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IL-4 Induces the Proteolytic Processing of Mast Cell STAT6

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IL-4 is a potent, pleiotropic cytokine that, in general, directs cellular activation, differentiation, and rescue from apoptosis. However, in mast cells, IL-4 induces the down-regulation of activation receptors and promotes cell death. Mast cells have been shown to transduce IL-4 signals through a unique C-terminally truncated isoform of STAT6. In this study, we examine the mechanism through which STAT6 is processed to generate this isoform. We demonstrate that STAT6 processing in mast cells is initiated by IL-4-induced phosphorylation and nuclear translocation of full-length STAT6 and subsequent cleavage by a nuclear serine-family protease. The location of the protease in the nucleus ensures that the truncated STAT6 has preferential access to bind DNA. IL-4-responsive target genes in mast cells are identified by chromatin immunoprecipitation of STAT6, including the IL-4 gene itself. These results suggest a molecular explanation for the suppressive effects of IL-4 on STAT6-regulated genes in mast cells. The Journal of Immunology, 2002, 169: 3811–3818.

Signal transducer and activator or transcription 6, a latent cytoplasmic transcription factor, is a member of a family of signaling molecules that mediate responses to cytokines. IL-4 (and IL-13) binding to its receptor results in the activation of Janus kinase (JAK) and JAK3, receptor-associated tyrosine kinases (1, 2). JAK1 and 3 directly phosphorylate tyrosine residues within STAT6 Src homology (SH)2 domains leading to dimerization and translocation to the nucleus where STAT6 can associate with regulatory elements that control IL-4 (and/or IL-13) responsive genes. Numerous studies using STAT6−/− mice have demonstrated the importance of this molecule in providing immunological competence (3–5). For example, the STAT6 signaling pathway is of particular significance in the development of Th2 immunity to extracellular pathogens. STAT6−/− mice are extremely vulnerable to these infections, and T cells from these mice are unable to produce adequate amounts of the Th2 cytokines IL-4, IL-13, and IL-5 (3–5). B cell isotype switching, immunity to some tumors (6, 7), and development of contact hypersensitivity (8) are also compromised in STAT6−/− mice.

Because IL-4 plays a central role in the regulation of immune responses, the magnitude and duration of STAT6-induced responses must be tightly controlled. At least two mechanisms have been described to accomplish this regulation. First, suppressor of cytokine signaling (SOCS) proteins are up-regulated by cytokine-induced STAT activation and either compete with STAT for binding to receptors or bind to and inhibit JAK kinase activity (reviewed by Chen et al. in Ref. 9). SOCS-1 is an IL-4-inducible protein and thus acts relatively late in the STAT6 signaling cascade to inhibit JAK1 and limit the duration of STAT6 activation (10). Second, alternate isoforms of STAT proteins have been described for several STAT family members (11–15). These β isoforms (identified for STATs 1, 3, 5A, and 5B) lack the transactivation domain (TAD) but retain DNA binding ability. Therefore, by competing with the transcriptionally active form of STAT for binding to DNA, they effectively inhibit the early transcription of cytokine-responsive genes.

The biological significance of STAT isoforms that act as dominant negative transcription factors is clearly evident from studies of STAT5, a signaling molecule activated in response to IL-2, IL-3, IL-5, stem cell factor, and IL-15 (16–18). The truncated STAT5β isoforms are expressed uniquely in myeloid progenitor cells and are responsible for maintaining an IL-3-refractive state (15). Upon differentiation, these cells lose expression of STAT5β and express only full-length STAT5, coincident with a gain of IL-3 responsiveness (19). The truncated STAT5 proteins are generated through proteolysis of full-length STAT5 by a nuclear protease found only in immature myelocytes (20). This protease, termed modulator of STAT activity (MSA), is a 25-kDa member of the serine protease family. Its recognition and cleavage site in STAT5 has been identified, and mutation of this site results in a STAT5 protein that resists proteolysis (19).

We previously identified a STAT6 isoform that shares characteristics with the β forms of the other STAT family members (21). In contrast to the 100-kDa full-length STAT6 molecule, STAT6β is truncated to ~65 kDa. Results from epitope mapping studies demonstrate that STAT6β lacks the C-terminal TAD, but it appears to retain a high affinity for DNA. STAT6β is expressed in a cell type-specific manner and is present in mast cells, but not in B or T cells. Interestingly, while IL-4-induced STAT6 is associated with an activating phenotype in B cells and T cells, it is often suppressive for mast cell gene expression. Mast cell receptors c-kit and FceRI are down-regulated in response to IL-4 stimulation (22–24) and the induction of several cytokines is inhibited upon long-term IL-4 exposure (23).

