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General Nature of the STAT3-Activated Anti-Inflammatory Response

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Although many cytokine receptors generate their signals via the STAT3 pathway, the IL-10R appears unique in promoting a potent anti-inflammatory response (AIR) via STAT3 to antagonize proinflammatory signals that activate the innate immune response. We found that heterologous cytokine receptor systems that activate STAT3 but are naturally refractory (the IL-22R), or engineered to be refractory (the IL-6, leptin, and erythropoietin receptors), to suppressor of cytokine signaling-3-mediated inhibition activate an AIR indistinguishable from IL-10. We conclude that the AIR is a generic cytokine signaling pathway dependent on STAT3 but not unique to the IL-10R. The Journal of Immunology, 2006, 177: 7880–7888.

Interleukin-10 plays an essential role in regulating both acute and chronic inflammation. In vitro and in vivo studies have established that the primary role of IL-10 is to inhibit the powerful stimulatory effects of TLR agonists such as LPS that act on macrophages and dendritic cells (1). The central molecular effect of IL-10 is to block the production of multiple inflammatory mediators, including cytokines, chemokines, and cell surface molecules crucial for the promotion of inflammation (2). The role of IL-10 in blocking inflammation through the selective reduction of expression of a wide range of mediators can be defined as an endogenous anti-inflammatory response (AIR) necessary to protect the body from excessive inflammation. The anti-inflammatory effects of IL-10 are essential: while conventionally housed mice lacking IL-10 have a chronic inflammatory disease of the intestines, they also exhibit profound, and often lethal, proinflammatory phenotypes when infected with pathogens that generate acute inflammatory responses (1, 3). The absence of IL-10 leads to runaway or chronic inflammation depending on the disease context, and the excessive inflammation correlates with greatly increased levels of cytokines and chemokines. Accordingly, the IL-10 signaling pathway has attracted attention for the potential to regulate chronic (e.g., arthritis, Crohn’s disease) or acute (e.g., sepsis) inflammation (4).

The molecular mechanisms involved in IL-10 signaling have escaped definition for over a decade. Four significant insights have, however, recently opened research into this apparently complex signaling pathway. First, STAT3 appears essential for all known aspects of the IL-10-regulated anti-inflammatory effect both in vivo and in vitro (5–9). Second, the effects of IL-10 are primarily mediated at the level of transcriptional inhibition (10). Third, STAT3 does not function directly but rather induces the expression of new genes that are responsible for executing the AIR: this response is relatively rapid consistent with STAT3 inducing expression of new genes that subsequently exert their effects on inflammatory genes (10). The identity of the STAT3-induced genes that mediate the AIR is an active area of investigation. Fourth, IL-10 is not a general inhibitor of transcription but rather functions to selectively block expression of genes involved directly or indirectly in inflammatory pathways (6, 10).

At the level of receptor activation, additional questions concerning IL-10 signaling have been refractory to explanation. Chief among these uncertainties is the use of STAT3 by multiple receptors, including receptors that are expressed concurrently with the IL-10R, but are unable to generate a strong AIR like IL-10. The IL-6R is one example of this type of receptor, and IL-6 is often regarded as a pleiotropic or proinflammatory cytokine (11). In keeping with a potential proinflammatory role of IL-6, a humanized anti-IL-6R mAb has shown considerable efficacy in treating Crohn’s disease, a condition of excessive gut inflammation (12). Like the IL-10R, the IL-6R uses Jak1 and primarily STAT3 to generate its signal transduction cascade. Both receptors are heterodimeric with one signal transduction chain that binds STAT3 and provides a platform for STAT3 activation. In the case of the IL-10R, the IL-10R α-chain has two defined docking sites for STAT3 (3), whereas gp130 provides five docking sites for STAT3 activation from the IL-6R (11). Like IL-10, STAT3 appears to be

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3 Abbreviations used in this paper: AIR, anti-inflammatory response; BMDM, bone marrow-derived macrophage; SOCS, suppressor of cytokine signaling; EPO, erythropoietin; EpoR, erythropoietin receptor; ELR, EpoR-leptin R hybrid.

