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Stat2-Dependent Regulation of MHC Class II Expression

Wenli Zhao,2* Edward N. Cha,2* Carolyn Lee,* Christopher Y. Park,3* and Christian Schindler4*†

MHC type II (MHC II) expression is tightly regulated in macrophages and potently induced by IFN-γ (type II IFN). In contrast, type I IFNs (IFN-Is), which are far more widely expressed, fail to induce MHC II expression, even though both classes of IFNs direct target gene expression through Stat1. The unexpected finding that IFN-Is effectively induce MHC II expression in Stat2−/− macrophages provided an opportunity to explore this conundrum. The ensuing studies revealed that deletion of Stat2, which uniquely transduces signals for IFN-Is, leads to a loss in the IFN-I-dependent induction of suppressor of cytokine signaling-1. Impairment in the expression of this important negative regulator led to a striking prolongation in IFN-I-dependent Stat1 activation, as well as enhanced expression of the target gene, IFN-regulatory factor-1. The prolonged activity of these two transcription factors synergized to drive the transcription of CIITA, the master regulator of MHC II expression, analogous to the pattern observed in IFN-γ-treated macrophages. Thus, IFN-I-dependent suppressor of cytokine signaling-1 expression plays an important role in distinguishing the biological response between type I and II IFNs in macrophages. The Journal of Immunology, 2007, 179: 463–471.

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*Abbreviations used in this paper: MHC I, MHC type I; MHC II, MHC class II; IRF, IFN-regulatory factor; IFNAR, IFN-α receptor; ISRE, IFN-stimulated response element; IFN-I, type I IFN; GAS, γ-activation site; BM, bone marrow; BMM, BM macrophage; WT, wild type; DC, dendritic cell; Socs, suppressor of cytokine signaling; ChIP, chromatin immunoprecipitation; Q-PCR, quantitative PCR; RPA, RNAse protection assay; ISGF-3, IFN-stimulated gene factor 3; USF-1, upstream stimulatory factor 1.

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expression through CIITA pIV. This was found to correlate with the loss in the IFN-α-dependent expression of Socs-1, an important negative regulator of IFN-I response (20). Thus, in WT macrophages, IFN-α- and Stat2-dependent expression of Socs-1 serve to prevent fortuitous CIITA transcription and MHC II expression.

Materials and Methods

Mice

Stat2−/−, Stat1−/−, IFNAR1−/−, and WT (19, 21, 22), all on a pure 129/Sv background, were housed under specific pathogen-free conditions. Stat1/Stat2 double knockout mice, obtained by crossing the appropriate strains, background, were housed under specific pathogen-free conditions. Stat1/

Cell culture

Peritoneal macrophages (nonelicited) were harvested by lavage and cultured in RPMI 1640 (Invitrogen Life Technologies) supplemented with 10% FCS (HyClone). Bone marrow (BM) macrophages (BM), harvested from femurs, were grown in RPMI 1640 supplemented with 20% t-cell-conditioned medium for 5–10 days on 10-cm petri dishes (19, 23). HEK-293T cells and L929 cells (t-cells), from American Type Culture Collection, were cultured in DMEM (Invitrogen Life Technologies), supplemented with 10% FBS. Splenocytes were harvested, filtered through a nylon mesh, counted, and then cultured in RPMI 1640/10% FCS. IFN stimulation entailed the addition of “universal” human IFN-α, (1000 U/ml; PBL), or murine IFN-γ (66 U/ml; PBL), as indicated. Ectopic suppressor of cytokine signaling-1 (Socs-1) expression entailed transfection of vector into HEK-293T to generate high-tier transactivators, as previously reported (23). BM cells, 24 h after harvest, were sequentially infected (three times) with pMIG or murine pSocs-1 retrovirus (provided by P. Rothman, University of Iowa, Iowa City, IA; Ref. 24). Infected cells were harvested by vigorous pipetting with cold PBS, counted, replated for 16 h, and then treated with IFN.

Flow cytometry

After IFN treatment (24–72 h), peritoneal and BMMs were fixed and stained with CD11b (M1/70; eBioscience), CD11c (HL3; BD Pharmingen), I-A^d (BD Pharmingen), streptavidin-PerCP (BD Pharmingen), or an isotype-matched control. Recovered (i.e., by gentle scraping) stained samples were analyzed on a FACSCalibur with CellQuest software (BD Biosciences), as previously reported (19).

