

Molecular mechanisms in allergy and clinical immunology

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Turning off signal transducer and activator of transcription (STAT): The negative regulation of STAT signaling

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Signal transducer and activator of transcription (STAT) proteins are a group of transcription factors that transmit signals from the extracellular milieu of cells to the nucleus. They are crucial for the signaling of many cytokines that are mediators of allergic inflammation. Considerable information is known about the activation of STATs and their role in gene transcription; comparably much less is known about how STAT signaling is regulated. Because STATs are critical for the induction of many genes crucial for the allergic cascade and immune host defense, understanding the regulation of these molecules will provide novel insights into allergic and immunodeficiency disorders and will likely identify new targets for therapeutic interventions. This review will summarize the current understanding of the regulation of STAT signaling, emphasizing recent observations. (*J Allergy Clin Immunol* 2004;114:476-89.)

Key words: Cytokine, Jak-STAT, STAT, methylation, suppressor of cytokine signaling, protein inhibitor of activated STAT, phosphatase, review

During allergic inflammation, preformed and newly synthesized cytokines are released that contribute to the pathology seen in allergic diseases. These cytokines have a wide range of activities on different cell types. They exert their effects by binding to specific cell-surface receptors and inducing the expression of relevant target genes. The discovery of signal transducer and activator of transcription (STAT) proteins more than 15 years ago

Abbreviations used

CIS: Cytokine-inducible SH2-containing protein
ERK: Extracellular signal-regulated kinase
GH: Growth hormone
Jak: Janus kinase
JH: Jak homology
LIF: Leukemia inhibitory factor
LMB: Leptomycin B
LMW-PTP: Low-molecular-weight protein tyrosine phosphatase
MKP: Mitogen-activated protein kinase phosphatase
NES: Nuclear export signal
PIAS: Protein inhibitor of activated STAT
PP2A: Protein phosphatase 2A
PTP: Protein tyrosine phosphatase
SCID: Severe combined immune deficiency
SHP: SH2 domain-containing tyrosine phosphatase
SOCS: Suppressor of cytokine signaling
STAT: Signal transducer and activator of transcription
SUMO: Small ubiquitin-like modifier
TC-PTP: T cell-protein tyrosine phosphatase
TC45: Nuclear isoform of T cell-protein tyrosine phosphatase

provided a key molecular link between the binding of a cytokine to its cell-surface receptor and the induction of specific genes. This link between the IFN receptor and gene transcription in the first report of STAT protein¹ resulted in the discovery of the Janus kinase (Jak)-STAT pathway. Many ILs, including IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-23; type I and type II IFNs; and members of the hematopoietin family use this pathway to transduce their signals. These distinct factors transmit their signals through 4 Jak and 7 STAT molecules. As shown in Fig 1, Jaks constitutively associated with the receptors are brought into proximity after ligand-receptor interactions, leading to transphosphorylation and resultant activation of Jaks. Activated Jaks then phosphorylate specific tyrosine residues in the cytoplasmic region of the receptor that can serve as docking sites for STAT monomers. STAT monomers

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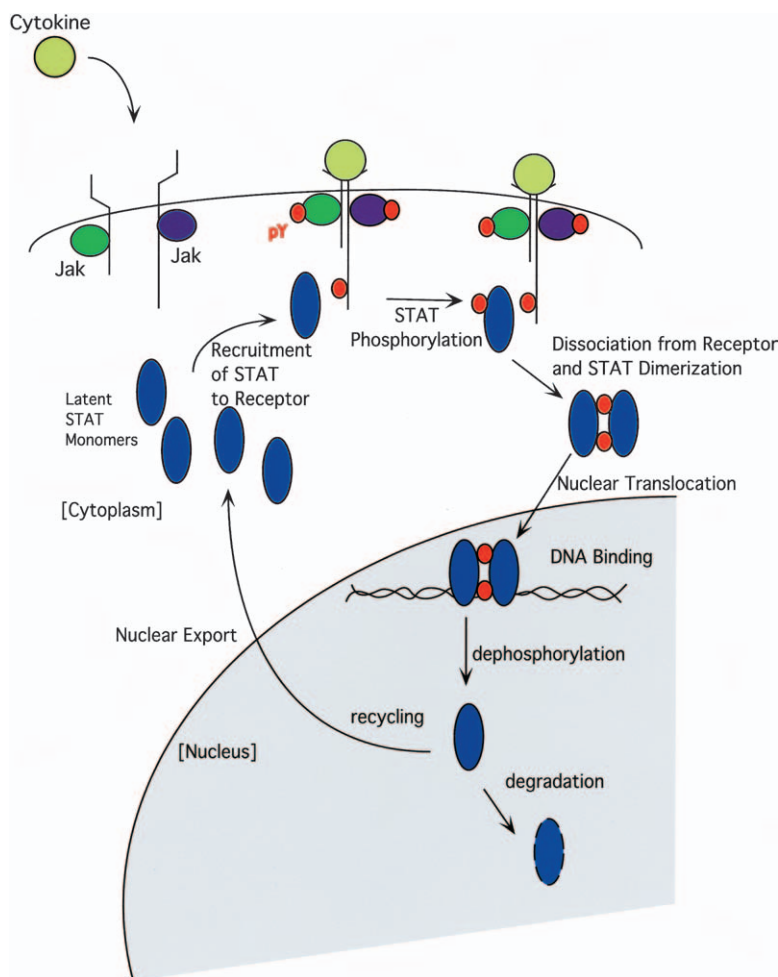


FIG 1. Overview of the Jak-STAT pathway.

associate with the phosphorylated tyrosine sites and become tyrosine phosphorylated through the action of Jaks. Activated STATs are released from the receptor, dimerize, translocate to the nucleus, bind specific canonical DNA elements, and initiate cytokine-specific gene transcription. Because the Jak-STAT pathway is central to so many fundamental biologic processes, it is not surprising that the regulation of STAT function is a tightly regulated process. Indeed, several mechanisms have been described that serve to regulate the Jak-STAT pathway at different points along the cascade. This review will summarize the current understanding of the regulation of STAT signaling.