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the main STAT used by IL-6 for signaling new gene expression (11). Therefore, a key question is why is the IL-6R not anti-inflammatory? A logical explanation for this discrepancy is that the IL-10R activates a unique pathway that is STAT3-dependent but has additional functions not found in other receptors that activate STAT3 (13, 14). An alternate explanation is that inhibitory pathways block the ability of the IL-6R to activate the AIR. We wondered whether the IL-10R was not unique in generating the AIR but rather that any cytokine could do so, provided its receptor activated STAT3 and was relieved from the inhibitory signals that block the AIR signal. To test this question, we used different heterologous receptor systems to generate signaling analogous to the IL-10R (see Fig. 1a). Our results suggest the STAT3-mediated AIR can be established by any STAT3-activating cytokine receptor. The results also provide an explanation for diverse reports in which unexplained anti-inflammatory effects have been observed and linked with STAT3 activity. Finally, we propose that the STAT3-mediated anti-inflammatory signaling pathway can operate in multiple cells and tissues.

Materials and Methods

Mice and macrophage isolation

Mice used in this study were as follows: IL-10−/−, IL-6−/−, and control mice (all on a 129/BL/6 background) were from The Jackson Laboratory. Stat3−/−, lysMcre mice (6) were originally provided by Dr. I. Förster (Technisches Universität München, Munich, Germany) and bred at St. Jude Children’s Research Hospital (Memphis, TN). Mice with point mutations in S727 of STAT3 and Y757 of gp130 have been described in detail (15, 16). The suppressor of cytokine signaling (SOCS) gene in Socs3−/−; lysMcre or Socs5−/−; lysMcre mice have been described previously (17). Socs3−/−, lysMcre mice were bred at St. Jude’s Children’s Research Hospital and used as a source of macrophages for the remaining experiments. Studies using Socs3−/− mice (18) were purchased from Taconic Farms. Bone marrow-derived macrophages (BMDMs) and peritoneal macrophages were isolated and cultured as described in detail. Socs1−/− mice were a gift from E. Parganas and J. Ihle (St. Jude’s Children’s Research Hospital, Memphis, TN) and interbred to yield Socs1−/− mice and controls as described previously (19). Institutional animal care and oversight committees approved all animal studies.

Stimulation conditions

BMDMs and peritoneal macrophages were generated and cultured as described previously (6). For the generation of supernatant for the detection of cytokines by ELISA, cells were plated at 0.5 × 10⁶ cells per well in triplicate in 1 ml of complete DMEM (Invitrogen Life Technologies) containing 10% FBS and stimulated with the following cytokines unless otherwise stated: LPS at 100 ng/ml from E. coli 0111:B4; Sigma-Aldrich), IL-10 at 10 ng/ml, IL-6 at 10 ng/ml, and IFN-γ at 2 ng/ml. All cytokines were from BD Pharmingen and diluted in DMEM at 1 μg/ml. After different time periods of stimulation (detailed in each experiment), supernatant was recovered (100 μl/well) and stored at −80°C until assayed. For immunoblotting, cells were grown at 0.75 × 10⁶ cells/well in complete DMEM and lysates were obtained after the respective incubation times with cytokines (IL-10 at 10 ng/ml, IL-6 at 10 ng/ml, and IL-22 at 10 ng/ml) and LPS. Cells were washed twice in ice-cold PBS and lysed in 150 μl of lysis buffer (50 mm HEPES, pH 7.5, 1 mm PMSF, 1% Triton X-100). Lysates were centrifuged at 4°C for 10 min to remove debris and DNA and supernatants were stored at −80°C.

