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 and impact various cell types critical to allergy including epithelial cells, mast cells, lymphocytes, dendritic cells, and eosinophils. Dysregulation of STAT signaling has been implicated in allergic disease, highlighting the importance of these ubiquitous molecules in allergic inflammation and the potential of these pathways as a target for therapeutic intervention. This review will summarize the current understanding of the roles of STAT signaling in allergic disease and the potential of targeting STATs for the treatment of allergic disorders, emphasizing recent observations. (J Allergy Clin Immunol 2007;119:529-41.)

Key words: Allergy, STAT, cytokine, JAK-STAT, 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 over 15 years ago 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 interferon (IFN) receptor and gene transcription in the first report of a STAT protein resulted in the discovery of the Janus kinase (JAK)-STAT pathway. Many interleukins and members of the hematopoietin family utilize this pathway to transduce their signals. These distinct factors transmit their signals via 4 JAK and 7 STAT molecules. JAKs constitutively associated with the receptors via conserved box-1 motifs are brought into proximity following 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 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, and initiate cytokine-specific gene transcription. The preferred binding sites for STAT transcription factors consist of the palindromic motif TTC(Xn)GAA, where the number of nucleotides separating the half-sites can be from 2 to 4 nucleotides. The JAK/STAT pathway is central to many fundamental biologic processes and is tightly regulated by several mechanisms along the signaling cascade, including suppressor of cytokine signaling and protein inhibitor of activated STAT, as summarized in Fig 1. JAK/STAT dysregulation has been implicated in many disease processes, including allergic inflammation. This review will summarize the role of STATs in allergic inflammation.
OVERVIEW OF STAT PROTEINS

Structure

The STAT protein family has 7 members: STAT1 through STAT6, including STAT5A and STAT5B. Recently, a STAT6 homologue (STAT6B), which may be generated by alternative splicing, was identified. STATs are activated in response to distinct stimuli and induce the transcription of genes that can elicit diverse biological outcomes. STAT homologs have been identified in simple eukaryotes, suggesting that they arose from a single gene. All STAT proteins share the same overall structure (Fig 2), including an N-terminal domain, a coiled-coil domain, a DNA-binding domain, a linker domain, an SRC homology 2 (SH2) domain, a conserved single tyrosine residue that is phosphorylated following activation, and a carboxy terminal that facilitates transcriptional activation. STATs are activated by tyrosine phosphorylation of the conserved tyrosine residue in the transactivation domain, and this modification serves as a molecular switch that alters their conformation, enabling their dimer formation through reciprocal tyrosine phosphorylation and SH2-domain interactions and specific binding to DNA. Although some STAT molecules form dimers in the cytoplasm, dimerization is essential for nuclear translocation and transcriptional activation. The assembly of STAT dimers is regulated by various mechanisms, including tyrosine phosphorylation, SH2-domain interactions, and DNA binding. The dephosphorylation and proteolytic degradation of STAT dimers are also important for the regulation of their activity. STAT proteins are involved in a wide range of biological processes, including immune responses, development, and cancer. Their function is tightly regulated at multiple levels, ensuring that they respond appropriately to various stimuli.
homodimers or homotetramers with each other in an unphosphorylated state, it is the specific conformation of tyrosine-phosphorylated dimers that enables STATs to bind to consensus sequences in target genes.5-9 The diverse functions of STATs have been elucidated by studies in mouse gene targeting models,10-21 human deficiency,22,23 and associated human diseases for STAT1,24-31 STAT2,31,32 STAT3,25,30,33,34 STAT4,29,35-37 STAT5,27,30,38-40 and STAT629,41-44 (Table I).

**STAT Phosphorylation**

Tyrosine phosphorylation of STATs is initiated predominantly by cytokine binding to cell-surface cytokine receptors. The intracellular domains of many cytokine receptors are constitutively associated with JAK tyrosine kinases via conserved box-1 motifs. There are 4 mammalian JAK kinases (JAK1, JAK2, JAK3, and Tyk2), which each consist of 7 conserved JAK homology (JH) domains. JAKs have both a pseudokinase domain (JH1) and a true catalytic kinase (JH2), leading to their being named after the mythologic Roman god Janus who had 2 faces. The other domains, JH3-JH7, mediate association with receptors.45 The JAK-STAT pathway is critical for the response to cytokines critical for allergic inflammation, including IL-4 and IL-13.