OVERVIEW OF JAK AND STAT PROTEINS

Jak kinases consist of 7 conserved Jak homology (JH) domains. Jaks have both a pseudokinase domain (JH1) and a true catalytic kinase domain (JH2), leading to their being named after the mythological Roman god Janus, who had 2 faces. The other domains, JH3 through JH7,

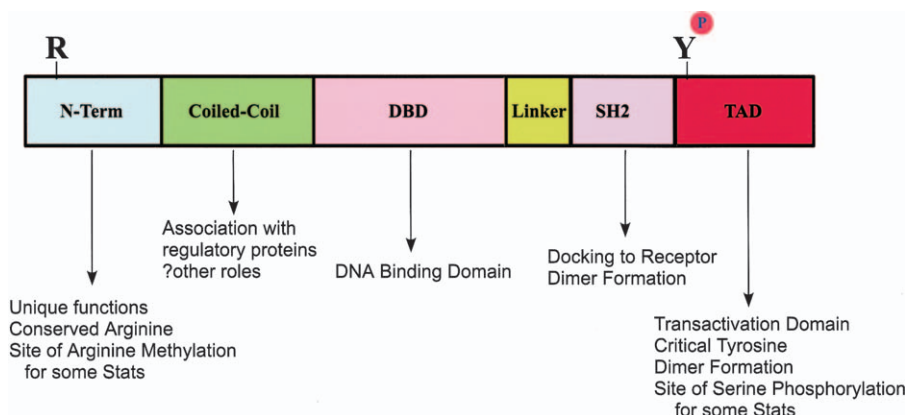
mediate association with receptors.² Although Jaks have largely overlapping functions, studies of Jak gene-targeted mice reveal characteristic signaling defects, as summarized in Table I.³⁻³⁰

Seven STATs have been identified (STATs 1-6, including 2 genes encoding STAT5) that are activated by a different group of cytokines and hematopoietins. Studies in null mice reveal essential activities for STATs, as summarized in Table I. For example, STAT4 is critical for IL-12 signaling and T_H1 cell differentiation, whereas STAT6 is critical for IL-4 and IL-13 signaling and T_H2 cell differentiation. STAT homologues have been identified in simple eukaryotes, suggesting that they arose from a single gene.³¹ All STAT proteins share the same overall structure (Fig 2). They have an N-terminal domain, a coiled-coil domain, a DNA-binding domain, a linker domain, an SH2 domain, and a C-terminal region that contains a critical tyrosine residue, as well as the trans-activation domain. In some STATs the C-terminal region also contains a serine residue that is phosphorylated, and this phosphorylation is also important for STAT function (discussed further below). STAT proteins exist as latent

TABLE I. Immunologic functions of Jak, STAT, and SOCS components

Gene	Phenotype of null mice	References
Jak kinase		
Jak1	Perinatal lethal: defects in lymphoid development	3
Jak2	Embryonic lethal: failure of erythropoiesis	4, 5
Jak3	Severe combined immune deficiency	8-10
Tyk2	Defective IL-12 and IFN signaling	6, 7
STAT		
STAT1	Decreased viral immunity, failure to thrive, impaired IFN signaling	11, 12
STAT2	Impaired IFN signaling	13
STAT3	Embryonic lethal: impaired responses to pathogens	14
STAT4	Impaired IL-12 signaling: defective T _H 1 cell differentiation	15
STAT5a	Impaired mammary gland development: impaired prolactin signaling	16, 17
STAT5b	Impaired GH signaling: defective NK cell function	18, 19
STAT5a + STAT5b	Decreased T-cell proliferation in addition to single knockout phenotypes: absent NK cells	20
STAT6	Impaired IL-4 and IL-13 signaling: defective T _H 2 cell differentiation	21, 22
SOCS		
CIS	Normal	28
SOCS1	Perinatal Death (IFN- γ dependent)	23, 24
SOCS2	Growth abnormalities	25-27
SOCS3	Embryonic lethal	28-30

NK, Natural killer.

**FIG 2.** Conserved structural domains of STAT family proteins and their functions. DBD, DNA binding domain; TAD, transactivation domain.

cytoplasmic monomers until cytokine stimulation, whereupon they dock onto phosphorylated tyrosine residues in receptor chains through their conserved SH2 domains. Then they are phosphorylated by receptor-associated Jaks on a conserved tyrosine residue in their C-terminal region. After phosphorylation, the STAT dissociates from the receptor and dimerizes (usually homodimers). The dimer is formed by the SH2 of one STAT interacting with the phosphorylated tyrosine in the C-terminal region of the opposing STAT, resulting in a stable bivalent interaction.³² The dimers then translocate to the nucleus and recognize and bind through the DNA-binding domain to members of the IFN- γ -activated sequence family of enhancers, resulting in modulation of downstream gene transcription. The basis for nuclear translocation of STATs is not well understood (discussed further below). The N-terminal domain contains a conserved arginine

residue in all STATs that has been shown to be methylated in STAT1³³ and STAT6.³⁴ However, the role of methylation is distinct for the 2 STATs (discussed below). It is likely that the N-terminus of each STAT family member has a unique function. This is further supported by recent studies examining STAT1 through STAT4 chimeric proteins; the N-terminal domains of STAT1 and STAT4 were not functionally interchangeable.³⁵

REGULATION OF STAT SIGNALING

As described above, STAT activation is central to the many biologic processes, and the regulation of this pathway occurs at several points along the cascade. A number of negative regulators of the Jak-STAT signaling pathway have been described, including tyrosine phos-