Immunoblotting

Immunoblotting for phosphorylated forms of STAT1 and STAT3 was performed on radioimmunoprecipitation assay lysates as described previously (20). Polyclonal Abs for phosphorylated forms of the STATs were from Cell Signaling Technology. Polyclonal Abs to STAT3 were a gift from J. Ihle (St. Jude Children’s Research Hospital). Polyclonal Abs to STAT1 were from Santa Cruz Biotechnology.

Microarray analysis

Affymetrix GeneChip analysis was performed at Hartwell Center for Bioinformatics and Biotechnology, St. Jude Children’s Research Hospital using murine GeneChip U74A version 2. Raw Affymetrix metric text data files (.met) were loaded into Spotfire Decision Site Functional Genomics module version 7.2. Data were filtered based on frequency modulation of detection p-value quality indices (to eliminate genes that could not be detected in any of the samples analyzed). Data were then log transformed and normalized to remove any intensity-dependent bias, and fold change was calculated. Statistical significance was calculated using the t test module of Spotfire through pairwise comparison of triplicate untreated (LPS only) vs treated (LPS and IL-10) experiments. Genes with significantly differentiating gene expression were identified based on fold change as well as p value, binned into different categories using the bin function in Spotfire, and differentially color-coded for identification. All microarray data has been deposited in the GEO database (accession no. GSE5589).

Real-time RT-PCR

Total RNA isolated from macrophages was reverse transcribed as described previously (6). Target gene expression was analyzed using SYBR Green or dye-labeled primer real-time RT-PCR as described previously (10). Primers for each target are available upon request from P. J. Murray (St. Jude Children’s Research Hospital, Memphis, TN).

ELISA

Microtiter plates (Maxisorb; Nunc) were coated with the respective anti-mouse capture Abs at 50 μl/well: IL-12 (2 μg/ml; BD Pharmingen), IL-10 (4 μg/ml; BD Pharmingen), IL-6 (2 μg/ml; BD Pharmingen), TNF-α (3 μg/ml; eBioscience) in coating buffer (0.1 M Na2CO3/H2O, 0.1 M NaHCO3) overnight at 4°C. The next day, plates were washed with ELISA washing buffer (1% Tween 20, 1 mM Tris base, and 154 mM NaCl) and blocked with 300 μl/well of ELISA buffer (10% FCS in PBS) and incubated for 2 h at room temperature. After washing, 50 μl/well of triplicates from cell culture supernatants and duplicate serial 2-fold dilutions of the respective standards (50 ng/ml starting concentration) were added and incubated overnight at 4°C. Plates were then washed three times, and biotin-conjugated detection Abs for IL-12 (1 μg/ml; BD Pharmingen), IL-10 (1 μg/ml; BD Pharmingen), IL-6 (1 μg/ml; BD Pharmingen), and TNF-α (1.6 μg/ml; BD Pharmingen) diluted in ELISA buffer were added at 50 μl/well and incubated for 45 min at room temperature. After washing the plates three times, streptavidin peroxidase (Sigma-Aldrich) diluted 1:1000 in ELISA buffer was incubated for 30 min at room temperature. Plates were soaked in washing buffer for 1 min and washed five times. Tetramethylbenzidine peroxidase substrate system (Kirkegaard & Perry Laboratories) was applied at 50 μl/well and reactions were stopped with 10% phosphoric acid (50 μl/well). Absorbance was measured at 405 nm in an ELISA reader (Molecular Dynamics). Cytokine concentrations were calculated according to the respective standard curves.

Transfection and selection of RAW cells

For transfection, the plasmids pEF-BOS-mIL-22R (21), pEF-BOS, and pCDNA3.1 were linearized with HindIII. Plasmids containing the Epo R-leptin R hybrid (ELR) or ELR mutant constructs were linearized with BgIII. RAW cells were transfected by electroporation as described previously (22). Cells were plated into 10-cm dishes with 10 ml of complete RPMI 1640 containing 10% FBS (HyClone) and 1% penicillin-streptomycin (Invitrogen Life Technologies) and incubated at 37°C overnight. Transfected cells were selected with G418 (200 μg/ml).