Biochemical studies

Whole cell and nuclear extracts were prepared from IFN-treated cells and evaluated by immunoblotting or EMSA, as previously reported (19, 25). Abs used in supershifts, immunoblotting studies and chromatin immuno-
Results

IFN-I induces MHC II in Stat2-null macrophages

The initial analysis of fibroblasts from the Stat2 knockout mice revealed a defect in IFN-α-dependent Stat1 activation and target gene expression (19). In contrast, IFN-α activated Stat1 and target genes normally in Stat2−/− macrophages, highlighting significant tissue-specific differences (19). The response was, however, unaffected by the loss of Stat2 in macrophages. This included induction of MHC II expression, a signature IFN-γ response (Fig. 1). IFN-α failed to induce MHC II expression in WT macrophages. Yet, IFN-α potently stimulated MHC II expression in Stat2−/− macrophages (Fig. 1A). Similar results were obtained with BMMs. Subsequent analysis of Stat1/Stat2 double knockout macrophages determined that analogous to IFN-γ, the aberrant ability of IFN-α to induce MHC II was dependent on Stat1 (Fig. 1B; Ref. 22). As anticipated, no differences were observed in the IL-4-dependent induction of MHC II in WT and Stat2−/− B cells (data not shown). These observations suggest that Stat2 normally serves to suppress the IFN-I-dependent MHC II expression in WT macrophages.

Comparison of IFN-dependent signaling in WT and Stat2−/− BMMs

To more thoroughly evaluate the unanticipated pattern of IFN response, extracts from WT and Stat2−/− macrophages were evaluated for STAT activation by immunoblotting and EMSA. As anticipated, IFN-α directed both the rapid (i.e., within 0.5 h) and transient (i.e., peaking at 0.5 h) tyrosine phosphorylation of Stat1 in WT macrophages (Fig. 2A). A similar pattern was observed for the IFN-α-dependent activation of Stat2. In contrast, IFN-γ directed a more robust and prolonged pattern of Stat1 phosphorylation. Stat2 was not activated (Fig. 2A). The pattern of Stat1 activation was, however, markedly different in IFN-α-stimulated Stat2−/− BMMs. Stat1 phosphorylation peaked rapidly, but remained activated at 12 and 24 h (Fig. 2A). In contrast, the pattern of IFN-γ-dependent Stat1 phosphorylation was not affected by the loss of Stat2.

A similar pattern of STAT activity was observed by EMSA. IFN-α stimulated a rapid, but transient activation of the ISRE-binding complex, ISGF-3, in WT macrophages. This activity actually peaked before the 2-h assay point (Fig. 2B; data not shown). As anticipated, the ISGF-3 complex (i.e., Stat1 plus Stat2 plus IRF-9) was effectively supershifted by a Stat2-specific Ab. IFN-γ also transiently induced GAS-binding activity (i.e., Stat1) in WT macrophages and this was readily supershifted with a Stat1-specific Ab. In contrast, IFN-γ failed to induce ISGF-3, but promoted strong and prolonged activation of GAS binding (Fig. 2B). Consistent with immunoblotting studies, a steady increase in Stat1 DNA-binding activity in IFN-α-stimulated Stat2−/− macrophages was evident over the 24-h assay period. This correlated well with a steady increase in the absolute level of Stat1 protein (Stat1 is an IFN target gene; Ref. 19; data not shown). As anticipated, ISGF-3 was not activated by IFN-γ and IFN-γ-dependent Stat1 activation was largely unaffected by the loss of Stat2 (Fig. 2). These results suggest that the transient pattern of IFN-α-stimulated Stat1 activation observed in WT macrophages is dependent on the presence of Stat2.

Next, the expression of IRF-1, a well-known GAS-driven Stat1 target gene, was evaluated (17, 19, 22, 33, 34). As previously reported, IRF-1 transcripts were rapidly induced by both IFN-α and IFN-γ in WT macrophages (Fig. 2C). Consistent with the profile of activated Stat1, expression was more transient in IFN-α-treated cells. Stat2−/− macrophages, however, exhibited an enhanced pattern of IRF-1 transcription at later time points (i.e., after 6 h of IFN-α stimulation), correlating with the increase in Stat1 activation. Again, there were no differences in response to IFN-γ. These studies demonstrated that IFN-α-dependent Stat1 activation and IRF-1 expression (IRF-1 is active upon expression) are both enhanced in Stat2−/− macrophages.