In this study, we set out to 1) characterize the STAT6 protein in mast cells with regard to the mechanism of isoform generation, phosphorylation status, and cellular localization, and 2) identify a role of STAT6β in mast cell gene transcription. We demonstrate that analogous to STAT5β, STAT6β is also generated by proteolytic processing. Although distinct from STAT5 MSA, the mast
cell STAT6 protease is a member of the serine protease family and is located in the nucleus. We show that mast cell STAT6 is phosphorylated in response to IL-4 and binds in vivo to a STAT6 binding site in the IL-4 promoter in mast cells. Mast cell STAT6 is also associated with the promoters of apoptosis regulator genes bax and bel-2. The restricted expression and binding of this isoform to IL-4 responsive genes in mast cells could account for the cell-type specific inhibitory effects of IL-4.

Materials and Methods

Cell culture and stimulation

CFTL-15, a murine IL-3 dependent mast cell line (C15; Ref. 25), WEHI-3B, and bone marrow-derived mast cells (BMMC) were harvested from BALB/c mice (The Jackson Laboratory, Bar Harbor, ME) and STAT6−/− BALB/c mice (The Jackson Laboratory) (4) and were differentiated with 25% WEHI-3B supernatant. BMMC were used after 4 wk in culture at 90% purity as determined by FACS analysis (FACScan; BD Biosciences, Mountain View, CA) and Ref. 28) were stimulated with M12 B cells or stored at −80°C.

Materials and Methods

Cell extract preparation

BMMC whole cell extracts were prepared in the presence of high concentrations of protease inhibitors (Roche, Indianapolis, IN) according to the published protocol (29). Briefly, after treatment with IL-4 (15 min at 37°C), cells were pelleted, washed in PBS, resuspended in lysis buffer (50 mM Tris, pH 8.0, 0.5% Nonidet P-40, 200 mM NaCl, 10% glycerol, 0.1 mM EDTA, 1.0 mM DTT, 100 mM sodium orthovanadate, and protease inhibitors), and incubated for 60 min on ice. Following high-speed microcentrifugation at 4°C (13,000 rpm) (model 5522; Forma Scientific, Marietta, OH), supernatants were collected and protein concentration was determined by the Bradford procedure (Ref. 30; Bio-Rad, Hercules, CA).

Cell extract preparation

Nuclear extracts were prepared following the procedure of Fiering et al. (31). Briefly, cells were harvested and washed once in ice-cold PBS and once in cold buffer A (10 mM HEPES, pH 7.8, 15 mM KCl, 2 mM MgCl2, 1 mM DTT, 0.1 mM EDTA, 1% protease inhibitors). Cells were then lysed in buffer B (buffer A plus 0.2% Nonidet P-40) on ice for 5 min with vortexing. Nuclei were pelleted by high-speed microcentrifugation and the supernatant was saved as the “cytosolic fraction.” Pelleted nuclei were resuspended in buffer C (50 mM HEPES, pH 7.8, 50 mM KCl, 0.1 mM EDTA, 0.5 mM glycerol, 1% protease inhibitors, and 10% volume of 3 M (NH4)2SO4) and incubated for 10 min. The solution was then centrifuged at 20,000 × g at 4°C for 10 min. A total of 0.3 M (NH4)2SO4 was adjusted to the supernatant and precipitated proteins were pelleted at 100,000 × g for 10 min. The proteins were resuspended in 100 µl buffer B per 106 cell equivalents and used for mixing experiments with M12 B cells or stored at −80°C.

Preparation of SDS lysates

To prepare SDS lysates, cells were pelleted and washed once with cold PBS. The cell pellet was then suspended directly in 1× SDS-PAGE loading buffer (50 mM Tris-HCl, pH 6.8, 5% [v/v] glycerol, 1% SDS, 1.5% DTT, 0.002% bromophenol blue) at the ratio of 2500 cells to 1 µl buffer. The viscous lysate was then sonicated (4 × 1 s pulses on power setting 3) (Sonic Dismembrator Model 100; Fisher Scientific, Pittsburgh, PA) and frozen at −80°C.