Generation of retroviral producer cells

Retroviral producer cell lines were generated as previously described (23–25), with some modifications (26). 293T cells were transiently transfected with 4 μg of pMiy vector with or without the IL-22R, ELR cDNAs (27), erythropoietin receptor (EpoR), or EpoR-S3 cDNAs (28, 29), together with packaging and envelope vectors using TransIt 293 transfection reagent (Mirus). GP plus E86 cells were transduced with virus and 6 μg/ml polybrene every 12 h for 3–4 days until a viral titer greater than 10⁵/ml after 24 h was obtained.

Retroviral-mediated transduction of stem cells

Retroviral transduction of murine bone marrow cells was performed as described previously (26, 30). Briefly, bone marrow was harvested from 5- to 10-wk-old donor mice 48 h after treatment with 150 mg/kg 5-fluorouracil (American Pharmaceutical Partners). Bone marrow cells were cultured for 48 h in complete DMEM with 20% FBS, 20 ng/ml murine IL-3, 50 ng/ml human IL-6, and 50 ng/ml murine stem cell factor (BioSource International). Cells were subsequently cocultured for a further 48 h with irradiated (1200 rad) retroviral producer cell lines plus 6 μg/ml polybrene and cytokines as previously detailed. Nonadherent, transduced bone marrow
cells were collected, red cell lysed using Gey’s solution, washed, and cells resuspended in PBS/2% FBS with 20 U/ml heparin. Cells were then subjected to FACS, gating on forward and side scatter and YFP positive cells. Cells were subsequently plated out with 500 ng/ml Fungizone and 25 g/ml gentamicin in complete DMEM with 20% FBS and L cell conditioned medium as a source of M-CSF. Following differentiation, mature cells were measured by flow cytometry for YFP along with surface markers (CD11b, F4/80) for mature macrophages.

Results

Elimination of SOCS3 binding allows the IL-6R to generate the AIR

We used microarray analysis to compare the ability of IL-10 or IL-6 to block inflammatory gene expression in primary macrophages. Consistent with previous data (6), IL-10 induced a measurable AIR, inhibiting ~10% of LPS-induced mRNAs in BMDMs, whereas IL-6 had no discernible inhibitory effects on inflammatory target gene expression (data not shown) when used at a concentration that gave equivalent STAT3 tyrosine phosphorylation to IL-10 (Fig. 1b). Within the pool of LPS-induced mRNAs blocked by IL-10 but not IL-6, we found the expected target mRNAs including IL-12p40, TNF-α, IL-1α, IL-1β, IL-6, and numerous chemokines (data not shown). A selection of established IL-10 targets were used as markers of the AIR for the experiments described below.

In continuous stimulation experiments, both IL-6 and IL-10 activate STAT3 tyrosine phosphorylation equivalently over the first 30 min (Fig. 1b). Thereafter, STAT3 phosphorylation induced by IL-10 decays slowly, whereas STAT3 phosphorylation induced by IL-6 has a faster decay, an effect dependent on SOCS3-mediated inhibition of IL-6 signaling (20, 31, 32). Similar findings were obtained using BMDMs stimulated with IL-10 or IL-6 in the presence of LPS as a proinflammatory costimulus (data not shown). Collectively, these studies showed STAT3 is activated by both IL-10 and IL-6, but the AIR is activated by the IL-10R and not the IL-6R. In additional studies, we found that the ability of the IL-10R to generate its inhibitory effects on inflammatory gene expression were independent of STAT1, SOCS1, or serine phosphorylation on S727 of STAT3 (data not shown) but strictly dependent on STAT3 (our unpublished data) as anticipated by published studies (6, 8, 9).

