the ability of IL-10 to suppress LPS-induced TNF-α and IL-6 production in a dose-dependent manner, whereas the control infection had no effect (Figs. 5, a and b).

A notable effect of infection with Ad STAT3 DN was the superinduction of LPS-induced TNF-α and IL-6 production after 4 h of stimulation (there was no effect of the Ad STAT3 DN virus alone; Fig. 5, a and b). This was a very reproducible finding, and was also observed when supernatants were harvested at later time points, such as 18 h after LPS stimulation (data not shown). The enhancing effect was not uniformly observed in all cytokines assayed, as LPS-induced VEGF production was unperturbed in response to Ad STAT3 DN expression (Fig. 5c). Similarly, LPS-induced IL-10 expression, which had previously suggested to be...
regulated by STAT3 (47), was unaffected by the expression of Ad
STAT3 DN (Fig. 5d). The ability of STAT3 DN to reverse the
inhibitory effects IL-10 was not limited to cytokine synthesis, as
IL-10-mediated suppression of LPS-induced (COX-2) expression
was also sensitive to the inhibition of STAT3 function (Fig. 5e)

**STAT3 DN-mediated elevation of LPS-induced TNFα occurs in
the absence of IL-10**

A consequence of LPS stimulation of macrophages is the produc-
tion of IL-10, which then feeds-back onto the cell to suppress
cytokine synthesis; this can be demonstrated by the addition of
anti-IL-10/IL-10R Abs to cultures (see Fig. 6). Therefore, it might
be expected that if this endogenous pathway were deactivated by
the expression of STAT3 DN, there would be enhanced levels of
TNF-α/IL-6 produced in response to LPS. To investigate this fur-
ther the experiments were performed in the presence of neutraliz-
ing anti-IL-10 and anti-IL-10 R1 Abs. This combination of Abs
totally ablated the effect of exogenously added IL-10 to block
TNF-α production (Fig. 6). The presence of the Ab mixture results
in an increase in LPS-induced TNF-α production from 0.6 ± 0.15
to 1.8 ± 0.04 ng/ml. However, even in the presence of the anti-
IL-10 Ab cocktail, the elevation of TNF-α production observed
with the overexpression of STAT3 DN was still evident. This
would suggest that either STAT3 has a direct suppressive effect on
LPS-induced TNF-α production or STAT3 DN was inhibiting an-
other autocrine anti-inflammatory mediator.

**Ad STAT3 DN only reverses IL-10-mediated TNF mRNA
suppression 2 h after LPS stimulation**

Our data on the role of STAT3 in IL-10 suppression of TNF-α
protein expression concurred with the previous studies by Riley et
al. (36, 37). We therefore decided to extend our study further by
investigating STAT3 at the level of suppression of TNF-α mRNA
by IL-10. Fig. 7a shows a short time course of LPS-induced
TNF-α mRNA accumulation (as analyzed by TaqMan real-time
PCR) in the presence or the absence of IL-10 added simulta-
nenously with the LPS. TNF-α mRNA was first detected at 20
min after LPS stimulation; surprisingly, even at this early time point,
IL-10 significantly \( p = 0.016 \) inhibited LPS-induced TNF-α
mRNA accumulation, suggesting that this effect is direct, as few
proteins are synthesized so rapidly. At later time points (2 h), the
level of inhibition of TNF-α mRNA by IL-10, became even more
significant \( p = 0.00087 \). Supernatants were harvested from these
same cultures, and TNF-α protein levels were assessed by ELISA
(Fig. 7b). TNF-α protein was detected within 1 h of stimulation,
and again even at this early stage IL-10 strongly suppressed
TNF-α production. The effect of expression of Ad STAT3 DN on
IL-10 suppression of TNF-α mRNA at these early time points was
then assessed. Time points of 1 and 2 h were chosen because these allowed us to concurrently monitor the effects on protein production as well as mRNA accumulation. At 1 h, IL-10 inhibited LPS-induced TNF-α mRNA accumulation by >50%; however, overexpression of STAT3 DN (ST3) had no effect on this inhibition (Fig. 7c). After 2 h, the level of suppression by IL-10 of TNF-α mRNA was >90%, but STAT3 DN did reverse the IL-10 suppression of LPS-induced TNF-α mRNA. The effects of STAT3 DN on TNF-α protein production mirrored the effects on TNF-α mRNA (Fig. 7d). These data suggest that in the early stages, IL-10 directly affects TNF-α mRNA accumulation/stability; however, a second STAT3-dependent mechanism develops as time progresses. It was also noted that the enhancing effect of STAT 3 DN on LPS-induced TNF-α protein expression was seen at the mRNA level only at the later time point. No enhancing effect of Ad STAT3 DN on TNF-α mRNA was observed in the absence of LPS.