Western blot analysis

Western blot analysis performed with rabbit polyclonal anti-STAT6 Abs raised against the C-terminal domain (M20X; Santa Cruz Biotechnology, Santa Cruz, CA), the DNA-binding domain (M20OX; Santa Cruz Biotechnology), phosphorylated tyrosine 641 of murine STAT6 (Cell Signal; Beverly, MA) and rabbit polyclonal anti-JAK3 and anti-upstream stimulatory factor (USF; Santa Cruz Biotechnology). Cell extracts (20 µg) were electrophoresed on a 10% SDS-PAGE gel and transferred to nylon membrane. The blot was blocked in 5% milk/TBST (10 mM Tris, pH 8.0, 150 mM NaCl, 0.5% Tween) overnight at 4°C. Abs (1 µg/ml) were added in 2% milk/TBST (the phospho-STAT6 Ab was diluted in 5% BSA/Tween 20 + TBS) and incubated 1 h at room temperature. HRP-conjugated donkey anti-rabbit secondary Abs (Amersham, Arlington Heights, IL) were added at a 1/5000 dilution in 2% milk/TBST and incubated 1 h at room temperature. Reactive proteins were visualized by ECL (DuPont, Boston, MA) and radiography.

Stat6 protease analysis

For analysis of STAT6 expression in cell lines, 10 µg of total RNA was electrophoresed on a 1% formaldehyde gel and transferred to nitrocellulose. The STAT6 DNA probe (bp 1–384 from STAT6 sequence; Accession no. AF481809) was labeled by random hexamer priming.

Rnase protection analysis

Total RNA samples from C15 and M12 cell lines were isolated using the RNA-STAT-60 reagent according to the manufacturer’s instructions (Tel-Test, Friendswood, TX). Rnase protection assays were performed using four different STAT6 cDNA fragments as templates for the synthesis of antisense RNA labeled with [32P]UTP (Riboprobe kit; Promega, Madison, WI). The probes correspond to the following sequences of STAT6 cDNA: GenBank accession no. AF481809; probe 1, bp 1718–2392; probe 2, 3 bp, 2422–2887; probe 4, 3 bp, 2134–2463. Full-length RNA probes were gel-purified and hybridized (5 × 106 cpm) to 10 µg of total RNA overnight at 45°C in 40 mM PIPES, pH 6.4, 400 mM NaCl, 1 mM EDTA, and 80% formamide in a total volume of 30 µl. Samples were incubated for 1 h at 37°C after the addition of 350 µl of digestion buffer (10 mM Tris-HCl, pH 7.5, 5 mM EDTA, 300 mM NaCl, 0.14 µg of RNAse T1, and 1 µg of RNAse A). Proteinase K (50 µg) and SDS (10 µl of 20% solution) were added and incubated for an additional 30 min at 37°C. Samples were extracted with phenol/chloroform, precipitated, resuspended in gel loading buffer, and analyzed by 6% denaturing PAGE.