Yoshimura and colleagues (33–36) have shown that IL-6 signaling in SOCS3-deficient macrophages allows the IL-6R to generate an AIR-like inhibitory signal following LPS stimulation. SOCS3 is an inducible E3 ligase that binds to Y757 in gp130 (Fig. 1a), the signal transduction chain of the IL-6R, and blocks STAT3 activation. We reasoned that if the absence of SOCS3 caused an IL-10-like effect upon IL-6 stimulation, then the gp130 Y757F mutation should recapitulate this finding. BMDMs were isolated from gp130Y757F/Y757F or control mice and stimulated with LPS, LPS and IL-10, or LPS and IL-6. We found that IL-6-stimulated BMDMs from gp130Y757F/Y757F mice behaved identically to IL-10-treated cells in terms of inhibiting cytokine mRNAs (Fig. 1c). Because SOCS3 cannot bind to gp130 in the mutant cells, these data support the hypothesis that SOCS3 is the primary regulator of the inability of IL-6 to stimulate the AIR, independently confirming the findings of Yoshimura and colleagues (32) who used an alternative system to test the requirement for SOCS3 function in
the context of gp130 binding. The stimulation of the AIR by IL-6 in the absence of SOCS3 suggests that SOCS3 regulates the function of negative regulatory signals that normally restrain the ability of IL-6 to promote the AIR. Therefore, the IL-6R has the potential ability to activate the AIR, suggesting that other receptors may share this property.

Ectopic expression of the IL-22R in macrophages activates an AIR indistinguishable from IL-10

We next asked whether any cytokine receptor could activate the AIR. We began by asking whether the IL-22R, a receptor that naturally activates STAT3 but in nonhemopoietic cells, could also...
activate the AIR. The IL-22R is a member of a subgroup of type II cytokine receptors that includes the IL-10R (37). The IL-22R is composed of the IL-22R α-chain and the IL-10R β-chain (Fig. 1a), which is widely expressed and used by several IL-10-like cytokines. The IL-22R α-chain is not expressed in hemopoietic cells but rather expressed in tissues such as skin and pancreas where it responds to IL-22 secreted by activated T cells (37). Because IL-22 activates STAT3 phosphorylation, we reasoned that the IL-22R could play a similar anti-inflammatory role to the IL-10R. We first selected RAW macrophage cell lines expressing the IL-22R α-chain and tested their response to IL-22. In these lines, the IL-10R β-chain is endogenously expressed and can therefore form an active IL-22R in the presence of the IL-22R α-chain. When stimulated with IL-22, the cell lines robustly activated STAT3 as expected (Fig. 2a). When stimulated with LPS in the presence of IL-10, IL-22, or IL-6, both IL-10 and IL-22 but not IL-6 were capable of suppressing cytokine gene expression to equal extents (Fig. 2, b and c). We next isolated primary macrophages transduced with retroviruses expressing the IL-22R α-chain cDNA. IL-22 induced STAT3 phosphorylation in the IL-22Rα-expressing macrophages as expected (Fig. 2d). When stimulated with LPS and IL-22, IL-22Rα-positive macrophages induced an AIR that could not be distinguished from the effects of IL-10 as measured by ELISA and quantitative RT-PCR for key IL-10-regulated inflammatory targets including KC, IL-12p40, TNF-α, and IL-6 (Fig. 2, e–h). The effects of both IL-10 and IL-22 were independent of SOCS3 because both cytokines inhibited inflammatory mediator expression equally in control or SOCS3-deficient macrophages (Fig. 2, f–h). These results support the notion that the IL-22R can activate, via STAT3, an anti-inflammatory pathway indistinguishable from IL-10 and therefore, like the IL-10R, must be free of the negative constraints that block the ability of the IL-6R to signal the AIR.