CIITA expression is negatively regulated by Stat2

The ability of CIITA to direct IFN-γ-dependent expression of MHC II in macrophages has been well-characterized (7–9). To determine whether CIITA also directs IFN-α-stimulated MHC II expression in Stat2−/− BMMs, Northern blotting studies were conducted (Fig. 3A). As anticipated, treatment with IFN-γ, but not IFN-α, led to the expression of CIITA transcripts in WT macrophages. Consistent with published studies, a more detailed Q-PCR analysis revealed that IFN-γ directed both rapid and prolonged CIITA expression in WT macrophages (data not shown; Ref. 35). In
Stat2−/− macrophages, however, IFN-γ and IFN-α both potently induced CIITA expression. Moreover, both basal and IFN-γ-dependent CIITA expression increased in Stat2−/− macrophages. Although the enhanced response to IFN was already evident at 6 h in Stat2−/− BMMs (Fig. 3A), it remained high in IFN-γ-stimulated cells and continued to increase in IFN-α-stimulated samples over 24 h (data not shown). These observations suggest that the unusual ability of IFN-α to induce MHC II correlates directly with the induction of CIITA, the master regulator of MHC II expression.

In mice, three “tissues-specific” promoters direct expression of three distinct CIITA isoforms (7, 8). Promoters pI and pIII are recognized for driving CIITA expression in dendritic and B cells, respectively, whereas pIV is largely responsible for the initial, robust response to IFN-γ in macrophages (9, 26, 35, 36). Promoter I has been shown to contribute to basal and prolonged IFN-γ-dependent CIITA expression in macrophages (35). Additionally, pII contributes to the IFN-γ response, under more restricted settings (32, 35, 37–39). To determine which of these promoters directed the IFN-α-dependent CIITA expression in Stat2−/− BMMs, an RPA was conducted with riboprobes specific for the different CIITA transcripts. Each probe protects a unique promoter-specific fragment, as well as one that is common to all transcripts (8, 29). Consistent with previous studies, this RPA analysis revealed that pIV was largely responsible for IFN-γ-dependent expression in WT macrophages (see Fig. 3B; Ref. 9). IFN-α failed to induce the expression of any protected transcripts in WT macrophages. Yet, in Stat2−/− macrophages, IFN-α-induced significant quantities of pIV-specific transcripts. There was also an increase in the level of pI-specific transcripts in these samples (Fig. 3B, upper band). Consistent with Northern blotting, pIV-specific transcripts were also significantly increased in IFN-γ-stimulated Stat2−/− BMMs (Fig. 3B). However, this did not correlate with a further increase in the level of surface MHC II (Fig. 1). Control studies with IFNAR1-null mice also revealed an increase in IFN-γ-dependent pIV transcripts, but again this failed to correlate with changes in MHC II expression (data not shown). As anticipated, deletion of Stat1 abrogated all IFN-dependent induction of CIITA. These studies suggest that, analogous to IFN-γ, pIV plays an important role in the IFN-α-dependent expression of CIITA in Stat2−/− BMMs.

Promoter IV-dependent CIITA expression in Stat2−/− BMMs

IFN-γ directs pIV-dependent CIITA expression through a cluster of three enhancer elements that bind Stat1, USF1, and IRF-1, respectively (9, 26). Whereas USF1 is constitutively active in macrophages, both Stat1 and IRF-1 are activated by IFN-γ. Having already determined that loss of Stat2 is associated with an enhanced IFN-α-directed activation of Stat1 (see Fig. 2), it became important to determine whether this correlated with an increase in IRF-1 protein. Consistent with IRF-1 transcription (Fig. 2C), IFN-α stimulated a vigorous and prolonged increase in the level of IRF-1 protein in Stat2−/− BMMs. This expression trailed Stat1 activation by ∼1.5 h and persisted for 24 h, contrasting the modest and
transient increase seen in WT macrophages (Fig. 4A). As previously reported, IFN-γ stimulated the robust and prolonged expression of IRF-1 in both WT and Stat2−/− macrophages (9, 17, 19, 26). In addition, enhanced IRF-1 expression in Stat2−/− macrophages correlated directly with a prolonged IFN-α-dependent expression of LMP-2, an IRF-1 target gene. This observation also underscores the direct relationship between IRF-1 expression and its transcriptional activity (Fig. 4B; Refs. 40 and 41).