**TABLE I. Functions of STAT proteins**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phenotype of knockout mice</th>
<th>Human deficiency</th>
<th>Dysregulation in human diseases</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>STAT1</td>
<td>Impaired IFN signaling</td>
<td>Mycobacterial and viral diseases</td>
<td>IBD, rheumatoid arthritis, celiac disease, Alzheimer disease, multiple sclerosis, ischemic heart disease, cancers</td>
<td>10, 11, 22, 24-31</td>
</tr>
<tr>
<td>STAT2</td>
<td>Impaired IFN signaling</td>
<td></td>
<td>BBD, carcinoid tumor</td>
<td>12, 31, 32</td>
</tr>
<tr>
<td>STAT3</td>
<td>Embryonic lethal, impaired IL-2, IL-6, and IL-10 signaling</td>
<td>BBD, psoriasis, multiple sclerosis, cancers</td>
<td>13, 25, 30, 33, 34</td>
<td></td>
</tr>
<tr>
<td>STAT4</td>
<td>Impaired IL-12 signaling</td>
<td></td>
<td>COPD, psoriasis, Zezary syndrome</td>
<td>14, 29, 35-37</td>
</tr>
<tr>
<td>STAT5A</td>
<td>Impaired PRL signaling</td>
<td></td>
<td>BBD, cancers, diabetes, ischemic heart disease</td>
<td>15-18, 23, 27, 30, 38-40</td>
</tr>
<tr>
<td>STAT5B</td>
<td>Impaired GH signaling</td>
<td>GH insensitivity with immunodeficiency</td>
<td>Asthma, atopic allergic rhinitis, ischemic heart disease, Hodgkin lymphoma</td>
<td>19-21, 29, 41-44</td>
</tr>
<tr>
<td>STAT6</td>
<td>Impaired IL-4 and IL-13 signaling</td>
<td></td>
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</tbody>
</table>

*IBD*, Inflammatory bowel disease; *COPD*, chronic obstructive pulmonary disease; *PRL*, prolactin; *GH*, growth hormone.
nucleus.46 The G-protein coupled receptor-mediated STAT3 activation requires protein kinase A (PKA), c-Jun N-terminal kinase (JNK), and PI3 kinase (PI3K).47 Angiotensin II and some chemokines, such as CC-chemokine ligand 5 (CCL5; also known as RANTES) signal via G-protein coupled receptors and activate STATs in a Jak-dependent manner.48-51 STAT3 and STAT5 can also be activated by non-receptor oncogenic tyrosine kinases that are of viral origin or are generated by chromosomal translocation, such as v-Src and breakpoint cluster region-Abelson, respectively.52 The activity of STAT3 and STAT5 is dysregulated in a variety of human tumors. STAT3 and STAT5 acquire oncogenic potential through constitutive tyrosine phosphorylation, and this activity has been shown to be required to sustain a transformed phenotype. Disruption of STAT3 and STAT5 signaling in transformed cells therefore represents an excellent opportunity for targeted cancer therapy. In contrast to STAT3 and STAT5, STAT1 negatively regulates cell proliferation and angiogenesis and thereby inhibits tumor formation. STAT1 and its downstream targets have been shown to be reduced in a variety of human tumors, and this pathway is another potential target for cancer therapy.53

In some STATs, the C-terminal region also contains a serine residue that is phosphorylated, and this phosphorylation has been found to regulate the transcriptional activity of STATs.54 Serine phosphorylation was first reported for STAT1 and STAT3 and mapped to Ser 727.55-58 STAT1 and STAT3 serine phosphorylation is required for maximal transcriptional activity,59 and serine phosphorylation of STAT C-termini may contribute to the specificity of signaling.60 Serine phosphorylation of STAT1 is independent of the tyrosine phosphorylation, although serine kinases may recognize tyrosine-phosphorylated STAT1 preferentially in response to IFN-γ.61