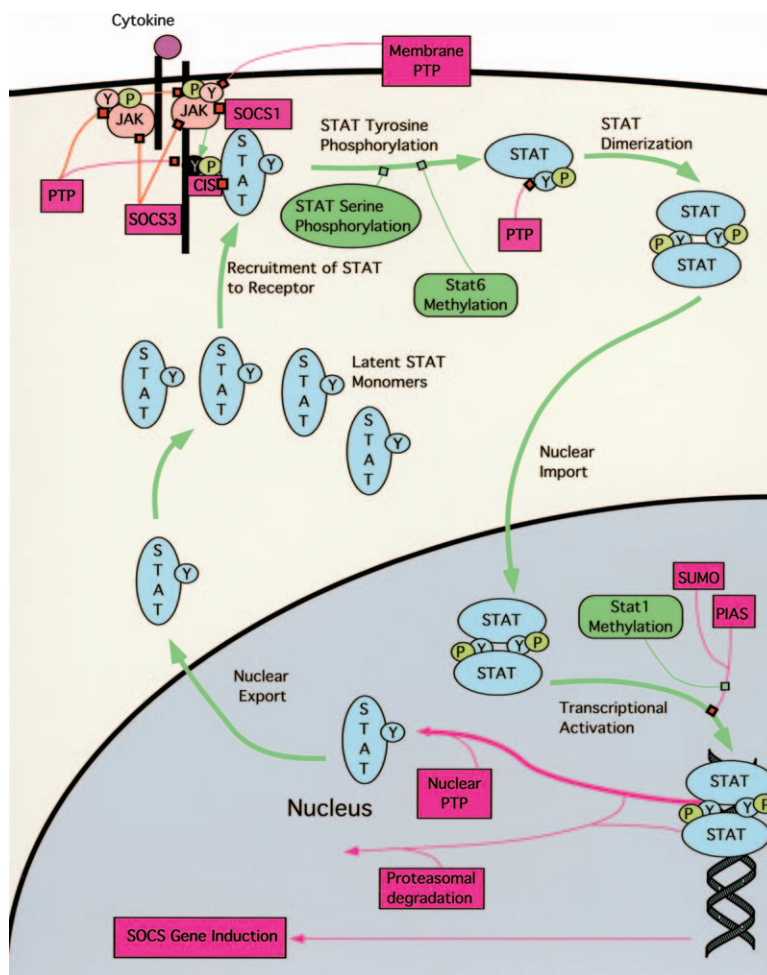


FIG 3. Complex regulatory networks acting on Jak-STAT signaling.

phatases, suppressors of cytokine signaling (SOCSs), and protein inhibitors of activated STAT (PIASs). STAT signaling is also regulated by STAT serine phosphorylation and arginine methylation. Finally, nuclear localization is a potential regulatory point for STAT function. Each of these regulation steps is discussed below.

TYROSINE PHOSPHATASES

Protein tyrosine phosphatase family

There are 3 groups of enzymes that can dephosphorylate tyrosine phosphorylated residues: classical protein tyrosine phosphatases (PTPs); dual-specificity PTPs (dephosphorylate both tyrosine and serine-threonine phosphorylated residues), including mitogen-activated protein kinase phosphatase (MKP) 1; and low-molecular-weight PTPs (LMW-PTPs). The classical PTPs are divided into 2 families, the transmembrane receptor-like PTPs, including CD45 and PTP ϵ , and nontransmembrane

PTPs, including SH2-domain-containing PTP1 (SHP1), SHP2, PTP1B, and T cell–protein tyrosine phosphatase (TC-PTP). The identification of 113 distinct vertebrate PTP domains has been reported, and they are classified into 17 subtypes by analysis of the sequence alignment. The sequence VHCSXGXGR[T/S]G is defined as the PTP signature motif.³⁶ Inhibition of PTP activity by using the PTP inhibitor pervanadate results in constitutive activation of STATs, which requires Jaks.³⁷ Several PTPs were studied for their roles in regulation of Jak-STAT signal transduction, including SHP1, SHP2, PTP1B, TC-PTP, CD45, PTP ϵ , MKP-1, and LMW-PTP.

SHP1

There are 2 naturally occurring mutations of murine SHP1. The *motheaten* (*me*) allele has an early frame shift, resulting in the absence of SHP1 activity, whereas the *motheaten viable* (*me^v*) allele encodes SHP1 mutant proteins with only approximately 20% of wild-type SHP1 activity. The mice with these mutations express a severe autoimmune and immunodeficiency syndrome.^{38,39} In

bone marrow–derived macrophages from *me/me* mice, which lack SHP1 activity, Jak1 and STAT1 tyrosine phosphorylation is dramatically increased and that of Tyk2 and STAT2 is largely not affected on IFN- α stimulation.⁴⁰

SHP1 also negatively regulates IL-4– and IL-13–dependent signal transduction. In bone marrow–derived macrophages from *me^v/me^v* mice, IL-4–induced STAT6 DNA-binding activity is remarkably enhanced compared with that seen in wild-type mice. Overexpression of SHP1 in NIH3T3 cells downregulates IL-4/IL-13 signaling and gene induction.⁴¹ Studies with wild-type or *me^v/me^v* primitive hematopoietic precursor cells indicate that SHP1 contributes to the early loss of tyrosine phosphorylated STAT6 induced by IL-4.⁴²

SHP1 associates with Jak2 and dephosphorylates Jak2 when coexpressed in Cos-7 cells. The N-terminal domain, and not the SH2 domain, of SHP1 is involved in interaction with Jak2.⁴³ Leukemia inhibitory factor (LIF)–induced Jak2 and STAT3 dephosphorylation is delayed in cells overexpressing an inactive SHP1 mutant.⁴⁴ SHP1 also downregulates IL-2–induced tyrosine phosphorylation of Jak1 and Jak3. Tyrosine phosphorylation of IL-2R β and associated Jak1 are both decreased in SHP1-transfected cells. An SHP1 mutant lacking phosphatase activity does not affect the tyrosine phosphorylation of the IL-2R complex.⁴⁵ However, in nonhematopoietic HeLa cells, expression of the SHP1-inactive mutant reduces IFN- γ –induced STAT1 activity by 30% to 50%, whereas the expression of native SHP1 increases STAT1 activity by 20% to 30%.⁴⁶ The mechanisms for the differential activity of SHP1 in hematopoietic and nonhematopoietic systems are unclear.