Leptin receptor signaling activates an AIR

We next used heterologous receptor systems that activate STAT3 but are more distantly related to either the IL-6- or IL-10-like cytokine receptors. As a first step, we used the leptin receptor signaling as a model system. The leptin receptor is regulated by the SOCS3, and the single binding sites for both STAT3 (Y1138) and SOCS3 (Y985) have been defined (38, 39). We expressed the leptin receptor cytoplasmic domain fused to the extracellular domain of the EpOr (ELR) (Fig. 1a) and tested the induction of the AIR response to cells stimulated with LPS, or LPS and erythropoietin (EPO). Retroviral-mediated transduction was used to introduce ELR or ELR Y985L into primary macrophages from control or from Soc3fl/fl, lysMcre mice (Fig. 3a). BMDMs expressing ELRs induced considerable STAT3 phosphorylation when stimulated with EPO (Fig. 3b). STAT3 phosphorylation was extended in the absence of SOCS3, or when ELR Y985L was expressed in wild-type cells consistent with the known properties of these receptors (Fig. 3b). When transduced wild-type BMDMs were stimulated with EPO, we found that even the wild-type ELR receptor induced the AIR for some inflammatory targets (Fig. 3). However, BMDMs expressing ELR Y985L inhibited the expression of cytokine mRNAs to a greater extent than ELR consistent with the proposed regulatory role for SOCS3 in blocking STAT3 phosphorylation from the leptin receptor cytoplasmic domain (Fig. 3c). Both ELR and ELR Y985L inhibited cytokine mRNA expression in Soc3fl/fl, lysMcre BMDMs as expected. A caveat of these experiments is that the ELR constructs are highly expressed in primary cells when driven by the retroviral long-terminal repeats. The high expression of ELR, even though regulated by SOCS3, can stimulate an AIR comparable to IL-10 for sensitive targets like IL-12p40 (Fig. 3c). IL-12p40 is an example of an inflammatory gene inhibited by supraphysiological concentrations of IL-6 (data not shown). Consistent with this notion, we found that the partial anti-inflammatory effects observed in BMDMs expressing the wild-type ELR were reduced when the EPO concentration was lowered to 1 from 4 U/ml (Fig. 3d), as judged by ELISA measurements of IL-12p40. In contrast, EPO stimulation at 1 or 4 U/ml inhibited IL-12p40 production in Soc3fl/fl, lysMcre BMDMs transduced with ELR (Fig. 3d, right). These data suggest
that a receptor (ELR) that activates STAT3 to sufficiently high amounts can also activate the AIR.

An EpoR that activates STAT3 instead of STAT5 can activate the AIR

As a final test of the general ability of STAT3 to activate the AIR, we used a synthetic receptor signaling system (Fig. 4a). The homodimeric EpoR normally activates STAT5 (Fig. 4b). However, when the two major STAT5 binding sites are replaced with STAT3 binding sites from gp130 (EpoR-S3), STAT3 activation in response to EPO stimulation ensues (Fig. 4b), with concurrent activation of STAT3-regulated genes such as Socs3 (28, 29). Using retroviral-mediated transduction we introduced the EpoR or EpoR-S3 cDNAs into bone marrow stem cells. Macrophages were differentiated from transduced cells and subjected to assays to measure the ability of EPO to induce the AIR compared with either IL-10 or IL-6. EPO stimulation of EpoR transduced cells had no ability to inhibit LPS-mediated induction of cytokine synthesis (Fig. 4, c–f). However, EPO stimulation of EpoR-S3 transduced cells stimulated the AIR, again indistinguishable from IL-10 (Fig. 4, c–f). These data support the idea that the STAT3-mediated AIR is generic and can be produced by any receptor that activates STAT3, provided the negative regulatory events that block receptors like the IL-6R are negated.

Discussion

Our results establish that the AIR is not unique to the IL-10R signaling pathway but can be generated from a wide variety of STAT3-activating receptors. The AIR is therefore a general STAT signaling pathway and is not a unique property of the IL-10R. By comparison, another stereotypical STAT signaling pathway is the STAT1-mediated IFN response. Like the AIR, the STAT1-mediated IFN response can be generated from receptors other than the IFN receptors by manipulating levels of key signaling components (20, 31, 40). The findings we reported have implications for understanding the AIR in cells other than IL-10-responsive macrophages. We suggest that the AIR is likely to operate in contexts in which cells are subject to stimulation through TLR pathways that must be rapidly controlled.