Time-dependent requirement for protein synthesis in IL-10-mediated suppression of TNF-α

The data presented above lead to the hypothesis that STAT3-independent inhibition occurs in the absence of de novo protein synthesis, whereas the later mechanism requires STAT3 as a transcription factor to drive transcription of an effector protein. Using cycloheximide, the requirement for protein synthesis in the STAT3-independent and -dependent mechanisms was investigated. TNF-α mRNA accumulation was inhibited by IL-10 at both 1 h (Fig. 8a) and 2 h (Fig. 8b) after LPS stimulation. Cycloheximide is known to induce the stabilization of mRNAs, so caution should always be taken when analyzing such data. A small induction of TNF-α mRNA by the drug alone was expected, but this was dwarfed by the LPS response. At 1 h (Fig. 8a), the presence of cycloheximide slightly increased the level of IL-10 suppression of TNF-α mRNA from 42 to 49%, but this was not significant. In contrast, at 2 h (Fig. 8b) IL-10 suppressed TNF-α mRNA by 60 ± 0.57%, whereas in the presence of cycloheximide, inhibition was no longer observed. Fig. 8c shows the mean percent inhibition of LPS-induced TNF-α mRNA by IL-10 in the presence or the absence of cycloheximide in five different donors. Clearly, at 1 h after LPS stimulation, the level of inhibition between the cycloheximide-treated groups is not different (p = 0.061), whereas at 2 h poststimulation there is a statistically significant difference (p = 0.00008).

Discussion

The activation of STAT3 is one of the most clearly defined events of IL-10 signaling. However, attempts to determine its role in the anti-inflammatory activity have not produced a consensus. Also the studies have, on the whole, been confined to investigating the IL-10 suppression of TNF-α expression, but not other anti-inflammatory mechanisms of the cytokine. Furthermore, all these studies have been performed in murine cells. Our study has addressed these outstanding issues by studying human macrophages and investigating multiple aspects of IL-10 anti-inflammatory activity. Although the data showed that STAT3 is required for the majority of IL-10 functions, the study also revealed that there was a potential STAT3-independent mechanism in the earliest stages of suppression of TNF-α production that correlated with the absence of any requirement for IL-10-directed de novo protein synthesis.
Where STAT3 was required, this correlated with a requirement for protein synthesis, suggesting that STAT3 does act as a transcription factor.

Several lines of evidence suggest that the ability of IL-10 to suppress cytokine synthesis requires de novo protein synthesis (33, 34). Equally, other publications suggest alternative mechanisms that do not require intermediate gene synthesis. These mechanisms involve IL-10-mediated inhibition of transcription factor NF-κB via suppression of IκB activity (32, 48) or by DNA binding (28). Alternatively, Kontoyiannis et al. (31) demonstrated an immediate effect of IL-10 on p38 mitogen-activated protein kinase activation. Our data would potentially agree with aspects of both mechanisms, which show both a direct and an indirect effect. However, our previous studies (29) have shown no effect on NF-κB or p38 mitogen-activated protein kinase, agreeing with other reports (49). Clearly, the field requires further clarification.

Given that this study shows that STAT3 is required for virtually all the anti-inflammatory effects of IL-10 in human macrophages, the most obvious mechanism would be for it to act as a transcription factor. Studies in murine macrophage cell lines produced contradictory data regarding the role of STAT3 in IL-10 signaling (36, 38). Both agree that removal of the STAT3 docking sites from IL-10R1 renders the receptor incapable of transducing the signal required to suppress cytokine synthesis. However, our data disagree with the findings of O’Farrel et al. (38). This may be a reflection of the inability of their DN STAT3 to completely inhibit all STAT3 activity or may be due to the different model systems used. Based on the data presented in this study, we show that STAT3 is required for all the anti-inflammatory actions of IL-10 except the immediate inhibition of TNF-α mRNA and protein production. The time-sensitive dependence of STAT3 in the inhibition of TNF-α mRNA by IL-10 is further supported by the cycloheximide studies. One would speculate that the initial rapid inhibition of TNF-α is a direct mechanism that does not require STAT3, whereas the sustained and more profound level of inhibition is mediated by a STAT3-dependent gene, the identity of which remains unknown. Given the limited knowledge of IL-10 signaling at this time, we are unable to even speculate what may be mediating the immediate STAT3-independent response, and we are currently trying to identify novel IL-10R-associating proteins.
is inducing another anti-inflammatory cytokine that also signals through STAT3. One possibility is that LPS directly inhibits the enhancing effect of the DN STAT3. We initially thought that this was simply because we were inhibiting the auto-crime negative feedback loop of the LPS-induced IL-10. However, when these same experiments were performed at early time points where no detectable levels of IL-10 were produced or in the presence of neutralizing anti-IL-10/anti-IL-10R Abs, we still observed the enhancing effect of the DN STAT3. This was unexpected, but it does suggest that IL-10 does indeed induce an anti-inflammatory cytokine that also signals through STAT3. Alternatively, STAT3 binding sites exist within the TNF promoter, and STAT3 may play a suppressive role that is alleviated by expression of the DN STAT3 (50).

