

● Introduction

Cytokine receptor signal transduction is negatively regulated by several mechanisms to ensure that the magnitude of the signal and its duration are optimal to generate a physiological response (1). Suppressors of cytokine signaling (SOCS) is a novel family of negative feedback regulatory proteins which attenuate cytokine receptor signal transduction (2). SOCS1, which is strongly induced by interferon gamma (IFN γ), is a critical regulator of IFN γ signaling. *SOCS1*^{-/-} mice, which die within 2-3 weeks after birth, are rescued in

lular bacterial pathogens and tumors (9). The major cellular target of IFN- γ is the macrophage, however

induces several other cell types in and around the inflammatory lesions to express MHC-II, allowing these cells to function as non-professional APCs. Perpetual activation of such non-professional APCs by IFN γ is one of the contributing factor to the persistence and autoimmune nature of chronic inflammatory diseases such as arthritis (13).

● IFN γ receptor signaling

The JAK-STAT pathway stimulated by IFN γ is the prototype signal transduction pathway utilized by several cytokines signaling via the hematopoietin receptor superfamily (14). These receptors, lacking intrinsic kinase activity,

an IFN γ -deficient background (3,4). While IFN γ induces SOCS1 expression within a few hours (5), several other effects of IFN γ are not manifested until several hours after cell stimulation (6). While exploring the possibility that SOCS1, in addition to attenuating the early signaling events, may actually participate in some of the late functions of IFN γ , we observed that SOCS1 is essential for the IFN γ -induced expression of major histocompatibility complex (MHC) class-II molecules (MHC-II) on fibroblasts (7). The significance of this finding in chronic inflammatory responses, where fibroblasts significantly contribute to the pathogenic processes (8) is discussed.

● Pleiotropic functions of IFN γ

Cytokines regulate several critical check points in the development of immune cells and control their functions during immune responses. IFN γ is a type-II interferon, secreted primarily by activated T helper type 1 (Th1) lymphocytes and natural killer cells (6). IFN γ elicits a wide range of cellular responses which are critical for generating effective protective immunity against viruses, intracel-

other cell types within and outside the immune system also express IFN γ receptor, and respond to IFN γ

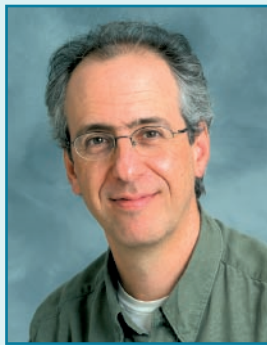
stimulation. IFN γ is cytostatic to many cell types including macrophages, fibroblasts and T cells (10). In macrophages, IFN γ not only augments their cytolytic functions but also modulates antigen processing by the proteasomes and presentation of the processed antigenic peptides by the MHC-I molecules to cytolytic CD8⁺ T lymphocytes (11). IFN γ also upregulates MHC-II expression in professional antigen presenting cells (APCs) such as dendritic cells (DC), macrophages and B cells to stimulate CD4⁺ T lymphocytes more efficiently (12). In addition, IFN γ

transduce signals by activating the receptor-associated Janus family kinases (JAKs). The IFN γ receptor is composed of two chains IFNGR1 and IFNGR2, which associate with JAK1 and JAK2, respectively (9) (Fig. 1). Following ligand-induced oligomerization of the receptor chains, the closely juxtaposed JAKs activate each other by transphosphorylation. Activated JAKs subsequently phosphorylate tyrosine residues on the receptor chains to recruit the signal transducers and activators of transcription 1 (STAT1) proteins.