To determine whether increases in IRF-1 and activated Stat1 observed in IFN-α stimulated in Stat2−/− BMMs were responsible for pIV-dependent CIITA expression, Stat1- and IRF-1-specific Abs were exploited in ChIP assays (Fig. 5). As previously reported, IFN-γ stimulated the rapid recruitment (i.e., within 0.5 h) of Stat1 to pIV in WT macrophages (26). Moreover, Stat1 binding was still evident at 24 h. In contrast, IFN-α drove the rapid, but transient recruitment of Stat1 to pIV, correlating well with the levels of activated Stat1 found in WT macrophages (see Fig. 2). However, upon loss of Stat2, IFN-α directed a robust and prolonged recruitment of Stat1 to pIV, analogous to the pattern observed in IFN-γ-stimulated cells.

As previously reported, IFN-γ also stimulated the robust recruitment of IRF-1 to pIV, first notable at 2 h, but persisting for the remainder of the assay (Fig. 5; Ref. 26). Again, this correlated directly with IRF-1 protein levels (see Fig. 4A). In contrast, the IFN-α-dependent IRF-1 recruitment to pIV in WT cells was modest and transient (i.e., only evident at 2 h), paralleling protein expression data (Fig. 4A). In Stat2−/− macrophages, however, IRF-1 recruitment to pIV was robust, analogous to the pattern observed in IFN-γ-stimulated macrophages. These observations suggest that differences in the levels of Stat1 activation and subsequent IRF-1 expression are sufficient to explain the enhanced, IFN-α-dependent expression of CIITA Stat2−/− macrophages. Moreover, the direct correlation between IRF-1 expression and pIV recruitment, suggests that posttranslational modifications to IRF-1 may not play an important role in this process (42).

How is IFN-α-dependent Stat1 activity prolonged in Stat2−/− macrophages?

To determine the mechanism by which Stat2 negatively regulates the IFN-α-dependent activation of Stat1, a number of additional studies were conducted. Based on the bimodal pattern of enhanced Stat1 phosphorylation observed in Stat2−/− BMMs (Fig. 2A), we determined whether secretion of activating ligand might account for this response (i.e., through an autocrine loop). ELISA studies failed to detect IFN-γ in any of the macrophage cultures, excluding this appealing candidate (data not shown).

Before screening for another Stat1-activating ligand, it was important to establish that the Stat1 activation persisted in Stat2−/− macrophages after IFN-α withdrawal. This would both confirm the existence of an autocrine loop and establish conditions for planned supernatant transfer experiments. Unexpectedly however, Stat1 activity was found to decay rapidly when IFN-α was withdrawn from WT and Stat2−/− macrophages (compare Fig. 6A with Figs. 2A and 4A). This provided strong evidence that the prolonged Stat1 activation observed in Stat2−/− macrophages was dependent on the continuous presence of IFN-α. A subsequent study, in which aliquots of variously aged IFN-α (i.e., in medium alone) were assayed on WT macrophages (i.e., for 1 h), confirmed that IFN-α retained full biological activity for at least 24 h at 37°C (Fig. 6B, first panel).

The requirement of continuous IFN-α stimulation for the prolonged Stat1 activity in Stat2−/− macrophages, suggested that Stat2 might normally direct the expression of a negative regulator. To determine whether this putative negative regulator functioned by inactivating IFN-α activity (e.g., through secretion of a ligand-specific protease or soluble IFNAR2; Ref. 43), another second set of supernatant transfer experiments were conducted. Aliquots of
variously aged IFN-α, collected from the culture supernatants of IFN-α-stimulated WT macrophages, were assayed on fresh BMMs (as above). Again, IFN-α retained full activity for at least 24 h in macrophage cultures, undermining the notion that the negative regulation was directed toward the outside of the cell (Fig. 6B, second panel).

Efforts to determine whether IFNAR down-regulation might explain the Stat2-dependent effect were hampered by the lack of sensitive murine receptor-specific Abs. However, these receptor levels do not appear to vary acutely in human cells (44). Therefore, we turned our efforts to determine whether the Stat2-dependent negative regulator required new protein synthesis. Consistent with older studies on latency (45), IFN-α-stimulated Stat1 signal decay was substantially impaired by cycloheximide pretreatment (i.e., in WT macrophages; data not shown), providing additional evidence for the existence of a Stat2-dependent negative regulator.