STAT cofactors

Various cytokines and growth factors use overlapping JAKs and STATs to induce their diverse and distinct functions. One mechanism by which transcription factors mediate specificity for the promoters they activate is via interaction with specific cofactors. Cofactors of DNA-binding transcriptional activators are essential for achieving the threshold level of gene activation required to overcome baseline repressive effects of nucleosomes and other heterochromatin constituents. The coregulatory mechanisms by which STATs assemble transcriptional machinery and selectively regulate specific gene expression are not clear, but coactivators have been shown to be important for STAT activation. The activation of STAT1 requires a DNA replication factor, eukaryotic minichromosome maintenance 5 (MCM5).62 The activation of STAT3 involves CREB-binding protein (CBP)/p300 through acetylation. STAT3 is acetylated at a lysine residue in the C-terminal transactivation domain by CBP/p300 and activated for its DNA-binding ability and transcriptional activity.63,64 A transcriptional cofactor for STAT6, collaborator of STAT6 (CoaSt6), was recently described.65 CoaSt6 interacts with STAT6 in vivo and amplifies IL-4-induced STAT6-dependent gene expression. Aside from the STATs that are activated via the JAK-STAT pathway, additional transcription factors and cofactors that are activated lead to activation of specific genes via direct and indirect interactions with STATs.66

STAT nuclear import and export

Nuclear translocation is crucial for the function of STATs. The movement of large molecules between the cytoplasm and nucleus is restricted and is a potential target for transcriptional regulation by controlling the access of transcription factors to the nucleus. Because STAT activation is critical to many pathways involved in the development of allergy and allergic inflammation, regulation of STAT nuclear trafficking is a potential target for therapeutic intervention.

Nuclear localization occurs both at the level of nuclear import and nuclear export.67 STATs are translocated into 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. Although the STAT proteins share similar structural features, their respective nuclear-cytoplasmic localization is distinctly regulated. The recognition signals are amino-acid sequences that function either as nuclear-localization signals (NLSs) or nuclear-export signals (NESs). These signals can function constitutively or conditionally depending on protein modifications (that is, phosphorylation) or association with another protein that can alter the conformation of the protein. Thus, association with different proteins can result in different localization patterns. STAT1 can form a complex with tyrosine-phosphorylated STAT2 and a non-STAT factor, IFN-regulatory factor 9 (IRF9).68 This complex, ISGF3, translocates from the cytoplasm to the nucleus and specifically binds to the IFN-stimulated response element (ISRE) in the promoters of responsive genes. STAT2 dimerizes with STAT1 after tyrosine phosphorylation and accumulates in the nucleus only in the presence of STAT1.69,70 STAT2 does not form phosphorylated homodimers. STAT1 is also tyrosine phosphorylated in response to IFN-γ and forms a homodimer that binds to a distinct DNA target known as the IFN-γ-activated site (GAS). Importin-α5 recognizes tyrosine-phosphorylated STAT1 either in the form of IFN-stimulated gene factor 3 (ISGF3) or as a homodimer.70 A single leucine residue at position 407 is required for binding to importin-α5 and transport into the nucleus.70 Interestingly, the binding site on importin-α for the phosphorylated STAT1 dimer is distinct from conventional NLS-containing proteins.52 It has been hypothesized that this would enable a STAT1 dimer to bind to importin-α5 that is already occupied with cargo and ensure that STAT1 dimers are readily moved to the nucleus independent of rate-limiting quantities of importin-α5. STAT2 does not form phosphorylated homodimers; nuclear import depends on heterodimerization with STAT1.65,66,71-73

Maintenance of STAT6 activity requires ongoing JAK kinase activity and a continuous cycle of activation,
Deactivation, nuclear export, and reactivation. Similarly, nuclear accumulation of STAT1 is transient, and within hours, the STAT1 protein recycles back to the cytoplasm. STAT5 also undergoes nucleocytoplasmic cycles upon erythropoietin (EPO) stimulation. The nuclear export is often 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. The NES interacts with chromosome region maintenance/exportin 1 (CRM1), and the interaction can be disrupted by antibiotic leptomycin B (LMB). Both LMB-sensitive and LMB-insensitive nuclear export exists for STAT1 and STAT4. Thus, non–LMB-sensitive nuclear export pathways for STATs exist, but have not been well defined.