Regulation of IFN γ -induced MHC Class-II Expression by Suppressor of Cytokine Signaling 1 (SOCS1)

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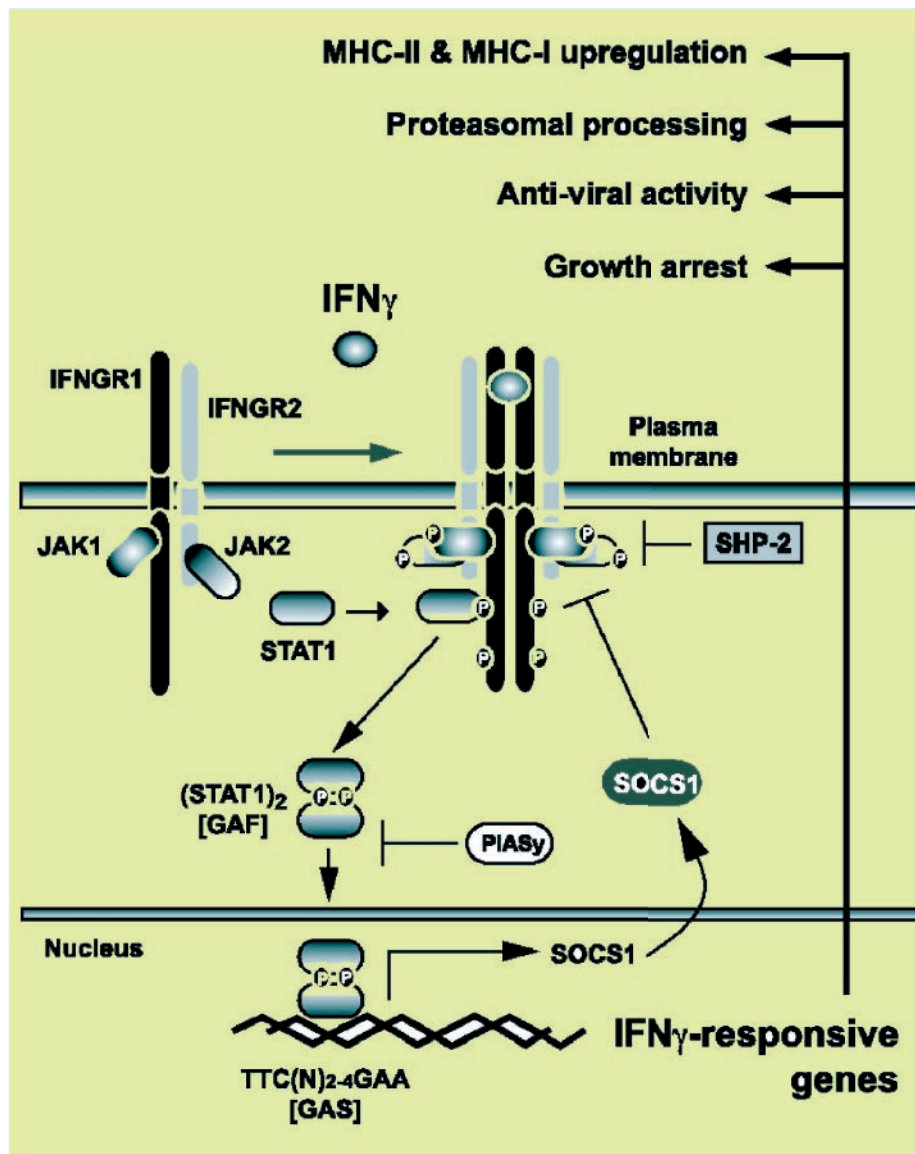


Figure 1: IFN γ receptor signaling and its regulation. Following ligand binding, the IFN γ receptor complex signals via JAK1, JAK2 and STAT1 to stimulate hundreds of genes which mediate a variety of IFN γ -mediated cellular functions. IFN γ -stimulated SOCS1 functions as an indispensable, feedback negative regulator of IFN γ receptor signaling

Phosphorylation of STAT1 is followed by their dimerization into gamma activating factor (GAF), which translocates into the nucleus, binds specific gamma activating sequences (GAS) within the promoter regions of IFN γ -responsive genes and stimulates their expression (15). Phosphorylated JAKs and IFNGR chains also interact with several other signaling molecules and adaptor proteins such as Pyk2, Fyn, c-Cbl, CrkL, CrkII, Vav and SHP-1 which participate in IFN γ signaling (9). Microarray analysis of IFN γ -induced genes in STAT1 null cells has revealed that at least part of the IFN γ -regulated genes is induced independently of STAT1 (16).