Recent evidence that Socs-1 associates with IFNAR1 and negatively regulates the antiviral response to IFN-α, prompted us to explore whether Socs-1 might be the Stat2-dependent negative regulator (20). Supporting this possibility, a well-conserved ISRE consensus site was identified in the Socs-1 promoter, ~75 bp upstream from the transcription start site (data not shown). Moreover, IFN-α vigorously induced Socs-1 expression in WT, but not Stat2−/− macrophages, despite robust Stat1 activation (Fig. 6C, top panels). In contrast, the even stronger IFN-γ-stimulated Socs-1 expression was Stat2 independent. Although Socs-1 appears to negatively regulate IFN-γ-dependent Stat1 activation (46, 47), this response is delayed (compare Fig. 6C and 2A). Control studies determined that the IFN-α and IFN-γ-dependent expression of Socs-3, another important negative regulator, is also Stat2 independent (Fig. 6C, bottom panels).

To provide more direct evidence that Socs-1 negatively regulates IFN-α-dependent Stat1 activation, Socs-1 was ectopically expressed in Stat2−/−BMMs (see Fig. 6D). Efficient ectopic expression of GFP by empty vector (pMIG; ~52% GFP-positive Stat2−/−BMMs) had no significant effect on IFN-α-dependent Stat1 activation. In contrast, ectopic expression of Flag-Socs-1 (pSocs1; ~42% GFP-positive Stat2−/−BMMs), which yielded two Socs-1 species (likely Socs-1 phosphoisoforms; Ref. 24), significantly abrogated the enhanced Stat1 activation in Stat2−/− macrophages. Based on other studies with pMIG-infected BMMs, we believe that GFP expression underestimates the extent of ectopic gene expression (W. Zhao and C. Schindler, manuscript in preparation). These observations provide evidence that Socs-1 is an important negative regulator of
IFN-I signaling. Moreover, they demonstrate that ISGF-3 directs the IFN-I-dependent expression of Socs-1, but not Socs-3.

Discussion
IFN-Is play a critical role in the innate response to both viral and nonviral pathogens (2, 4, 48). IFN-I expression is controlled through a complex autocrine loop that directly interfaces with pattern recognition receptors from the Toll and nucleotide domain-binding families (49). This autocrine loop is basally active in most cell types, but superseded by an alternate pathway in plasmacytoid dendritic cells (DCs) (2, 4, 19, 49, 50). The important role IFN response plays in immunity is further underscored by the numerous strategies viral pathogens have evolved to impede IFN-I expression and/or activity (51, 52).
In contrast, the expression of IFN-γ, which shares Stat1 activation with IFN-I, is much more restricted (6). This tight regulation has been attributed to its potent inflammatory activity, which includes MHC II induction (5, 7, 8, 46). An unknown regulatory process prevents the fortuitous expression of MHC II by the more pervasively expressed type I IFNs. The surprising observation that IFN-α induces MHC II when Stat2 is deleted, suggested this transcription factor might control the expression of a negative regulator. Even though Stat2−/− mice do not succumb to an obvious autoimmune disease, defective immune regulation is likely to account for their foreshortened lifespan (19).

Over the last decade, a number of elegant studies have served to elucidate the mechanism(s) by which IFN-γ regulates MHC II expression (7–9, 26, 35, 53). In macrophages, the acute response to IFN-γ is largely directed by promoter pIV of CIITA, the master regulator of MHC II expression. This entails the sequential recruitment of Stat1 and IRF-1 to specific elements in pIV. Although studies have also revealed roles for pI and pIII in the response to IFN-γ, the mechanism is not fully elucidated (26, 32, 35, 53).

Because of the overlap in IFN-signaling pathways and the important role CIITA plays in response to IFN-γ, studies on the aberrant IFN-α-dependent expression of MHC II quickly focused on CIITA and two critical transcription factors, Stat1 and IRF-1. Biochemical studies revealed that loss of Stat2 was associated with a dramatic increase in IFN-I-dependent Stat1 activation and subsequent expression of its target gene, IRF-1 (Figs. 2 and 4). As with IFN-γ, IFN-α was determined to induce the rapid, robust, and sequential recruitment of Stat1 and IRF-1 to CIITA pIV in Stat2−/− BMMs, culminating in robust CIITA and MHC II expression (Fig. 5). This contrasted the considerably more modest and subthreshold pattern of Stat1/IRF-1 recruitment in IFN-α-stimulated WT macrophages. These observations underscored a critical role for the level of Stat1 in directing biological response. Additionally, because IFN-dependent CIITA expression correlates directly with phospho-Stat1 and IRF-1 levels, a role for additional reported IRF-1 modifications seem less likely (42).