SHP2

Kinetic studies of STAT1 activation with regulators of PTP indicate that more than one PTP is involved in regulation of constitutive and IFN- γ –dependent STAT1 activity.⁴⁷ The DNA-binding activity of STAT1 and STAT2 is significantly increased in SHP2^{−/−} mouse fibroblast cells. The activation of both STATs by IFN- α or IFN- γ is diminished in the SHP2^{−/−} cells when SHP2 is reintroduced. SHP2 is constitutively associated with IFN- α/β and IFN- γ receptors.⁴⁸ It associates with STAT1 in A431 cells after IFN- γ or epidermal growth factor treatment and acts as a dual-specificity protein phosphatase, dephosphorylating STAT1 at both Tyr701 and Ser727. The interaction of SHP2 with STAT1 through its C-terminal PTP domain and the SH2 domain of STAT1 destabilizes STAT1-DNA complexes and inhibits STAT1-mediated gene expression.⁴⁹

SHP2 specifically interacts with tyrosine phosphorylated STAT5a through its C-terminal SH2 domain and dephosphorylates STAT5a.^{50,51} Prolactin induces SHP2 nuclear translocation and the binding of the SHP2/STAT5 complex to the β -casein gene promoter.⁵² By using a substrate-trapping mutant of SHP2, it was shown that tyrosine phosphorylated STAT5 is associated with SHP2 on IL-2 stimulation.⁵⁰ In SHP2-deficient cells dephosphorylation of STAT5a is dramatically delayed, but dephosphorylation of STAT3 is unaffected.⁵¹ In contrast, in cutaneous T-cell lymphoma cells, constitutive STAT3 and IL-2–induced STAT5 tyrosine phosphorylation are not affected by expression of wild-type SHP2 or inactive SHP2.⁵³

SHP2 also dephosphorylates Jak2 and contributes to the Jak2 stability by decreasing its association with SOCS-1. Specifically, SHP2 regulates prolactin/STAT5–mediated signaling by dephosphorylating Tyr-1007 of Jak2, the SOCS-1 recruitment site.⁵⁴

PTP1B

PTP1B, a cytosolic PTP, dephosphorylates and deactivates prolactin-activated STAT5a and STAT5b.⁵⁵ PTP1B also dephosphorylates Tyk2 and Jak2, which contain the (E/D)-pY-pY-(R/K) consensus, but not Jak1, which does not have this motif.⁵⁶ The substrate-trapping mutant of PTP1B interacts with Tyk2 and Jak2 in response to IFN- α , and in PTP1B-deficient cells STAT1 phosphorylation is enhanced in response to IFN- α .⁵⁶ Similarly, overexpression of PTP1B results in reduced STAT5-dependent gene expression.⁵⁷

TC-PTP

The nuclear isoform of TC-PTP (TC45) dephosphorylates IL-6–induced activated STAT3 in 293T cells. TC45 interacts with the C-terminal domain of STAT1. Overexpression of TC-PTP in myeloid leukemia M1 cells suppresses IL-6–induced growth arrest.⁵⁸ In TC-PTP null mouse embryonic fibroblasts, IFN- γ –induced tyrosine dephosphorylation of STAT1 is defective. Only the nuclear (not the cytoplasmic) isoform of TC-PTP can rescue the defect in STAT1 dephosphorylation. Dephosphorylation of STAT3, but not STAT5 or STAT6, is also affected in TC-PTP^{−/−} cells.⁵⁹

CD45

CD45 is the most studied transmembrane PTP involved in Jak/STAT signal transduction. CD45 negatively regulates cytokine receptor signaling as a Jak phosphatase.⁶⁰ CD45 is a Jak phosphatase in human B cells; it inhibits IL-4–induced Jak1 and Jak3 phosphorylation and negatively regulates class-switch recombination to IgE. Recombinant CD45 directly dephosphorylates Jak1 and Jak3 *in vitro*.⁶¹

PTP ϵ C

Recently, a cytosolic form of transmembrane PTP ϵ C, PTP ϵ C, was found to negatively regulate IL-6– and LIF-induced Jak-STAT signaling. It inhibits IL-6– and LIF-induced tyrosine phosphorylation of Jak1, Tyk2, and the downstream targets gp130 and STAT3.⁶² The expression of an inactive mutant of PTP ϵ C results in enhanced tyrosine phosphorylation of STAT3 and STAT1 on IL-6 stimulation. It also enhances the IL-6–induced STAT1 activation. Expression of PTP ϵ C inhibits IL-10–induced STAT3 phosphorylation but has no effect on the IFN- β – or IFN- γ –induced STAT1 phosphorylation.⁶³

Dual-specificity phosphatases

MKP-1, a nuclear dual-specificity phosphatase, dephosphorylates tyrosine phosphorylated STAT1 in vascular smooth muscle cells⁶⁴ but does not have a role in tyrosine dephosphorylation of STAT3 in these cells.⁶⁵ Calcineurin (PP2B) is responsible for the STAT3 tyrosine dephosphorylation in angiotensin II-induced vascular smooth muscle cells. In contrast, protein phosphatase 2A (PP2A) is responsible for the STAT3 serine dephosphorylation in response to angiotensin II.⁶⁵

LMW-PTP

LMW-PTP can associate and dephosphorylate constitutively active STAT5 in DAM1 megakaryocytic cells. Their interaction requires the C-terminal domain of STAT5.⁶⁶

SUPPRESSOR OF CYTOKINE SIGNALING

SOCS family proteins are inducible inhibitors of cytokine signaling, which are represented by several structurally related proteins. SOCS proteins were discovered by 3 groups using distinct approaches to search for proteins capable of inhibiting cytokine responses,⁶⁷ interacting with Jaks,⁶⁸ and containing homology to STAT SH2 domains.⁶⁹ Thus SOCSs are also known as Jak2-binding protein, STAT-induced STAT inhibitor, and cytokine inducible SH2-containing protein (CIS). The first member of this family, CIS, was discovered as an immediate early gene induced by multiple cytokines.⁷⁰ There are now 8 members of this family, SOCSs 1 through 7 and CIS, and their expression profiles vary.⁷¹

SOCS proteins form a negative feedback loop whereby SOCS genes are induced after cytokine stimulation and inhibit cytokine signaling.⁷²⁻⁷⁴ An important feature of SOCS-mediated regulation is that SOCSs are induced by a large number of cytokines, and then they can inhibit multiple pathways, resulting in considerable crosstalk.^{71,72} This leads to an intersecting web of inhibition in which an SOCS is induced by a cytokine and results in feedback inhibition of the inducing cytokine and crosstalk inhibition of other cytokines. For example, SOCS-1 has been shown to be critical for the crosstalk between IL-4 and IFN- γ *in vivo*.⁷⁵

It is important to understand how SOCS expression is induced, what the key structural elements are in SOCS proteins, and what mechanisms of Jak-STAT inhibition SOCS proteins demonstrate to understand the role of SOCS proteins in the inhibition of Jak-STAT.