Several NES elements of STAT1 have been reported. 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. A functional NES of STAT1 is located in the DNA-binding domain of STAT1 (amino acids 392-413). Interestingly, this region includes the Leu407, which is required for nuclear import of STAT1. Thus, the nuclear import and export sequences are overlapping and may be counter-regulatory based on the conformation of this region.

Dephosphorylation of STAT1 and dissociation from DNA results in the recognition of NES by CRM1 and nuclear export of STAT1. 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. The nucleocytoplasmic shuttling of unphosphorylated STAT1 is controlled by nucleoporins, Nup153 and Nup214, and CRM1-dependent nuclear export.

Three NES elements were identified in STAT3 (amino acids 306-318, 404-414, and 524-535). The first 2 NES correspond to the NES in STAT1, whereas the third is novel in STAT3. The NES 306-318 is involved in nuclear export in stimulated cells, whereas NES 404-414 and 524-535 are involved in basal nuclear export. The NES 201-210, which has been shown possessing nuclear export activity for STAT1, does not have any nuclear export activity for STAT3. Constitutive nucleocytoplasmic shuttling is present for STAT3 in unstimulated cells, and it is independent of tyrosine phosphorylation. The shuttling of STAT3 is balanced by the nuclear export and import signals. Unlike STAT1, the C-terminal region of STAT3 (amino acids 321-771) contains the nuclear export activity, whereas the N-terminal part (1-320) has the nuclear localization signal.

Various importins mediate the nuclear import of STAT3. The arginine residues in the coiled-coiled domain of STAT3 are involved in both nuclear translocation and CRM1-mediated STAT3 nuclear export and activation of STAT3. Importin-α3 mediates STAT3 nuclear import independent of tyrosine phosphorylation.

**STAT trafficking determines cytokine sensitivity and responses**

Clearly, nuclear import and export of STAT are important determinants of the response to cytokine, but recent evidence suggests that modulation of STAT trafficking is one mechanism by which cytokine responses may be modulated and even fine-tuned. Cytokine sensitivity has been found to be determined by the nuclear export of the relevant STAT proteins. The rate of nucleocytoplasmic cycling of STATs is a novel mechanism by which response to cytokine is determined. Cytokine responses are affected when nuclear trafficking of STAT is diminished. IFN-γ production and cell proliferation were both impaired by the impairment of IL-12–dependent STAT4 nuclear translocation.

**Dephosphorylation and proteolytic processing of STATs**

Dephosphorylation of STATs involve tyrosine phosphatases. Several types of tyrosine phosphatases have been identified to dephosphorylate STAT proteins, including SH2 domain-containing tyrosine phosphatase 1 (SHP1), SHP2, protein tyrosine phosphatase 1B (PTP1B), T cell–protein tyrosine phosphatase (TC-PCP), CD45, PTPeC, dual-specificity phosphatases, and low-molecular-weight protein tyrosine phosphatase. Phosphorylated STATs dimerize in a parallel way and are subject to dephosphorylation. However, a recent study revealed during the activation-inactivation cycle of STAT1 that the parallel dimerized STAT1 proteins not bound to DNA were subject to a conformational change of their dimer from a parallel to antiparallel arrangement, which is more efficiently dephosphorylated. This is another potential mechanism by which STAT signals may be regulated.

In addition to dephosphorylation by tyrosine phosphatases, proteolytic processing is another important step in the deactivation and downregulation of STATs. The dephosphorylation and proteolytic processes coordinate to regulate the deactivation of STAT proteins. Ubiquitin/proteasome-mediated protein degradation is the major pathway for the inactivation of STAT5A, but in the cytoplasm, tyrosine dephosphorylation is the dominant mechanism of inactivation of STAT5A. A ubiquitin E3 ligase, STAT-interacting LIM protein (SLIM), was found to regulate proteosome-mediated protein degradation and dephosphorylation of STAT1 and STAT4. Overexpression of SLIM resulted in decreased STAT1 and STAT4 activity, whereas SLIM deficiency led to increased STAT1 and STAT4 activity and IFN-γ production. In addition to downregulating STATs through degradative pathways, proteolysis of STATs can also contribute to the formation of novel STAT isoforms. Isoforms of STAT3, STAT5, and STAT6 generated by proteolysis have been reported. These isoforms are referred as STATγ, whereas the isoforms generated by pre-mRNA alternative splicing are referred as STATβ. STATβ isoforms have been found for STAT1, STAT3, STAT4, and STAT5. The STATβ acts as a
dominant-negative variant of STAT5, whereas the STAT3β and STAT4β are not dominant-negative factors. STAT3β activates specific STAT3 target genes and STAT4β activates specific IL-12–induced gene expression. Some STAT isoforms are involved in disease progression in acute myeloid leukemia. The STAT3γ displayed distinct expression comparing to STAT3α and STAT3β during granulocytic differentiation. The STAT5γ generated by protein processing has distinct function in activation of IL-3 target genes. Proteolytic processing of STAT6 in mast cells generates truncated C-terminal proteins (STAT6γ) that lack the transactivation domain, which can negatively regulate STAT6 function, decreasing IL-4–induced gene expression.