● Regulation of IFN γ receptor signaling by SOCS1

Multiple mechanisms operate at the levels of ligand binding, receptor expression and stability, JAK activation and STAT functions to ensure that the intensity and duration of cytokine receptor signals are tightly regulated (1). Whereas the activity of JAK kinases can be regulated through dephosphorylation by phosphatases, it has become clear in recent years that inhibition of JAKs by SOCS proteins constitutes the major pathway of attenuating cytokine receptor signal transduction (2). There are at least eight known members of the SOCS family, which are differentially induced following stimulation by various cytokines.

SOCS1 gene is rapidly induced more than 100-fold following IFN γ stimulation (16-18). Over-expression of SOCS1 completely blocks IFN γ -mediated growth arrest, anti-viral activity, differentiation and MHC-II expression through inhibition of JAK kinase activation, and STAT1 phosphorylation (17,19-21). SOCS1 deficient mice are normal at birth, however they succumb within 2-3 weeks to acute inflammation and massive mononuclear infiltration of visceral organs (3,4,21,22). The pathological lesions in SOCS1 deficient mice are manifestations of uncontrolled IFN γ signaling, as treating these mice with neutralizing anti-IFN γ antibodies prolonged their survival, while breeding them into IFN γ ^{-/-} background prevented perinatal death (3,4). Even though IFN γ signaling could be inhibited by at least two other molecules, SHP-2 and PIAS (23-25), the SOCS1-dependent negative feedback regulation is critical to attenuate IFN γ signaling.

SOCS1 functions as a negative regulator of IFN γ signaling through the inhibition of JAK kinases (17,19-21). Like all other SOCS proteins, SOCS1 contains a central SH2 domain and a C-terminal 'SOCS box' motif (Fig. 2A). The N-terminus of SOCS1 protein is different from other members of the SOCS family. The SOCS1 SH2 domain, which binds directly to the activation loop phospho-tyrosine residue of JAKs, is essential for the inhibitory activity of SOCS1 on JAK kinases (Fig. 2B) (26-28). In addition, the pre-SH2 region of SOCS1 occludes the substrate binding pocket of JAK kinase allowing SOCS1 to function as a pseudosubstrate inhibitor. The SOCS box confers SOCS1 the ability to target SOCS1-interacting proteins such as JAKs and Vav to the ubiquitination machinery (Fig.2B) (29-33). This later role of SOCS1 seems to be essential for the complete function SOCS1, as mice lacking the C-terminus SOCS1 accumulate inflammatory lesions upon aging (34). In addition to the transcriptional regulation of SOCS1 gene (35), the expression of SOCS1 protein is regulated at the levels of translation and post-translational degradation (36,37).