Chromatin immunoprecipitation (ChIP) assays

Formaldehyde cross-linked chromatin was prepared from 4 × 109 cells for each sample. Abs (5 µg per immunoprecipitation [IP] sample) were pre-cleared with 40 µg each sonicated salmon sperm DNA and yeast tRNA in IP dilution buffer (50 mM Tris-HCl, pH 8.0, 0.5% Nonidet P-40, 200 mM NaCl, 0.5% SDS, and 10% volume of 3 M (NH4)2SO4) and incubated 900 µl IP dilution buffer (0.015% SDS, 1.1% Triton X-100, 2 mM EDTA, 16.7 mM Tris-HCl, pH 8.0, 167 mM NaCl) for 15 min at 4°C on a rocking platform. Chromatin (100 µl; one-fifth of the original sample) was added to the Ab solution for a further 2-h incubation at 4°C. Protein A/G agarose beads (Santa Cruz Biotechnology) were simultaneously pretreated with yeast tRNA (for each sample, 20 µl beads and 200 µg yeast tRNA). The pretreated beads were added to the Ab chromatin mixture and incubated for an additional hour at 4°C. The beads were washed extensively: three times with IP dilution buffer, three times with 1× dialysis buffer (2 mM EDTA, 50 mM Tris-HCl, pH 8.0, 0.2% Sarkosyl), three times with IP wash buffer (100 mM Tris-HCl, pH 9.0, 50 mM LiCl, 1% Nonidet P-40, 1% deoxycholic acid), and once with 10 mM Tris 0.1 mM EDTA. The Ab complexes were boiled with 1 µl of buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1% SDS) and 1 µl of digestion buffer (100 mM NaCl, 1% SDS). The DNA was phenol/ chloroform extracted and then precipitated with 20 µg glycogen as a carrier. The DNA was resuspended in 50 µl 10 mM Tris 0.1 mM EDTA and 1 µl (or 10-fold dilutions) was used for PCR analysis. Conditions for PCR were 40 cycles (94°C for 30 s, 50°C for 30 s, 72°C for 1 min) using HotStarTaq (Qiagen, Valencia, CA). PCR primers: IL-4 promoter (forward): GGTTGACCTCTTGCTGTTTAA; (reverse): GCTATACA ATGCAAATGCTGCG; IL-4 intron (forward): GAGATCCTGCGAGA CATCTTCTCTCCCTTTCC; (reverse): CATCTGTCAGACATCCGAG TAC; promoter (forward): GCTAGGGCCTGTCGTTGTT; (reverse): TTCCTGATCTGGTCGCTTCTACA; bel-2 promoter (forward): AGCT GCTTGGCTCCTCCATCA; (reverse): GCTACATGATACTGCTTGGTG; (reverse): GCCCTACCAAGCTTCCATT; bel-2 promoter (forward): AACCTCCCCACCACTTCTCT; (reverse): CCTTCTCCTCGGAATTTAATCT.

Results

STAT6β is induced by IL-4 treatment of mast cells

In previous studies we established that mast cells express both a 100- and a 65-kDa isoform of STAT6, and it is the smaller form...
that preferentially binds DNA in vitro (21). Because STAT6 requires activation by IL-4 signaling to dimerize, translocate to the nucleus, and bind DNA, we sought to determine the relationship between the 65-kDa isoform (herein referred to as the β form of STAT6) and IL-4 activation of mast cells. BMMCs, as well as B cells and WEHI-3B myeloid cells, were treated with IL-4 for 2 h before cell lysis. To denature any proteases that could result in artifactual proteolysis during the extract procedure, cells were directly lysed in 1× SDS-PAGE sample buffer. The extracts were analyzed by Western blot using M200, an Ab that detects both full-length and β forms of STAT6. As shown in Fig. 1, IL-4 treatment increases the presence of the β form in mast cells, but not in B cells or WEHI-3B.

**STAT6β is phosphorylated on Y641 and translocates to the nucleus in response to IL-4**

STAT6 typically resides in the cytoplasm of resting cells, possibly associated with scaffolding proteins (32). IL-4-induced activation leads to phosphorylation of Y641, translocation of STAT6 to the nucleus, and subsequent DNA binding (33–35). Using a commercially available Ab specific for the Y-641 epitope in Western blot analysis, we observed rapid phosphorylation of STAT6β upon exposure of the mast cells to IL-4 (Fig. 2A). Phosphorylation persisted over a 24-h period (Fig. 2B), similar to the long kinetics observed in B cells (33). Cytoplasmic and nuclear fractions were also isolated and examined for the presence of STAT6β. As shown in Fig. 2C, after IL-4 treatment of mast cells, the STAT6 isoform is located primarily in the mast cell nucleus, consistent with its phosphorylated state. These results indicate that STAT6β is subject to phosphorylation and can translocate to the nucleus in a manner similar to the full-length protein.

**STAT6 is proteolytically processed in mast cells**

STAT5 isoforms are proteolytically processed in immature myeloid cells by MSA, a serine family protease that is located in the nucleus (36). Due to the rapid kinetics of STAT6β expression, its induction by IL-4 treatment, and its nuclear location, we considered the possibility that STAT6β could be generated by a similar mechanism in mast cells. To address this possibility, mast cells were cultured for 16 h with a protease inhibitor mixture. After confirming cell viability, whole cell extracts were prepared. As shown in Fig. 3, the addition of protease inhibitors to live cells prevents the generation of the truncated STAT6 protein, indicating that the generation of the truncated STAT6 molecule is an active and specific process that occurs in viable cells. In contrast, simply increasing the concentration of protease inhibitors (up to five times the recommended dose) in the lysis buffer when preparing mast cell extracts does not prevent the appearance of STAT6β (37), because the STAT6 protein is processed in live mast cells before lysis.