**STAT FUNCTIONS**

**STATs and regulatory T cells**

Regulatory T cells (Tregs) have been characterized as a distinct subset of CD3⁺CD4⁺ T cells constitutively expressing the alpha chain of the high-affinity IL-2 receptor, CD25. Recently, a unique transcription factor, forkhead box P3 (FOXP3), was found to be specifically expressed in murine and human Tregs. Studies have also shown that ectopic expression of FOXP3 in CD4⁺ T cells was sufficient to confer suppressive capabilities to CD4⁺ CD25⁺ T cells. Functionally, CD3⁺ CD4⁺ FOXP3⁺ Tregs act as natural inhibitors of normal immune responses. Defects in Tregs have been associated with a variety of immune pathologies. FOXP3-deficient mice develop intense multiorgan inflammation that is associated with allergic airway inflammation. The resultant hyperimmunoglobulinemia E and eosinophilia observed in these mice can be reversed by concurrent STAT6 deficiency.

Numerous studies have demonstrated that IL-2 is important in the maintenance of Tregs. Neutralizing antibody-mediated IL-2 depletion in mice results in autoimmune diseases associated with reduced numbers of FOXP3-expressing Tregs in the periphery. Disruption of IL-2 signaling in mice by knocking out IL-2, CD25, IL-2Rβ, or STAT5 revealed an essential role for IL-2 in the thymic development and peripheral maintenance of Tregs. Although IL-2 is not required for FOXP3 expression, it has been shown to upregulate FOXP3 expression in Tregs via activation of STAT3 and STAT5. Natural Tregs constitutively express CD25 (IL-2Rα), so they express the complete high-affinity IL-2 receptor complex (IL-2Rα, β, and γc) and can presumably respond to physiologically low concentrations of IL-2 and effectively compete away IL-2 binding from naive T cells to receive IL-2-mediated survival signals.

Activation of STAT5 is a primary mechanism of Treg development stimulated by IL-2. Tregs were reduced in STAT5A/5B deficient mice, indicating that STAT5 is required for the maintenance of tolerance in vivo. STAT5A has been shown to be involved in the development of Tregs that modulate T helper cells differentiation to T helper 2 cells. The role of STAT5 is further highlighted by the recent observation that Tregs are decreased in human STAT5B deficiency.

Cytokine signaling pathways are an integral part of Treg biology. IL-2 and other γc-dependent cytokines are critical for the thymic development and peripheral maintenance of FOXP3-expressing natural Tregs. The signaling pathway downstream of IL-2, including STAT5, is an important potential target for therapeutic intervention directed at Tregs. Other cytokines, including IL-10 and TGF-β, are important in the development of acquired Tregs.

**STATs and inflammation**

Disregulation of STAT pathways can yield allergic inflammation. STAT6 is a key mediator of allergen-induced airway inflammation and plays an essential role in TH2 cell trafficking, chemokine production, mucus production, airway eosinophilia, and airway hyperresponsiveness. STAT6 induces the expression of numerous genes involved in allergic inflammatory responses, including eotaxin-1 and eotaxin-3, arginase I, and P-Selectin. A recent study on adipocyte fatty acid-binding protein aP2 showed that it is required in the allergic airway inflammation and its expression is induced via STAT6-dependent mechanisms.