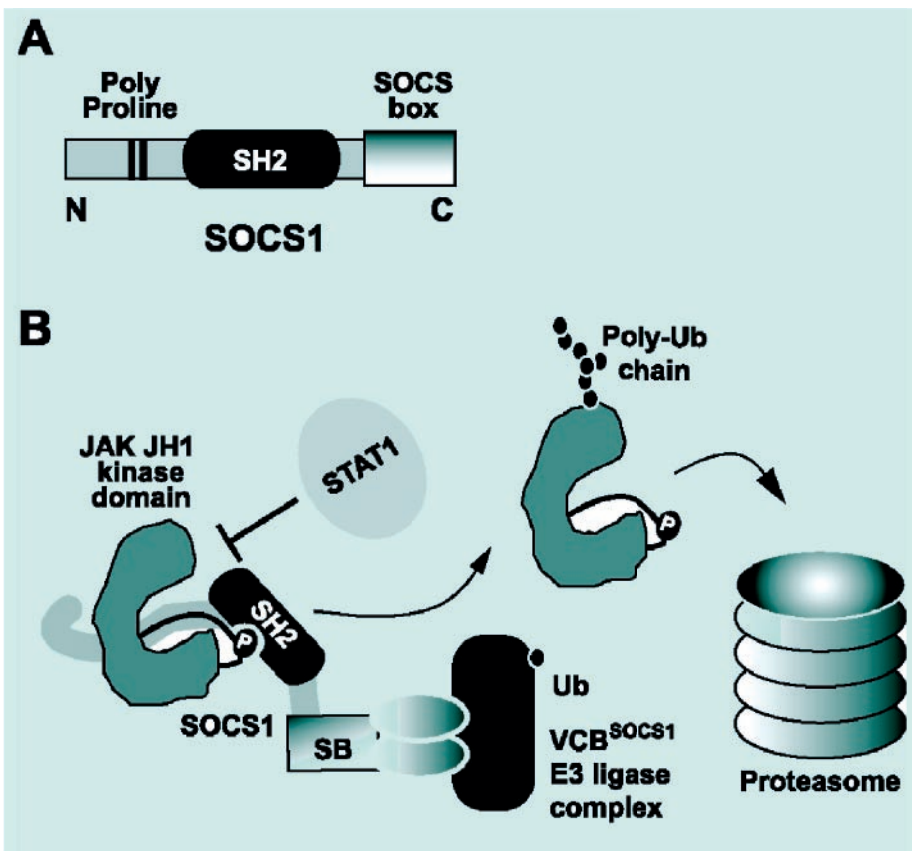


Figure 2: SOCS1 functions as a pseudosubstrate inhibitor of JAKs. SOCS1 contains a central SH2 domain and a C-terminal SOCS box. The SH2 domain binds to the phospho-Tyr residue within the activation loop of activated JAKs allowing a short stretch of amino acids N-terminal to the SH2 domain to occupy the substrate binding pocket of the kinase. This prevents STAT1 from gaining access to the kinase. The interaction of the SOCS box with components of the E3 protein-ubiquitin ligase machinery facilitates JAK ubiquitination and subsequent degradation by proteasomes.

The promoter region of CIITA contains four promoters in human (I, II, III, IV), of which I, III and IV are conserved in the mouse. While dendritic cells (DCs) and B cells constitutively express CIITA using promoter I and II respectively, promoter IV confers inducible expression in macrophages and non-professional APCs such as fibroblasts, endothelial cells, epithelial cells, neuronal cells and muscle cells (42,44,45). IFN γ -induced CIITA expression requires STAT1-dependent transcriptional activation of IRF-1, as CIITA induction is markedly reduced in mice lacking the IRF-1 gene (46). IRF-1 is a member of the IRF (interferon regulatory factors) family of transcription factors, which constitute one of the early response genes containing the STAT1 binding GAS in their promoter regions (6). CIITA does not bind to DNA directly, but functions to orchestrate the assembly of multiple transcription factors such as the RFX complex (RFX5, RFXAP and RFXANK), X2BP/CREB and NF-Y (NF-YA, NF-YB, NF-YC) to the S, X, X2 and Y boxes at the promoter region of the MHC-II genes encoding the α and β chains (12,43). Genetic defects in the expression of the RFX factors or CIITA in human causes inherited MHC-II deficiency (bare lymphocyte syndrome, BLS) leading to severe immunodeficiency, recurrent infections and childhood mortality (42).

The induction of MHC-II by IFN γ appears to be additionally regulated by translational control mechanisms. Stimulation of a B cell line (A20) with IFN γ caused a three fold increase in surface expression of MHC-II in a murine B cell line without increasing the amount or stability of mRNA for MHC-II genes I-A α or I-A β (47). This increase in MHC-II expression is accompanied by a marked increase in the amount of mRNA bound to polyribosomes, suggesting translational regulation of MHC-II expression. Neither the factors nor the mechanisms that contribute to more efficient translation of I-A α and I-A β mRNA following IFN γ stimulation have been elucidated.