Induction of SOCS expression

Expression of SOCS proteins is upregulated in response to many types of cellular activation. Diverse signaling pathways, including pathogen-associated molecular patterns (eg, CpG and LPS), T_H1 and T_H2 cytokines (IL-4, IL-13, and IFN- γ), and other ILs and growth factors, induce the expression of SOCS molecules.^{71,76,77} Thus

SOCS-dependent regulation enables environmental factors, pathogens, and other factors important in innate immunity, growth, and inflammation to modulate the Jak-STAT pathway.

Structure of SOCS proteins

All SOCS proteins are characterized by a central SH2 domain flanked by an N-terminal domain of variable length and a C-terminal SOCS box. The SH2 domain is required for association with other proteins, such as the phosphorylated tyrosine residue in the activation loop of Jak2 in the case of SOCS1, and is essential for the inhibitory functions of SOCS in those that have been studied.^{68,78} Mice expressing a mutant SOCS1 that lacks the SOCS box have a similar phenotype to SOCS1-deficient mice with premature death, inflammatory infiltrates, and increased IFN- γ responses, indicating that the SOCS box is also crucial to the inhibitory functions of SOCS.⁷⁹ The SOCS box is not unique to the SOCS family of proteins and is present in other proteins that lack the SH2 domain of SOCS.⁸⁰

Mechanisms of Jak-STAT inhibition

Several distinct mechanisms have been described by which SOCS proteins can inhibit cytokine signaling. The initial description of SOCS1 was as a Jak-binding protein.⁶⁸ SOCS1 binds directly to tyrosine phosphorylated Jak2 and inhibits Jak activity.⁸¹ SOCS3 also inhibits Jak activity, but in contrast to SOCS1, inhibition of Jak signaling by SOCS3 requires binding of SOCS3 to the activated receptor.^{78,82} Both SOCS1 and SOCS3 have contained a kinase inhibitor region at the N-terminus that is essential for Jak inhibition.^{83,84} Unlike SOCS1 and SOCS3, CIS inhibits STAT activation by competing with STATs for binding to receptor docking sites. CIS is able to prevent STAT5 from associating with cytokine receptors, blocking STAT5 activation.^{70,85}

The conserved SOCS box has been demonstrated in *in vitro* models to bind to elongins B and C, which are part of the E3 ubiquitin ligase complex that ubiquitinates proteins and targets them for proteasomal degradation.^{86,87} This raises the possibility that SOCS proteins might inhibit signaling by serving as adaptor proteins for the E3 ubiquitin ligase complex, which could then mediate ubiquitination of SOCS-associated proteins. Indeed, inhibitors of proteasomal degradation have been shown to result in prolonged activation of Jaks and STATs.^{88,89} Furthermore, SOCS1 and SOCS3 have been implicated in the targeting of insulin receptor substrate 1 and insulin receptor substrate 2 for ubiquitin-mediated degradation,⁹⁰ and SOCS1 has been implicated in the ubiquitin-dependent degradation of Jak2.⁹¹

An interesting feature of SOCS proteins is that they do not affect the activation of the Jak-STAT pathway but rather the deactivation. As a result, prolonged STAT activation induced by cytokines is observed in the absence of SOCS proteins. There is some evidence that prolonged STAT activation might lead to altered responses.^{92,93}

CIS

CIS was the first of the SOCS family proteins described.⁷⁰ It is induced by many factors, including growth hormone (GH), leptin, erythropoietin, prolactin, and several cytokines, and CIS inhibits signaling of erythropoietin, IL-2, IL-3, GH, and prolactin.^{70,94-96} CIS has redundant roles, as evidenced in the gene-targeted mice, which lack any phenotypic changes.⁹⁷ However, transgenic mice overexpressing CIS demonstrate a phenotype similar to that seen in STAT5a^{-/-} and STAT5b^{-/-} mice, supporting the importance of CIS in the regulation of STAT5.⁹⁸

SOCS1

SOCS1 gene-targeted mice are healthy at birth but die before weaning because of fatty degeneration of the liver, monocytic infiltration of the organs, and severe loss of T and B lymphocytes.^{23,24} Notably, the phenotype observed in the SOCS1^{-/-} mice is IFN- γ dependent and can be corrected by crossing mice with IFN- γ ^{-/-} mice or by administering anti-IFN- γ antibodies.^{99,100} The phenotype can also be rescued by crossing the mice with RAG2-deficient mice.¹⁰⁰

SOCS2

The SOCS2 knockout mice revealed a critical role for SOCS2 in GH and insulin-like growth factor signaling. SOCS2-deficient mice are significantly larger than their wild-type counterparts because of loss of SOCS2-dependent attenuation of GH signaling.^{25,26} Interestingly, mice overexpressing SOCS2 are also significantly larger than wild-type mice,²⁷ suggesting that SOCS2 might have a dual role.

SOCS3

Mice deficient in SOCS3 exhibit embryonic lethality, with erythrocytosis and placental defects.²⁸⁻³⁰ Conditional SOCS3 gene targeting studies have demonstrated an important role for SOCS3 in the negative regulation of IL-6.^{101,102}

The roles of the other SOCS family members, including SOCS4, SOCS5, SOCS6, and SOCS7, have not been as well defined.

PIAS PROTEINS

The PIAS family proteins are a group of 5 STAT-interacting proteins, PIAS1, PIAS3, PIASy, PIASx α , and PIASx β (PIASx α and PIASx β are identical except for their COOH-terminus).¹⁰³⁻¹⁰⁶ The first member of this family, PIAS1, was discovered to interact with STAT1 in a yeast 2-hybrid system screening for STAT-interacting proteins.¹⁰⁶ Other family members were identified on the basis of sequence homology to PIAS1. The PIAS proteins are constitutively expressed, and little is known about their regulation.

The PIAS proteins are evolutionarily conserved from yeast to humans. With the exception of a variable C-

terminal domain, the family members are highly homologous (50%-60% at the amino acid level). Key structural elements of PIAS members include an LXXLL nuclear receptor box,¹⁰⁷ a zinc-binding RING-like domain,¹⁰⁸ and, in PIAS1, PIAS3, and PIASx, a serine-threonine-rich region. Interestingly, PIAS structures do not include known phosphotyrosine-binding domains, despite the activation-dependent nature of its interaction with STAT.