The role of STAT6 in the development of allergic inflammation is complicated because both STAT6-dependent and STAT6-independent pathways are involved, and STAT6 can positively or negatively regulate gene expression. The STAT6-signaling pathway is mainly induced by IL-4 or IL-13, but other factors can affect STAT6 signaling. IFN-γ inhibits STAT6-dependent signaling and gene expression in human airway epithelial cells. Protein kinase C zeta (PKCζ)⁻/⁻ mice showed impaired tyrosine phosphorylation and nuclear translocation of STAT6 and decreased allergen-induced allergic airway responses. STAT6 is essential for the development of allergic airway inflammation induced by acute allergen exposure but only partially mediates airway inflammation in a chronic model. Gene targeting studies showed that STAT6-deficient mice were protected from the IL-13–induced pulmonary responses, but developed airway hyperresponsiveness and peribronchial fibrosis during chronic fungal asthma. IL-5 has been reported to reconstitute allergen-induced airway inflammation and airway hyperresponsiveness in STAT6-deficient mice supporting alternate pathways.

STAT6 is also involved in other inflammatory or immune-related diseases. STAT6-mediated TGF-β immunity is critical for the development of autoimmunity in Graves hyperthyroidism. STAT6 expression is increased in vascular smooth muscle cells from coronary arteries of ischemic heart disease, suggesting a role of STAT6-mediated inflammation in atherosclerosis. The deficiency of STAT6 and CD28 results in chronic ectoparasite-induced inflammatory skin disease.

The importance of STAT1 in allergic inflammation was demonstrated by the inhibition of allergen-induced airway inflammation and airway hyperresponsiveness by decoy
oligonucleotide specific for STAT1.\textsuperscript{150} STAT3, STAT4, and STAT5 all play important roles in inflammation. STAT3 negatively regulates STAT1-mediated inflammatory gene activation in response to type I IFNs.\textsuperscript{151} STAT3 is constitutively activated in intestinal T cells from patients with Crohn’s disease and growth hormone reduces STAT3 activation in Crohn’s colitis.\textsuperscript{152,153} Conditional knockout of STAT3 in endothelial cells revealed that STAT3 is a critical anti-inflammatory mediator in endothelia.\textsuperscript{154} Cardiomyocyte-restricted knockout of STAT3 results in higher sensitivity to inflammation, increased cardiac fibrosis, and age-related heart failure.\textsuperscript{155}

IL-12 and IL-18 attenuate airway hyperresponsiveness through STAT4 activation.\textsuperscript{156} and STAT4 has been shown to modulate allergen-induced chemokine production and airway hyperresponsiveness.\textsuperscript{157} Mucosal adenovirus mediated IFN-γ gene transfer attenuated allergen-induced airway inflammation and airway hyperresponsiveness in an IL-12- and STAT4-dependent manner.\textsuperscript{158} STAT4 is also involved in the development of airway inflammation in smokers and patients with chronic obstructive pulmonary disease.\textsuperscript{35}

STAT5A is indispensable in STAT6-independent Tp2 cell differentiation and allergic airway inflammation.\textsuperscript{159} STAT5 is critical for IgE-induced mast cell activation as STAT5-deficient mast cells display decreased IgE-mediated degranulation, leukotriene B₄ production, cytokine secretion, and survival signals.\textsuperscript{160} Reduced colonic STAT5 expression may contribute to persistent mucosal inflammation in colitis.\textsuperscript{161}

STATs and apoptosis

STATs have been implicated in the regulation of apoptosis. IFN-γ suppresses TNF-related apoptosis inducing ligand-mediated apoptosis through JAK-STAT pathways.\textsuperscript{162} STAT1α overexpression promotes apoptosis with enhanced release of cytochrome c from the mitochondria and increased caspase-3 activity.\textsuperscript{163} In contrast, inhibition of STAT3 induces apoptosis in prostate cancer cell lines.\textsuperscript{164} Serine phosphorylation of STAT3 is required for the suppression of apoptosis by the Bcl-2 family member Mcl-1.\textsuperscript{165} The function of STAT3 in anti-apoptosis is suppressed by GRIM-19, a death-regulated gene product, which interacts with STAT3 and inhibits STAT3 nuclear translocation.\textsuperscript{166} STAT5 is critical for the development and survival of mast cells as STAT5A/B-deficient cells displayed enhanced apoptosis.\textsuperscript{167} Similarly, the expression of dominant-negative forms of STAT5 in a pre-B cell line resulted in enhanced apoptosis.\textsuperscript{168} IL-4 mediates apoptosis of developing mast cells and monocyte/macrophages through a STAT6-dependent mitochondrial pathway.\textsuperscript{169}