● Differential regulation of IFN γ -induced cellular responses by SOCS1

IFN γ induces about 200 genes which differ in their onset and duration of expression (6). Some genes are expressed very early after IFN γ stimulation. Several other IFN γ -responsive genes are induced much later because their expression requires the protein products of the early genes. The MHC-II genes fall into the latter category as their induction requires the expression of IFN γ -induced transcription factors (38). Paradoxically, SOCS1, which is induced within an hour after IFN γ stimulation (17), does not seem to inhibit many IFN γ -responsive genes that are induced only several hours after IFN γ stimulation. Since SOCS1 can also function as part of a protein ubiquitination machinery (39), the apparent persistence of IFN γ signaling despite an early induction of SOCS1 suggested that SOCS1 may positively regulate some of the

temporally distant effects of IFN γ . Consistent with such a possibility, IFN γ stimulated sustained STAT1 phosphorylation in SOCS1 deficient mouse embryo fibroblasts (MEFs) and strongly inhibited their proliferation, but failed to induce MHC-II (Table 1) (7), suggesting that SOCS1 may be required for IFN γ -induced MHC-II upregulation.

● Regulation of MHC class-II gene expression

MHC-II genes encode a group of highly polymorphic cell surface glycoproteins, which are composed of α and β chains. The N-terminal domains of MHC-II α and β chains form a peptide binding groove which presents antigenic peptides generated from exogenous pathogens to CD4⁺ helper T lymphocytes (40). Expression of MHC-II is regulated primarily at the transcriptional level (38,41) (Fig. 3), and is dependent on a transcriptional coactivator protein, MHC class-II transactivator (CIITA) (42,43).