PIAS1 and PIAS3 proteins interact directly with STAT1 and STAT3, respectively and inhibit their association with DNA.^{104,106} Coprecipitation studies have demonstrated that PIAS1 is associated with STAT1 and that this association is dependent on the cytokine-dependent tyrosine phosphorylation and dimerization of STAT1.¹⁰⁶ PIAS1 interacts with STAT1 dimers and blocks STAT1 DNA-binding and STAT1-dependent gene induction.¹⁰⁶ These PIAS1-STAT1 interactions are inhibited by the arginine methylation (discussed further below) of STAT1³³ and are dependent on the C-terminus region of PIAS1, which has critical interactions with the N-terminal region of STAT1. Elimination of this C-terminal domain in PIAS blocks the inhibitory effect of PIAS on STAT1 activity.¹⁰⁹ Similarly, PIAS3 binds to STAT3 homodimers and STAT1-STAT3 heterodimers, but not STAT1 homodimers, and blocks DNA-binding activity.¹⁰⁴

In contrast, neither PIASx nor PIASy prevent their target STATs from binding DNA and mediate their effects through distinct mechanisms. PIASx was recently shown to bind to activated STAT4 complexes after IL-12 stimulation and inhibit STAT4-dependent gene induction.¹¹⁰ However, PIASx did not block DNA-binding activity. Interestingly, pharmacologic inhibition of histone deacetylase abolished PIASx-mediated inhibition of STAT4-dependent gene induction.¹¹⁰

Over the past few years, a novel mechanism by which PIAS proteins might regulate STAT activity has become evident. Siz1p was identified in a yeast 2-hybrid screen¹¹¹ as a protein that interacted with the small ubiquitin-like protein modifier (SUMO) 1 and was found to be necessary for SUMOylation of target proteins analogous to the role of E3 ubiquitin ligase.¹¹² The RING domain of Siz1p is similar to the conserved RING-like domain of PIAS proteins, indicating that PIAS proteins might function as E3-like SUMO ligases in mammals. Indeed, E3 SUMO ligase activity has been demonstrated for all PIAS family members.¹¹³ PIAS1 has also been shown to target SUMO to many proteins, including the androgen receptor p53 and c-Jun.^{114,115} SUMOylation is a reversible posttranslational modification involving formation of an isopeptide bond between the C-terminus of SUMO with an ϵ -amino of a lysine residue in the target protein, which is usually embedded in a consensus sequence large hydrophobic residue-lysine-x-glutamine. SUMOylation has been implicated in the regulation of protein-protein interactions, protein stability, protein localization, and protein activity.^{116,117} Recently, it was shown that STAT1 can be SUMOylated on Lys703 and that this is promoted by PIAS1, PIAS3, and PIASx.^{118,119} The role of

SUMOylation in the regulation of Jak-STAT is still not well understood. In addition, the recent association of STAT6 with a detergent-soluble inhibitor of phosphorylated STAT demonstrates that there might be similar molecules interacting with other STATs that have yet to be fully defined.¹²⁰

ARGININE METHYLATION

STAT1 function is regulated by arginine methylation.³³ An arginine residue at the N-terminus of STAT1, Arg31, is conserved among STAT proteins. Protein arginine methyltransferase 1 associates with STAT1 and can methylate STAT1 *in vitro*. Mutation of Arg31 alters STAT1-mediated transcriptional activity in response to IFN- α . The mechanism for this involves the interaction of PIAS with STAT1. PIAS1 binds to STAT1 dimers and prevents STAT1 DNA binding. Arginine methylation of STAT1 inhibits the association of STAT1 with PIAS to increase STAT1 DNA-binding activity.³³ Recently, it was found that dephosphorylation of STAT1 by TC45 is regulated by arginine methylation and that this regulation involves PIAS1.¹²¹ Arg31 is a critical residue for the interaction of STAT1 with both PIAS1 and TC45. Inhibition of STAT1 arginine methylation results in prolonged tyrosine phosphorylation of STAT1, which is mediated by increased binding of PIAS1 to STAT1 and concomitant decreased association of STAT1 with TC45.¹²¹

Arginine methylation has also recently been shown to regulate STAT6 function.³⁴ STAT6 is also methylated at the conserved arginine residue at the N-terminus. Inhibition of methylation or mutation of the arginine to alanine results in decreased tyrosine phosphorylation and diminished DNA-binding activity in response to IL-4 stimulation. This is different from the observation with STAT1 that inhibition of methylation results in inhibited DNA-binding activity but not tyrosine phosphorylation on IFN- γ stimulation. Further studies indicated that the reduced tyrosine phosphorylation of STAT6 Arg27Ala mutant results from the impaired phosphorylation step rather than the enhanced dephosphorylation step.³⁴

SERINE PHOSPHORYLATION OF STATs

In addition to tyrosine phosphorylation, which is essential for the activation of STATs, serine phosphorylation has been found to regulate the transcriptional activity of STATs.¹²² Serine phosphorylation was first reported for STAT1 and STAT3 and mapped to Ser727.¹²³⁻¹²⁶ STAT1 and STAT3 serine phosphorylation is required for maximal transcriptional activity.¹²⁴ Serine phosphorylation of STAT1 is independent of the tyrosine phosphorylation, although serine kinases might recognize tyrosine phosphorylated STAT1 preferentially in response to IFN- γ .¹²⁷ Serine phosphorylation of STAT1 and STAT3 can be induced by the combination of IL-12 and

IL-2.¹²⁸ Serine phosphorylation of STAT C-termini might contribute to the specificity of signaling.¹²⁹

Studies with gene-targeted mice expressing mutant S727A STAT1 indicate that serine phosphorylation of STAT1 promotes inflammatory responses. S727A STAT1 mice display reduced survival after bacterial infection because of an impaired ability to clear the bacteria from the spleen and liver.¹³⁰ Furthermore, expression of IFN- γ -induced genes is significantly reduced in macrophages expressing the S727A STAT1. Thus STAT1 serine phosphorylation results in increased STAT1 activity, contributing to macrophage activation and IFN- γ -mediated immune responses.¹³⁰ Studies with mice lacking STAT3 S727 phosphorylation show lethality and decreased growth.¹³¹