STAT POLYMORPHISMS

Most STAT genetic polymorphism studies have focused on STAT6. Several polymorphisms have been identified in STAT6 genes. Variation in the dinucleotide (GT) repeat sequence in exon 1 of the STAT6 gene has been associated with atopic asthma and increased total serum IgE level in a British population.\textsuperscript{170} Studies done in vitro examining the functional significance of this variation showed that the GT repeat modulates promoter activity by altering the binding stability of nuclear factors.\textsuperscript{170} In a Caucasian sib-pair study, the GT repeat in exon 1 of STAT6 was associated with eosinophilia and total serum IgE levels, but not with asthma.\textsuperscript{171} In a Japanese population, the GT repeat polymorphism in exon 1 in combination with homozygosity for the 2964G allele was significantly overrepresented in subjects with allergy.\textsuperscript{172} However, in a study of Chinese patients with atopic dermatitis, no association of the STAT6 GT repeat polymorphism was found.\textsuperscript{173} Specific haplotypes of a polymorphic CA repeat in the proximal promoter region and in the 5’ untranslated region of STAT6 were found to be associated with asthma in an Indian population.\textsuperscript{174} A separate study on 6 polymorphisms of the STAT6 gene in a German population revealed significant associations of specific haplotypes and single nucleotide polymorphisms (SNPs) with elevated serum IgE, specific sensitization, and/or asthma risk.\textsuperscript{175} In Finnish families with asthma, polymorphisms of STAT6 or STAT4 were not found to be associated with asthma or elevated serum IgE levels.\textsuperscript{176} In a British patient population, a STAT6 3’ untranslated region polymorphism was associated with the risk and severity of nut allergy.\textsuperscript{177} Cumulatively, there is strong evidence for genetic associations of STAT5 with atopic disease. The mechanisms by which these STAT polymorphisms confer risk for different atopy phenotypes is not yet clear, although several have been found to have functional significance.\textsuperscript{184} Of poly morphisms are also associated with other human diseases. One study showed that the STAT6 G2964A polymorphism is associated with Crohn’s disease in German patients, but other studies did not find this relationship in Dutch or Chinese populations.\textsuperscript{178-181}

In addition to STAT6, polymorphisms of STAT3 and STAT4 have also been studied in atopic disorders. Analysis of STAT3 polymorphisms revealed association of STAT3 SNPs with lung function (FEV₁) in adults and children with asthma.\textsuperscript{182} Among 12 SNPs of STAT4 analyzed, 1 SNP in intron 11 and 2 haplotypes were found to be associated with Dermatophagoides farinae (Der p)- or Dermatophagoides pteronyssinus (Der f)-specific IgE, suggesting a role for these STAT4 polymorphisms in the production of IgE in response to mite allergens in asthma.\textsuperscript{183} No association of polymorphisms of STAT4 with asthma or elevated serum IgE level was identified in Finnish families with asthma.\textsuperscript{176}

PHARMACEUTICAL TARGETING OF JAK-STAT

Progress in our understanding of inflammatory signaling pathways has identified new targets, including pathways involving JAK-STAT. Inhibitors have been developed that might be used clinically for inflammatory diseases.\textsuperscript{184} Of the 4 JAKs, JAK3 has been the focus of most interest in terms of drug development because of its selective...
expression in T cells and its activation by IL-2. JAK3 is critical for γc signaling (utilized by IL-2, IL-4, IL-7, IL-9, and IL-15) and constitutively associates with the γc chain. JAK3 deficiency accounts for approximately 7% to 14% of severe combined immunodeficiency cases. Importantly, JAK3 deficiency does not result in widespread pleiotropic defects, so a highly specific JAK3 inhibitor should also have limited and precise effects. An orally available selective JAK3 inhibitor has been developed.