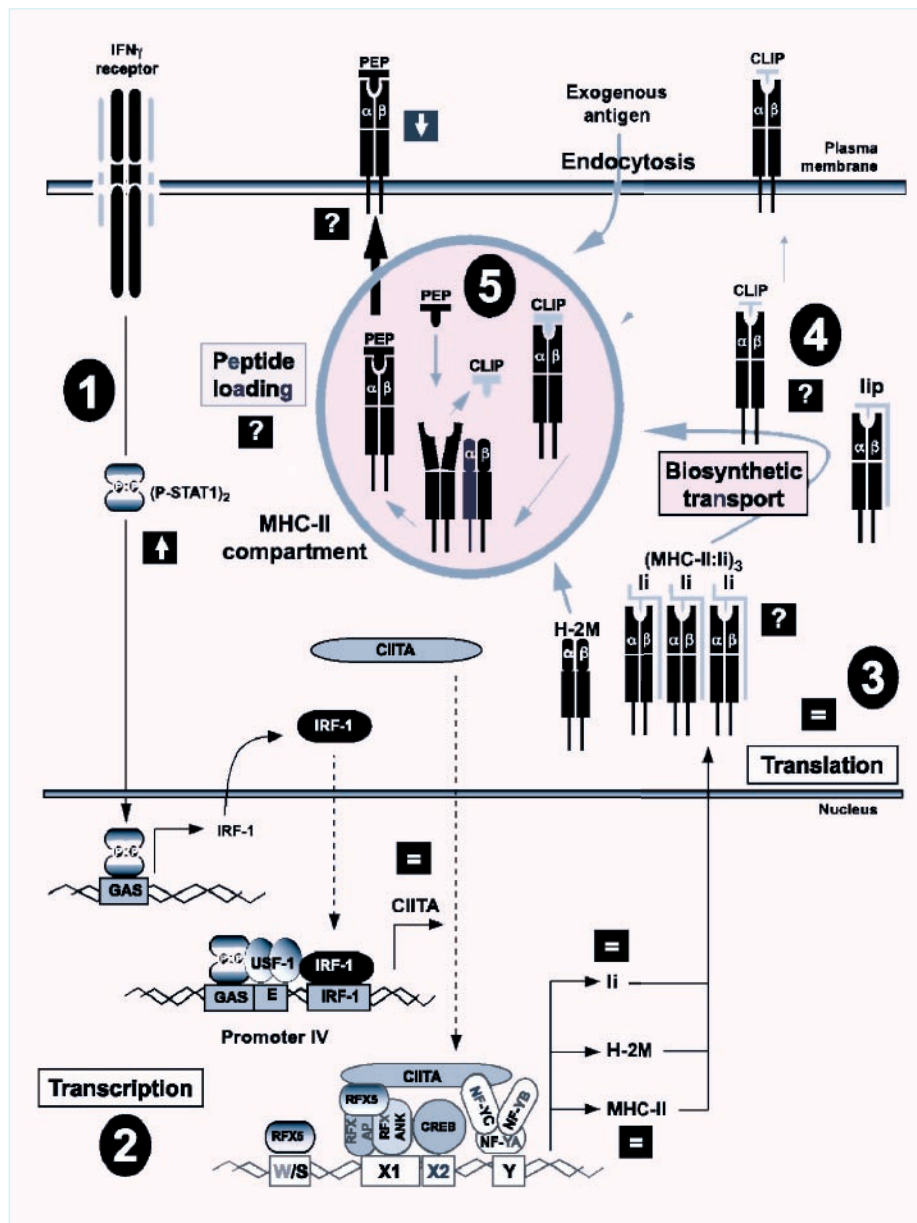


Figure 3: Role of SOCS1 in the regulation of MHC-II expression. Induction of MHC-II following IFN γ stimulation is regulated at the levels of (1) JAK-STAT signaling, (2) sequential transcription of IRF-1, CIITA, and MHC-II, Ii and H-2M genes, (3) translation of MHC-II mRNA, (4) assembly of MHC-II-Ii complexes within the endoplasmic reticulum and targeted delivery to the MHC-II compartment, (5) exchange of the CLIP peptide within the peptide-binding groove of MHC-II with peptides (PEP) derived from endocytosed antigens, and export of the MHC-II:peptide complexes to the cell surface (see text for details). Modulation of these control points by SOCS1 as revealed by cells lacking SOCS1 gene is indicated within black squares as increased (\uparrow), not affected (=), unknown (?) or decreased (\downarrow). While the JAK-STAT signaling is elevated in SOCS1 deficient cells and the MHC-II genes are transcribed and translated normally, the surface expression of MHC-II is defective due to decreased protein stability, probably due to degradation during biosynthetic transport or within the MHC-II compartment.

● Assembly and cell surface expression of MHC-II

The biosynthetic transport of the MHC-II α and β chains, and their assembly into functional MHC-II $\alpha\beta$ heterodimers with a foreign antigenic peptide bound to the antigen binding groove require the expression of the ‘invariant chain’ (Ii) and

the MHC-II-like H-2M molecules (Fig. 3) (48,49). The promoter regions of Ii and H-2M contain the same cis-regulatory elements as the MHC-II gene promoters in a conserved orientation, and require all the transcription factors that are essential for the expression of MHC-II genes (12,43). Ii binds to the MHC-II $\alpha\beta$ heterodimer in the endoplasmic

reticulum (ER), and trimerization of Ii generates a multimeric complex containing three molecules of each protein. A part of the Ii polypeptide occludes the peptide binding groove of MHC-II and protects it from being occupied by peptides derived from misfolded cellular proteins in the ER. In addition, Ii facilitates targeted delivery of the MHC-II to the low-pH endosomal compartment, where stepwise proteolytic cleavage of Ii by cathepsins leaves a short class II-associated invariant chain peptide (CLIP) occupying the peptide binding groove. Subsequently, destabilization of the MHC-II:CLIP complex by H-2M facilitates the loading of foreign antigenic peptides (PEP, in the Fig.3). Peptides with a high affinity for MHC-II generate stable MHC-II:peptide complexes which are transported to the cell surface.