Both IL-12 and IFN- α induce serine phosphorylation of STAT4 at Ser721. STAT4 serine phosphorylation is important for IL-12-induced IFN- γ production during normal T_H1 differentiation.^{132,133} STAT4 serine phosphorylation is not required for the DNA binding but might be necessary for the STAT4-mediated transcription.¹³⁴

STAT5a and STAT5b are also serine phosphorylated. Human STAT5a is phosphorylated at Ser726 and Ser780 (Ser725 and Ser779 for murine STAT5a), whereas the human STAT5b is phosphorylated at Ser731 (Ser730 for murine STAT5b).¹³⁵⁻¹³⁸ Ser725 of murine STAT5a is constitutively phosphorylated, whereas the phosphorylation of Ser730 of murine STAT5b is induced by prolactin. Serine phosphorylation of STAT5a and STAT5b is not required for DNA-binding and transcriptional activation.¹³⁵ Murine STAT5a is also constitutively phosphorylated at Ser779, but the S725A mutant of murine STAT5a has sustained prolactin-induced DNA-binding activity and tyrosine phosphorylation.¹³⁶ IL-2 induces serine phosphorylation of both STAT5a and STAT5b. Serine phosphorylation of human STAT5a at Ser780 does not enhance IL-2 signaling.¹³⁸ In GH-stimulated cells murine STAT5b is phosphorylated at Ser730, and the S730A mutant of murine STAT5b has decreased transcriptional activity.¹³⁷

STAT6 is phosphorylated at low levels on serine residues in nonstimulated cells, and IL-4 stimulation results in increased serine phosphorylation of STAT6, which involves an H7 (serine-threonine kinase inhibitor)-insensitive kinase.¹³⁹ This serine phosphorylation occurs independently of tyrosine phosphorylation. STAT6 lacks the consensus extracellular signal-regulated kinase (ERK) phosphorylation site (PXSP/PSP) at the C-terminus. The majority of serine phosphorylation of STAT6 is located at the C-terminus between amino acids 719 and 789, a region containing 4 serine residues conserved in human subjects and mice.¹⁴⁰ A recent study showed that STAT6 is phosphorylated at Ser756 in the transactivation domain, which is independent of tyrosine phosphorylation.¹⁴¹ Both IL-14 and IL-13 induce STAT6 serine phosphorylation in PHA-activated human T cells. The phosphorylation of Ser756 is not essential for the STAT6 nuclear translocation or DNA-binding activity in non-T cells, such as STAT6-transfected HEK293 and COS-7 cells. In addi-

tion, the serine phosphorylation alone is not sufficient for the STAT6 DNA-binding activity in the absence of tyrosine phosphorylation.

Several kinases were identified to phosphorylate STATs at serine residues.^{132,142-146} Serine phosphorylation of STAT1 requires activated Jak2.¹⁴² STAT3 is phosphorylated at Ser727 by protein kinase C δ in response to IL-6.¹⁴⁵ Serine and tyrosine phosphorylation of STAT3 are independently regulated, and serine phosphorylation involves ERKs.¹⁴³ ERKs, but not p38 or c-jun N-terminal kinase, are involved in STAT3 serine phosphorylation in epidermal growth factor-stimulated cells, whereas IL-6 induces ERK-independent serine phosphorylation of STAT3. Serine phosphorylation of STAT3 negatively regulates its tyrosine phosphorylation.¹⁴⁴ Mitogen-activated protein kinase kinase/p38 is involved in STAT4 serine phosphorylation.¹³²

In contrast to the serine phosphorylation, very little is known about serine dephosphorylation. PP2A, SHP-2, or both has been implicated in the serine dephosphorylation of STAT1, STAT3, and STAT6.^{49,123,147,148} PP2A treatment significantly reduces the formation of STAT1 dimers associated with the IFN- γ activation site.¹²³ Inhibition of PP2A increases serine phosphorylation of STAT3 and STAT6.^{147,148} Inhibition of PP2A also increases serine phosphorylation of STAT6 and significantly inhibits the DNA-binding activity of STAT6, whereas the inhibition of PP1 and PP2B has no such effect. Because inhibition of PP2A does not affect IL-4-induced tyrosine phosphorylation of Jak1 and STAT6, PP2A might regulate IL-4 signaling downstream of Jaks.¹⁴⁸ In SHP-2^{-/-} mouse fibroblast cells, both tyrosine and serine phosphorylation of STAT1 in response to IFN- γ is enhanced and sustained.⁴⁹

NUCLEAR LOCALIZATION OF STATs

The nuclear import requirements for the STAT proteins remain to be identified. Nuclear localization occurs both at the level of nuclear import and nuclear export.¹⁴⁹ STATs are translocated into the nucleus in response to cytokine stimulation to activate gene expression and subsequently exported back to the cytoplasm. Thus nuclear import and export are important potential regulatory points. The nucleus is the site for inactivation of STAT1, and the dephosphorylation of STAT1 is required for the nuclear export.¹⁵⁰ The dephosphorylation of STAT1 is required for the nuclear export, and DNA-binding protects STAT1 from dephosphorylation in a sequence-specific manner.¹⁵¹ Maintenance of STAT6 activity requires ongoing Jak kinase activity and a continuous cycle of activation, deactivation, nuclear export, and reactivation.¹⁵² STAT5 also undergoes nucleocytoplasmic cycles on erythropoietin stimulation.¹⁵³ The nuclear export is mediated by the leucine-rich nuclear export signals (NESs) that consist of short sequences of hydrophobic amino acids with the consensus of LX(1-3)LX(2-3)LXL.^{154,155} The NES interacts with chromosome region maintenance-exportin

1,^{156,157} and the interaction can be disrupted by the antibiotic leptomycin B (LMB).^{158,159}