Different types of STAT3 signals

Our data suggest that the overall amount of STAT3 phosphorylation generated from a given receptor is secondary to the type of STAT3 signal. Both IL-6 and IL-10 rapidly elevate tyrosine (Fig. 1b) and serine phosphorylation (data not shown) of STAT3 followed by expedient transit to the nucleus (our unpublished observation and Ref. 41). However, measurement of STAT3 activation by immunoblotting, EMSA, or microscopy does not necessarily give a strong correlation with the downstream process of specific gene activation (our unpublished data). The initial activation of STAT3 by the IL-10R or the IL-6R precedes Socs3 gene expression and the subsequent inhibitory effects of SOCS3 on gp130. The anti-inflammatory effects of IL-10 can be detected within 30 min when the STAT3-dependent mediators of the AIR are expressed and exert their effects on proinflammatory gene expression (10). Therefore, these kinetic correlates suggest that the initial wave of activated STAT3 from the IL-6R is blocked from inducing the AIR in a SOCS3-independent way. Support for this concept comes from STAT3 DNA binding studies performed in the presence of proinflammatory stimuli. These studies found that the initial STAT3 activated by the IL-6R in the presence of TLR4 or IL-1R signaling caused a block in DNA binding activity of STAT3 that was not observed when STAT3 was activated by the IL-10R (42–44). Therefore, it is possible that STAT3 located in the environment of gp130 is subject to additional modifications that inhibit STAT3 function. Experimental confirmation of this idea will likely require mass spectrometric measurements of STAT3 modifications immediately following receptor activation.

Regardless of the inhibitory effects on early STAT3 signaling from the IL-6R, SOCS3 is a dominant factor that creates the distinction between STAT3 signaling to the AIR or non-AIR signaling. Results from macrophages deficient in SOCS3 (32) or from mutant receptors unable to bind SOCS3, support the idea that the absence of negative regulatory effects on cytokine receptors by SOCS3 is a key step in establishing the AIR pathway. However,
we do not yet understand the biochemical mechanisms behind SOCS3 inhibitory effects on the IL-6R nor do we understand the kinetic and quantitative aspects of STAT3 activation from one receptor relative to another. The AIR requires STAT3 to activate the expression of a gene whose product is responsible for inhibiting the transcription of inflammatory genes (10). This mechanism suggests that STAT3 activated by IL-10 targets different pools of genes relative to STAT3 activated by IL-6. SOCS3 is an example of STAT3 target gene common to both receptors while the mediators of the AIR would be specific to STAT3 activated by IL-10. Two types of experiments could resolve this issue: genome-wide localization studies of STAT3 activated by different cytokines or isolation of the gene or genes responsible for the AIR and the demonstration that their expression is specific to IL-10 type of STAT3 signaling. In this scenario, SOCS3 acts to block the AIR component of STAT3 signaling in responsive receptors. In the absence of SOCS3, the AIR signal is amplified.

STAT3 is required for all known facets of the AIR in keeping with previous published data (6, 9). More surprising was that STAT3 generated from heterologous cytokine receptors could also mimic IL-10. From these data we draw two conclusions that resolve contradictory results concerning the uniqueness of IL-10. First, the IL-10R is unlikely to generate additional pathways that operate either independently or synergistically with STAT3 as has been previously proposed to explain IL-10 anti-inflammatory effects (13, 14). Elegant chimeric receptor studies and constitutive expression of a dominant active STAT3 confirm this idea (L. Williams and B. Foxwell, unpublished observations). Second, because STAT3 can generate the AIR without obvious or detectable additional signals, receptors other that the IL-10R could potentially act in an anti-inflammatory manner and thereby regulate inflammation in other cells or tissues.