● SOCS1 is required for IFN- γ induced MHC-II protein expression

To verify whether SOCS1 is required for the induction of MHC-II by IFN γ , we reconstituted *SOCS1*^{-/-} cells with wild type SOCS1 (WT SOCS1) or SOCS1 with R105K mutation (SOCS1R105K) that abolishes its binding to JAKs. Constitutive expression of WT SOCS1 completely inhibited IFN γ signaling in SOCS1 deficient cells and rendered them less susceptible to IFN γ -induced growth arrest (Table 1). As expected, SOCS1R105K failed to inhibit STAT1 phosphorylation or suppress IFN γ -induced growth arrest, however it was able to restore MHC-II expression in SOCS1 deficient cells (7). This observation strongly suggests that SOCS1 antagonizes IFN γ -induced growth arrest, but plays an agonistic function in IFN γ -induced MHC-II expression. Since the expression of CIITA and MHC-II α and β genes was not affected in *SOCS1*^{-/-} cells, SOCS1 seems to be required for the post-transcriptional regulation of MHC-II expression. Even though a role for SOCS1 in translational control of MHC-II protein expression is not excluded, over expression of I- $A\alpha$ and I- $A\beta$ cDNA in wild type and *SOCS1*^{-/-} cells revealed that the stability of both MHC-II α and β chains is reduced in the absence of SOCS1 (7). The post-translational stage at which SOCS1 is required for either the transport and/or assembly of MHC-II, and the mechanism involved in the destabilization of MHC-II in the absence of SOCS1 remain to be elucidated.

Fibroblast lines	Phospho-STAT1	Growth arrest	MHC-II surface expression	Transcription			MHC-II protein stability
				CIITA	I-A α , I-A β	II	
SOCS1 ^{+/+}	++	+	+++	+++	+++	+++	+++
SOCS1 ^{-/-}	+++	+++	+	+++	+++	+++	+
SOCS1 ^{-/-} + Vector	+++	+++	+	+++	+++	+++	NT
SOCS1 ^{-/-} + WT SOCS1	-	+	-	-	-	+++	NT
SOCS1 ^{-/-} + SOCS1R105K	++	++	+++	+++	+++	+++	NT

Table 1: Requirement of SOCS1 for IFN γ -induced MHC-II expression. SOCS1 deficient fibroblasts are defective in surface expression of MHC-II following IFN γ stimulation despite sustained STAT1 phosphorylation and normal transcription and translation of MHC-II genes. Reconstitution of SOCS1^{-/-} cells with wildtype SOCS1 totally abolished IFN γ receptor signaling, whereas expression of the R105K mutant, which cannot bind JAK kinase, restored MHC-II surface expression. The defect in SOCS1^{-/-} cells is due to decreased stability of MHC-II proteins. NT, not tested.

● Implications of SOCS1-dependent MHC class-II expression on fibroblasts

In chronic inflammatory lesions, interactions between lymphoid and non-lymphoid cells, and cross-modulation of their functions by various cytokines influence the outcome of the inflammatory response and eventually the development of protective immunity, or the breakdown of peripheral immunological tolerance. In recent years it has become clear that abnormal fibroblast activation can retard the resolution of acute inflammation and perpetuate a chronic inflammatory state. Activated fibroblasts not only secrete several proinflammatory cytokines, chemokines and prostaglandins but also modulate the behavior of T and B cells. Cytokine-induced MHC-II on fibroblasts can activate T cells (44), which in turn secrete cytokines that stimulate fibroblast proliferation leading to tissue fibrosis. For example, in the dermis of patients with progressive systemic sclerosis and in arthritic joints. IFN γ -induced MHC-II is believed to contribute to T cell activation and chronic inflammation (13,50). We propose that induction of SOCS1 in fibroblasts by a variety of cytokines in the milieu of chronic inflammatory lesions may contribute to MHC-II expression in these cells, and thus to subsequent pathological events.

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