As shown in this study, one of the genes induced by IL-10 that requires STAT3 is SOCS3. Previous studies in murine macrophages have claimed that SOCS3 is required for the anti-inflammatory activity of IL-10 (43, 51). However, the recent generation of macrophage-specific SOCS3-null mice has suggested that SOCS3 is not required for the anti-inflammatory activity of IL-10 (52, 53). Indeed, SOCS3 appears to prevent IL-6 acting as an anti-inflammatory cytokine by blocking its prolonged signaling from the IL-6R. The fact that although both IL-10 and IL-6 induced STAT3 phosphorylation and SOCS3 expression, they have distinct effects on macrophages has always been a puzzle. However, these new observations would suggest that it may be a quantitative, rather than qualitative, difference in signaling that defines the anti-inflammatory response. Our own studies would support this, as we have observed in human macrophages that whereas IL-10 induced a prolonged activation of STAT3 and SOCS3 expression, the equivalent IL-6 responses are much more transient. This is consistent with the SOCS3-null macrophage data, which demonstrated that SOCS3 is able to specifically inhibit IL-6, but not IL-10, STAT3 activation (52). We have shown that IL-10 rapidly induces SOCS3 protein expression in human macrophages and that this expression absolutely requires STAT3. The appearance of detectable levels of SOCS3 correlates with the ability of IL-10 to inhibit TNF-α mRNA accumulation. This may merely be coincidental, but it does confirm that SOCS3 is present at the relevant time to mediate IL-10’s effects on TNF-α mRNA. However, 1 h poststimulation with LPS, IL-10 can still inhibit TNF-α mRNA/TNF-α protein expression in the presence of the STAT3 DN. As we have shown that SOCS3 expression will be induced in such circumstances, this means that this initial phase of inhibition is also independent of SOCS3.

We were surprised that inhibition of STAT3 had such a profound effect on such a large number of quite distinct effector functions. Recently published data from Lang et al. (54) using STAT3-null macrophages also reported that STAT3 was essential for most, if not all, IL-10-induced genes. The question that still needs to be addressed is whether STAT3 is acting solely as a transcription factor inducing the expression of an intermediate gene that is regulating these effects or whether STAT3 is acting as a signaling intermediate and no gene transcription is actually required, as our data suggested that multiple mechanisms are involved. The DN STAT3 we have used (Tyr705→Phe) is no longer phosphorylated in response to IL-10; therefore, the effects we observed may due to the inability of STAT3 to bind/activate further signaling intermediates. In the case of SOCS3 (55), p75 TNF-R (56), TIMP-1 (57), and FcR1 (23) up-regulation, it is most likely the STAT3 is acting as a transcription factor, as STAT3 consensus sites are found in the 5′ promoter regions of these genes. Similarly, there may be STAT3 consensus sites in the SLAM and TCPTP promoters. However, in the case of MHC class II down-regulation, IL-10 does not effect MHC class II gene transcription or translation, but, rather, it inhibits surface expression of MHC class II by post-translational processes involved in exocytosis as well as the recycling of internalized MHC class II (44). How STAT3 participates in these effects requires further clarification. New STAT3 mutations currently under construction within our laboratory will hopefully address the requirement for STAT3 to function within the nucleus vs the cytoplasm.

The previous studies defining a role for STAT3 in IL-10 anti-inflammatory effects have been contradictory. In this study we show that in all but one case STAT3 is pivotal to IL-10-mediated effector functions. As IL-10 is not alone in its ability to induce STAT3 or, indeed, SOCS3, the focus of our research must be to...
define what other signaling pathways interact with STAT3 to elicit IL-10’s unique anti-inflammatory properties.

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