Anti-inflammatory signals generated by the IL-27 and IL-22 receptors

Experiments were performed to show that the IL-22R, when expressed in macrophages, could generate the AIR. This result stands in contrast to the argument that IL-22 has a proinflammatory function (45–47). The evidence for the latter comes primarily from studies describing IL-22-mediated induction of defensin genes (45, 47). We suggest that IL-22 is more likely to act as an anti-inflammatory cytokine; because T cells are the primary source of IL-22 and nonhemopoietic cells express the IL-22R, but not the IL-10R α-chain, the AIR may be compartmentalized by tissue type. Perhaps the IL-22 system is required for generating an AIR in a variety of tissues and thereby acts in concert with IL-10 during systemic infection with the overall goal of regulating excessive inflammation? Examination of the inflammatory response in IL-22-deficient or IL-22Rα-deficient mice will be decisive in resolving this issue.

IL-27 is an additional example of a cytokine that activates STAT3 and has a clear anti-inflammatory function (48). Mice lacking WSX-1, a component of the IL-27R complex, die when infected with Mycobacterium tuberculosis (49) or Trypanosoma cruzi (50). In the case of M. tuberculosis, bacterial overgrowth did not cause death because bacterial numbers in the lungs of WSX-1−/− mice declined faster than controls (49, 51). Rather, excessive production of IL-12 and TNF-α is strongly linked to faster death of WSX-1−/− mice compared with control mice. Similarly, large increases in systemic cytokines were observed in WSX-1−/− mice infected with T. cruzi or Leishmania donovani (50, 52) and similar increases in cytokine production from T cells were also observed (53, 54). These examples appear to be an IL-10-independent AIR and support the concept that the STAT3-regulated AIR could be used in diverse settings.

Generation of the AIR in diverse pathological scenarios

Mice lacking STAT3 in hemopoietic lineage cells are extremely sensitive to LPS challenge (7, 8). This response is expected because the anti-inflammatory effects of IL-10 are absent. However, when mice lacking STAT3 in endothelial cells were similarly challenged, they were also sensitive and rapidly died (55), suggesting that STAT3 was also required in endothelial cells to withstand systemic endotoxin challenge. However, there is no strong evidence that endothelial cells express the IL-10R, can respond to IL-10, or that IL-10 plays a physiological role in nonhemopoietic cells. Therefore, the STAT3 AIR is required to protect endothelial cells from excessive inflammation but is controlled not by the IL-10R but rather another receptor that acts like IL-10 in nonhemopoietic cells. It is possible that there are multiple receptors that can activate STAT3, in a similar way to the IL-10R, but are expressed on nonhemopoietic cells and similarly control inflammatory responses when exposed to TLR agonists or other proinflammatory stimuli.

Additional evidence for a central role of STAT3 in regulating the AIR comes from diverse pathological models of which two are notable. First, loss of STAT3 function in keratinocytes leads to an inflammatory disease in the skin (56). These mice suffer from spontaneous ulcers and a variety of other inflammation linked pathologies showing that STAT3 is essential for regulating pathways to control inflammation in the skin. A second example comes from the experimental induction of fulminant hepatitis induced by Con A injection. Hong et al. (57) have shown that STAT1-deficient mice are protected from disease concurrent with an increase in STAT3 activity. Using different methods to manipulate cytokine signaling, they were able to show that STAT3 activity is essential for the protection of the liver against inflammation. Intriguingly, IL-22 seems to play a major protective role in the Con A hepatitis model (58). Both of these examples stress the concept that STAT3 activity, be it regulated by IL-10 or other cytokines in other tissues, is essential for anti-inflammatory pathways.

In conclusion, the AIR is likely to be found in a variety of normal and pathologic settings where TLR or other inflammatory signals must be controlled. Pathogens have developed a variety of pathways to exploit the AIR including the promotion of IL-10 production (59) or the direct activation of the STAT3 AIR (60). The vagus nerve neuroendocrine pathway that contributes to the suppression of inflammation in sepsis has also been traced to increased STAT3 activation (61) as have the ability of some solid tumors to escape immune surveillance (62, 63). The challenge will be to identify the genes activated by the AIR and determine how they regulate the selective transcription of inflammatory genes.

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