Mast cells also were preincubated with individual protease inhibitors before IL-4 treatment and cell lysis to determine the class of protease that acts on STAT6. Treatment with a mixture of protease inhibitors or with 1 mM PMSF and 1 mM EDTA, but not...
mast cell protease is cleaved by a mast cell protease. Mast cell extracts were analyzed by Western blot using the M200 Ab. As shown in Fig. 5A, full-length STAT6 is cleaved by a mast cell protease. Mast cell extracts were then fractionated before mixing with B cells to localize the proteolytic activity. Incubation with the insoluble (uncleared) nuclear fraction and the whole cell extract, but not the cytoplasmic fraction, leads to the generation of STAT6β (Fig. 5B). Freeze-thawing the extracts or including PMSF/EDTA in the lysis buffer inhibits the STAT6 protease (Fig. 5A and data not shown). The results demonstrate that the proteolytic activity is localized in the nucleus of mast cells. Furthermore, STAT6 does not have to be activated (phosphorylated) to be processed by the STAT6 protease. This conclusion is based on the fact that STAT6 derived from resting B cells can be cleaved. The compartmentalization of the STAT6β isoform in the nucleus explains why both full-length and truncated STAT6 are observed in whole mast cell extracts (Fig. 3B and Refs. 21 and 37).

**STAT6β binds to the mast cell IL-4 promoter in vivo**

We have previously postulated that STAT6β is involved in negatively regulating mast cell IL-4 production. A STAT6 binding site is present in the IL-4 proximal promoter, and mutational studies have demonstrated that it confers transcriptional repression (39). STAT6 binding to this element could act to limit IL-4 transcription by interfering with positive transcriptional activators such as NFAT (40, 41). Alternatively, preferential binding of STAT6β, which presumably lacks the ability to transactivate because of the truncation of the parent protein, may directly interfere with transcription. EMSA analysis has demonstrated that STAT6β preferentially associates with the IL-4 promoter STAT6 in vitro (21).
To test the ability of STAT6 to bind to the IL-4 promoter in vivo, ChIP experiments were performed with mast cell chromatin and STAT6 antisera. Mast cell chromatin precipitated with anti-STAT6 but not normal rabbit IgG yields PCR-amplified IL-4 promoter fragments (Fig. 6A, upper panels). This finding indicates that STAT6 binds to the IL-4 proximal promoter sequences in vivo in mast cells. STAT6 was not associated with this site in B cells, Th1, or Th2 cells, a result consistent with our previous observations that IL-4 transcription in mast cells uses cell-specific regulatory mechanisms (39, 42, 43). As a control, PCR was also performed using primers for the IL-4 second intron, which contains a binding site for STAT5 (44). This region was not amplified from STAT6-associated chromatin (Fig. 6A, lower panels), suggesting that STAT6 is specifically associated with the promoter element of the IL-4 gene. In addition, stimulation of the mast cells with ionomycin (which activates IL-4 transcription) appears to result in decreased STAT6 binding to the IL-4 promoter (Fig. 6B, lanes 3 and 4), a finding that supports the hypothesis that the STAT6 isoform acts as a negative repressor.

Finally, we also examined STAT6 involvement in apoptosis gene regulation in mast cells. Recently, STAT6 has been implicated in induction of mast cell apoptosis through IL-4/IL-10 co-signaling (45) and protection of mast cell apoptosis through IL-15 signaling (46). The ratio of anti-apoptotic proteins (such as bcl-2 and bcl-x) to proapoptotic proteins (such as bad and bax) can determine the probability of a cell’s survival (reviewed by Zornig et al. in Ref. 47). Three of these genes (bcl-2, bcl-x, and bax) have been shown to regulate mast cell apoptosis (45, 46, 48). Thus, ChIP was performed on chromatin from IL-4-stimulated mast cells using the STAT6 Ab M200 and primers specific for the apoptosis genes bcl-2, bcl-x, and bax. As shown in Fig. 6C, we observed no detectable STAT6 binding to the bcl-x promoter, a region that does contain one consensus STAT6 site and can bind STAT6 in vitro (46). However, we did observe STAT6 binding to the proapoptotic gene bax, and to the prosurvival gene bcl-2. The demonstration of in vivo binding of STAT6 to these genes supports the hypothesis proposed by others (45, 46) that STAT6 can regulate apoptosis in mast cells.