The drug, CP-690550, has an IC50 of 1 nmol/L and is approximately 30-fold and 100-fold less potent for JAK2 and JAK1, respectively. CP-690550 showed remarkable efficacy in the prevention of graft rejection in a primate model, and the efficacy observed with CP-690550 surpassed that obtained with cyclosporine A in the same model. CP-690550 potently blocks IL-2 signaling and responses, but does not effect T-cell receptor signaling. Indeed, the combination of a calcineurin inhibitor, which would block signals emanating from the T-cell receptor, and CP-690550 reveal synergistic effects. CP-690550 did not cause granulocyte or platelet deficiency. There was a trend in the reduction of CD8 T cells, but no significant decline in total T lymphocytes. CP-690550 is currently being evaluated in models of autoimmunity.

IL-4 is a critical cytokine in promoting Th2 differentiation and the allergic response. Because IL-4 utilizes the γc chain and JAK3 for signaling, a JAK3 inhibitor would be expected to antagonize IL-4. Thus, a JAK3 antagonist may be useful in allergic disorders. CP-690550 was also found to inhibit delayed-type hypersensitivity, and its effects were reversible when the drug was discontinued. IL-9 has been reported to contribute to the development of asthma and has been suggested as a potential target for asthma treatment. Antagonizing the effects of IL-9 with a JAK3 antagonist could be of use in treating asthma, although the importance of this cytokine in asthma is controversial. Aside from JAK3, another possible JAK to target would be Tyk2. Tyk2 is involved in IL-13 signaling and is necessary for the induction of IL-13–mediated goblet cell hyperplasia in the airways.

At present, targeting STATs has met with less success than targeting JAKs, because it has been simpler to identify small molecules that interfere with JAK catalytic activity. However, because of their crucial selective functions, targeting STATs remains an attractive goal. Potential mechanisms to block STAT activity include blocking their recruitment to cytokine receptors, dimerization, nuclear import, DNA binding, dephosphorylation, or nuclear export (Fig 3). In terms of allergic disease, 2 STATs that might be useful to target are STAT4 and STAT6. These STATs are crucially important for the differentiation of Th1 cells. IL-4 activates STAT6 and promotes the differentiation of Th2 cells that promote allergic responses. Conversely, IL-12 activates STAT4 and drives the differentiation of naive T cells into Th1 cells that produce IFN-γ. As discussed above, constitutive activation of STAT3 and STAT5 has been noted in several tumors. Targeting these STATs for the treatment of cancer is of high interest.

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, and these regulatory mechanisms are potential targets to modulate STATs. The importance of STATs is further highlighted by their role in the mechanisms by which other agents mediate their effects. Cyclooxygenase-2 (COX-2) inhibitors prevent experimental allergic encephalomyelitis, a Th1-cell–mediated
autoimmune disease model of multiple sclerosis, by reducing IL-12 production and decreasing IL-12-induced T-cell responses, through inhibition of STAT3 and STAT4 tyrosine phosphorylation. The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, Atorvastatin (Pfizer, New York, NY), induces STAT6 phosphorylation and T_{H}2 cytokine secretion while inhibiting STAT4 phosphorylation and T_{H}1 cytokine secretion. The direct anti-inflammatory effects of statins are achieved by reducing IL-6-induced phosphorylation of STAT3 in hepatocytes. Paclitaxel (Myers Squibb Co, New York, NY), a microtubule stabilizer used in anticancer therapy, significantly decreases the nuclear translocation of STAT protein in adipocytes without effecting tyrosine phosphorylation. Given the central role of the JAK-STAT signaling in biologic processes involved in all phases of the immune response and the successful generation of a selective JAK inhibitor, STATs will continue to be the focus of considerable attention as potential therapeutics.

CONCLUSION

STAT proteins are critical for many intersecting and divergent pathways that contribute to allergic inflammation. Their expression, and consequently their impact, is ubiquitous, impacting a variety of cell types critical to allergy including epithelial cells, mast cells, lymphocytes, dendritic cells, and eosinophils. Dysregulation of STAT signaling has been found in human and animal studies of allergic disease. There is considerable potential of these pathways as a target for therapeutic intervention. Some progress has already been made in targeting JAK-STAT; however, considerable work remains for us to fully delineate the mechanisms by which these molecules are regulated and the optimal targets for intervention.

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