Having attributed the aberrant IFN-I-dependent CIITA expression to enhanced Stat1 activation, it became important to identify the mechanism by which this occurred. Initial studies focused on identifying a secreted (i.e., autocrine) factor. This effort unexpectedly determined that increased Stat1 activity was dependent on the continuous presence of active IFN-α in culture supernatants (Fig. 6A). Studies with cycloheximide revealed that negative regulation required new protein synthesis and underscored an important ligand-dependent increase in total Stat1 (i.e., Stat1 behaves like a GAS-driven gene; Ref. 10; data not shown). Thus, loss of a negative regulator and increased Stat1 expression are both likely to account for elevated Stat1 activity in IFN-α-treated Stat2−/− BMMs.

After excluding secretion in negative regulation, our attention focused on an intracellular regulator. Although a number of reported negative regulators were considered, most could be excluded. For example, PIAS-1 is not an IFN-stimulated gene. Moreover, it neither regulates Stat1 activation nor exhibits specificity for IFN-I responses (23, 54, 55). Likewise, T cell protein tyrosine phosphatase is not an IFN-stimulated gene and fails to exhibit specificity for IFN-I responses (55, 56). However, recent genetic studies revealed that Socs-1 negatively regulated IFN-α-dependent antiviral activity (20). Consistent with this, a highly conserved ISRE site was identified in the proximal Socs-1 promoter. In addition, Socs-1 was robustly induced by IFN-α stimulation in WT, but not Stat2−/− BMMs (Fig. 6C). This contrasted an even stronger IFN-γ-dependent Socs-1 induction, which was notably less effective at antagonizing IFN-γ-dependent Stat1 activation. Future studies will explore whether IFNAR features a Socs-1 recruitment site to account for the unique and rapid sensitivity to IFN-α (vs IFN-γ; Ref. 57). Finally, ectopic expression of Socs-1 abrogated the prolonged IFN-α-dependent Stat1 activation in Stat2−/− BMMs (Fig. 6D).

The analysis of IFN-α-dependent regulation of CIITA expression in Stat2−/− macrophages yielded a number of additional observations that warrant discussion. First, IFN-γ-directed enhanced CIITA expression in cells where the IFN-I autocrine loop was disabled (i.e., Stat2−/− and IFNAR1−/− BMMs; Refs. 2, 19, 50). This, however, did not yield increased MHC II expression (Fig. 1). Moreover, this enhanced CIITA expression could not be attributed to Socs-1, because IFN-γ-dependent expression of this regulator was normal in Stat2−/− and IFNAR1−/− BMMs (Fig. 6C; data not shown). Rather, another, yet unidentified negative regulator, whose basal expression is controlled by the IFN-I autocrine loop (2, 4, 19), is likely to account for this effect. Second, differences in the ability of IFN-α and IFN-γ to drive Socs-1 expression (Fig. 6C), despite equivalent levels of Stat1 activation (e.g., in Stat2−/− BMMs), suggests that a factor in addition to Stat1 is required for IFN-γ-dependent Socs-1 expression. Third, intrinsic differences in the kinetics of IFN-α- and IFN-γ-dependent Stat1 activation (i.e., rapid vs prolonged) are likely to play an important role in distinguishing the nature of the biological responses directed by these two distinct classes of IFNs. Fourth, even though Stat2−/− BMMs acquire the ability to express high levels of MHC II in response to IFN-I, these mice did not exhibit overt evidence of autoimmunity. This likely reflects the complex and redundant mechanisms that regulate the balance between tolerance and inflammation. For example, a potentially enhanced capacity of Stat2−/− macrophages to present Ags may be associated with enhanced tolerance. Fifth, multiple promoters assure that the regulation of CIITA expression varies between cell types (e.g., DCs vs B cells vs macrophages; Refs. 7, 8, 39, 53), explaining why the aberrant pattern of CIITA/MHC II expression is not phenocopied in B cells or DCs (data not shown). Sixth, we failed to observe differences in the levels of other regulators that have previously been implicated in the control of CIITA or IRF-1, including IRF-2, IRF-8, and Blimp-1 (data not shown; Refs. 58–61).

In summary, these studies have identified Socs-1 as an important negative regulator of the biological response to IFN-I. Analogous to the receptor specific role Socs-2 plays in regulating the response to growth hormone (62) and Socs-3 plays in distinguishing over-activation of the JAK/STAT pathway, recent advances and future challenges. Gene 285: 1–24.


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