The STAT6-specific antisera used in these ChIP experiments can associate with both full-length and truncated STAT6, and a STAT6 isoform-specific Ab does not yet exist. Therefore, we cannot definitively demonstrate that the STAT6β isoform (rather than full-length STAT6) is associated with mast cell chromatin. However, the STAT6 isoform is the predominant species in the mast cell nucleus (Fig. 2), and the isoform preferentially binds DNA in vitro despite the presence of full-length STAT6 (21). To provide further evidence for the idea that STAT6β preferentially binds STAT6-regulated genes in mast cells in vivo, parallel immunoprecipitations were performed using two STAT6 Abs: M200X, which binds both full-length and truncated STAT6β, and M20X, which associates with full-length STAT6 only (21). As shown in Fig. 7, both samples resulted in amplification of the IL-4 promoter sequence, suggesting that both full-length and truncated STAT6β are present in the mast cell nucleus. However, dilutions of the immunoprecipitated chromatin material demonstrated that the M200-associated sample contains at least 10-fold more template, consistent with our Western blot analysis (Fig. 2C) showing that most of the STAT6 protein in the nucleus is processed to the β isoform.
Discussion

In this study, we demonstrate that a C-terminally-truncated isoform of STAT6, termed STAT6β, is generated by posttranslational processing in mast cells and is associated with the promoter of the IL-4, bax, and bcl-2 genes in vivo. Although both STAT6 isoforms exist in mast cells, it is the full-length STAT6 that predominates in resting cells. We propose that IL-4 interaction with its receptor activates the conventional JAK-STAT signaling pathway in mast cells. The full-length molecule is phosphorylated on tyrosine 641, dimerizes, and translocates to the nucleus where it is subject to proteolysis by a protease sequestered in the nucleus giving rise to STAT6β. Thus, the generation of STAT6β is dependent on IL-4-induced nuclear translocation.

This model can account for the apparently conflicting data previously reported by other groups. Suzuki et al. (49) observed that the 65-kDa STAT6 isoform is phosphorylated by IL-4 treatment of mast cells. Conversely, Masuda et al. (50) did not detect the STAT6 isoform in resting MC/9 mast cells or BMMC and proposed that the use of a different lot of M200 Ab in Western blot analysis may be responsible for failure to detect STAT6β. However, without exposure to IL-4, MC/9 mast cells and BMMC would not express appreciable amounts of STAT6β. Notably, small amounts of STAT6β are sometimes detected in our resting mast cell lysates. This may be a result of a procedural artifact that allows the protease access to STAT6 during cell lysis. Alternatively, resting mast cells can express and release small amounts of IL-4 (51), thus activating STAT6β in a subset of cells. Regardless, our results clearly show that mast cell exposure to IL-4 is necessary for optimal STAT6β generation. Interestingly, in vivo administration of IL-4 induces the appearance of activated STAT6β in murine lymph nodes (52). Although the cellular source of this STAT6 was not identified in this study, mast cells have been demonstrated to be present in lymph nodes after immunologic stimulation (53).

The C terminus of STAT6, a region containing a defined TAD (54), is absent in STAT6β. Yet DNA binding activity is retained, suggesting that this molecule may act as a dominant-negative regulator of transcriptional activity by binding to the regulatory elements of STAT6-regulated genes and blocking the binding and/or action of positive-acting transcription factors. The regulation of STAT6 by the phosphatase sulfhydryl-2 domain-containing tyrosine phosphatase-1 (SHP-1) is also unique in mast cells: SHP-1 suppresses STAT6 activation in B cells but does not affect mast cell STAT6 (55), possibly due to the loss of a SHP-1 recognition site in the truncated STAT6β molecule. These features of the STAT6β isoform and its differential regulation are consistent with the known effects of IL-4 signaling in mast cells. In cell types where the full-length STAT6 protein is expressed, IL-4 exerts positive effects on differentiation and effector function. For example, in B cells, IL-4 induces expression of class II MHC Ags, Ig isotype switching, and proliferation. Endothelial cells induce VCAM-1 expression (56) when exposed to IL-4. The differentiation of CD4+ T cells to Th2 cells, a pathway essential for effective immune responses to extracellular pathogens, is also dependent on IL-4 (reviewed by Nelms et al. in Ref. 57). However, IL-4 acts to suppress the expression of several mast cell genes. Long-term exposure to IL-4 induces the down-regulation of FceRI and c-kit expression and significantly reduces the activation-dependent expression of IL-4, IL-5, IL-13, and IL-6 (22–24). The dominant-negative STAT6 isoform may play a direct role in this process.