Several NES elements of STAT1 have been reported.¹⁶⁰⁻¹⁶³ STAT1 contains a leucine-rich NES sequence (amino acids 197-205), and Jak1 is required for the STAT1 nuclear export mediated by this NES. STAT1 is exclusively located in the cytoplasm in Jak1-deficient cells, even in the Y710 and S727 phosphorylated forms.¹⁶⁰ A conserved leucine-rich NES (amino acids 302-314) in the coiled-coil domain of STAT1 is required for STAT1 nuclear export after IFN- γ treatment.¹⁶² A functional NES of STAT1 is located in the DNA-binding domain of STAT1 (amino acids 392-413). Dephosphorylation of STAT1 and dissociation from DNA results in the recognition of NES by chromosome region maintenance-exportin 1 and nuclear export of STAT1.¹⁶¹ A leucine-rich NES (amino acids 400-410) in the STAT1 DNA-binding domain contains LMB-sensitive nuclear export activity. This NES is not important for the nuclear export of unphosphorylated STAT1, but it is involved in the nuclear export of tyrosine-phosphorylated STAT1.¹⁶³

Three NES elements were identified in STAT3 (amino acids 306-318, 404-414, and 524-535).¹⁶⁴ The first 2 NES elements are corresponding to the NES in STAT1, whereas the third is novel in STAT3. LMB treatment blocks the nuclear export of STAT3 in both stimulated and unstimulated cells, indicating the existence of basal nuclear export in the absence of tyrosine phosphorylation. NES 306-318 is involved in nuclear export in stimulated cells, whereas NES 404-414 and 524-535 are involved in basal nuclear export. NES 201-210, which has been shown in nuclear export activity for STAT1, does not have any nuclear export activity for STAT3.¹⁶⁴

Constitutive nucleocytoplasmic shuttling is present for STAT3 in unstimulated cells, and it is independent of tyrosine phosphorylation.¹⁶⁵ Nuclear export of STAT3 is decreased in response to IL-6, which contributes to the nuclear accumulation of STAT3. The shuttling of STAT3 is balanced by the nuclear export and import signals. The C-terminal region of STAT3 (amino acids 321-771) contains the nuclear export activity, whereas the N-terminal part (1-320) contains the nuclear localization signal.¹⁶⁵ Both constitutive and IFN- γ -induced nucleocytoplasmic shuttling of STAT1 also exists.¹⁶³ In addition, both LMB-sensitive and LMB-insensitive nuclear export exists for STAT1 and STAT4.^{162,166}

STAT SIGNALING IN HUMAN ALLERGIC DISEASE

The Jak-STAT pathways are critical to many homeostatic processes, including growth, hematopoiesis, and innate and acquired immune responses. Deficiencies and dysregulation of the Jak-Stat pathway have been implicated in a number of disease states, although complete defects in the Jak-STAT pathway are uncommon in human subjects and are associated with severe immune dysregulation. It has been demonstrated that components

of the Jak-STAT pathway have significant contributions in asthma.¹⁶⁷ Markedly increased levels of STAT6, which is crucial for IL-4 and IL-13 signaling and T_H2 cell differentiation, were detected in the airways of patients with asthma.^{168,169} Furthermore, the density of STAT6-positive cells was found to be higher in atopic than nonatopic asthmatic individuals. In a recent study of individuals with severe asthma, STAT6 levels were increased in the airways and specifically in bronchial epithelial cells.¹⁶⁹ In addition to asthma, alterations in STAT signaling can lead to other atopic diseases.

Recently, a role for SOCS3 in regulating the onset and maintenance of allergic disease was reported. A strong correlation was found between the expression of SOCS3 and the pathology of T_H2-mediated allergic immune diseases, including atopic dermatitis and asthma.¹⁷⁰ This is further supported by the finding that SOCS3 transgenic mice display increased airway hypersensitivity and T_H2 responses.

Genetic studies have also provided a link between STAT6 and allergic disorders. Linkage between polymorphisms of the first exon of STAT6 and atopic diseases (asthma, food allergy, and atopic dermatitis) has been described in a Japanese population.^{171,172} These associations of alterations in Jak-STAT elements with human atopic diseases support the central nature of these pathways for homeostasis.

Human immune deficiency

Dysregulation of the Jak-STAT signaling pathway has been demonstrated to cause human immunodeficiency.¹⁷³ Severe combined immunodeficiency (SCID) with complete absence of T-cell immunity can be caused by defects in the common gamma chain (γ c),¹⁷³⁻¹⁷⁶ its associated Jak3,^{177,178} or the IL-7 receptor α chain.¹⁷⁹ The γ c is involved in Jak-STAT signaling for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. Defects in γ c lead to loss of Jak-STAT signaling dependent on these cytokines and results in SCID with a T-B⁺NK⁻ phenotype. Mutations in Jak3 lead to a similar phenotype. Common γ chain deficiency is inherited as an X-linked trait, whereas Jak3 deficiency is autosomal recessive. Finally, IL-7 receptor defects are also associated with diminished T-cell development and SCID. Defective IL-7 signaling is associated with a T-B⁺NK⁺ phenotype. The phenotype of IL-7 receptor SCID is altered from γ c and Jak3 deficiency because of intact signaling through other γ c/Jak3-dependent receptors.

Defects in STAT1 have been shown to be associated with increased susceptibility to mycobacterial infections, decreased IFN responses, and increased susceptibility to viral illnesses.^{180,181}

Jak-STAT PATHWAYS AS TARGETS FOR THERAPEUTIC INTERVENTION

The Jak-STAT pathway is critical for a variety of functions in hematopoietic and nonhematopoietic cells and has been shown in animal and clinical studies to be

implicated in the development of atopic disorders, including atopic dermatitis, allergic rhinitis, and asthma. Given the considerable redundancy of Jaks and STATs and their central importance in diverse cell types and biologic processes, it is likely more relevant to modulate Jak-STAT function than to attempt to block it as therapeutic interventions are developed. Several regulatory steps, including SOCS proteins, PIAS proteins, PTPs, posttranslational modifications, nuclear localization, and degradation have been identified and are potential sites of intervention. To effectively target these molecules and the requisite enzymes and associated proteins, it is critical to first understand their regulation.

CONCLUSIONS

The multiple levels of regulation along the Jak-STAT activation cascade underscore the biologic importance of this pathway. Herein we have reviewed regulatory steps that act at different points along the cascade (including at the level of Jak activity), recruitment of STATs to the receptor docking sites, tyrosine phosphorylation, serine phosphorylation, methylation, SUMOylation, dimerization, nuclear localization, DNA binding, degradation, dephosphorylation, and nuclear export. An overview of these complex regulatory networks is shown in Fig 3. Although major advances have been made in our understanding in this area, many gaps remain, and much work is still needed.

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