We have postulated that mast cell IL-4 expression is regulated through a negative feedback mechanism (37). Previous data from our laboratory confirms that the STAT6 binding site in the IL-4 promoter acts as a negative regulatory element (39), and work by others has shown that IL-4 signaling induces a down-regulation of further IL-4 expression by activated mast cells (23). Our demonstration that STAT6 binds to the IL-4 promoter in mast cells in vivo provides further data to support this hypothesis. We did not detect STAT6 binding to the IL-4 promoter in B cells or Th1 cells, consistent with the “closed” conformation of the IL-4 gene in these non-IL-4-producing cell types (58). We also did not detect STAT6 binding to the IL-4 promoter in Th2 cells, however, a recent report using ChIP analysis demonstrated that STAT6 does bind to the IL-4 promoter in Th2 cells after IL-4 stimulation (59). The difference in our results may reflect the fact that our chromatin was derived from resting Th2 cells and Avni et al. (59) used IL-4 treated Th2 cells, which would induce STAT6 binding to DNA.

Of particular interest is our finding that STAT6 associates with the proximal IL-4 promoter in resting, but not activated, mast cells. As mentioned above, mast cells continuously produce and release small amounts of IL-4 that may result in a low level of STAT6β in the nucleus. Powerful cell stimulation (such as the ionophore used in our studies) may recruit strong transactivators of IL-4, such as NFAT, which could displace STAT6β on the IL-4 promoter. We propose that the binding of STAT6β to the promoter limits active transcription in resting cells and may regulate the duration of IL-4 production after cell activation. Thus, STAT6 could facilitate a negative feedback response in mast cells. This response may be important physiologically during the generation of Th cell responses where early sources of IL-4, such as those from mast cells, can drive Th2 cell differentiation. Timely suppression of mast cell IL-4 may regulate the magnitude of the Th2 response. Consistent with this idea, Morris et al. (60) recently showed that treatment of mice with IL-4 before Ag challenge induces a rapid increase in IFN-γ and a suppression of Th2 cytokines. This finding is supportive evidence that a negative IL-4 feedback loop does exist in vivo.

Given the predominance of STAT6β in IL-4-activated mast cells, it is not clear how STAT6 can positively affect gene expression in these cells. Some insight into this question may be gleaned from studies of the effect of IL-4 on mast cell apoptosis. The mechanisms that regulate mast cell programmed cell death are distinct from those that control lymphocyte apoptosis in several ways: 1) IL-4 alone does not protect mast cells from apoptosis (61, 62) as it does in B and T cells (63–70); 2) a combination of signals resulting from exposure to both IL-4 and IL-10 induces genes that
promote apoptosis, events that are STAT6-dependent (45), and 3) IL-15 (which in most cell types activates STAT5) protects mast cells from apoptosis through STAT6 induction of the bcl-x gene (46). These data indicate that mast cell STAT6 does play a positive role in apoptosis gene regulation, but only if STAT6 is activated through another receptor (IL-15) or if STAT6 is induced coincident with other cytokine signals. Other studies have also revealed instances where the negative effects of IL-4 on mast cell gene transcription are abrogated if other cytokine signaling pathways are activated simultaneously. For example, if mast cells are exposed to both IL-4 and IL-6 or stem cell factor, the cells proliferate and express Th2 cytokines (71). Thus, the mast cell-specific STAT6 protease may act to render mast cells insensitive to IL-4-induced gene activation alone. Only through costimulation of mast cells or alternative pathways of STAT6 activation will STAT6 mediate gene activation in mast cells. Our preliminary experiments show in vivo binding of mast cell STAT6 to the promoter of the induced gene activation alone. Thus, the mast cell-speci
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