Role of the JAK-STAT pathway in PDGF-stimulated proliferation of human airway smooth muscle cells

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AIRWAY SMOOTH MUSCLE (ASM) volume is increased three- to fourfold in patients with asthma (29). Both hyperplasia and hypertrophy of ASM cells are thought to be responsible for this change in volume. Increased ASM mass is a feature of airway remodeling and contributes to the nonspecific bronchial hyperreactivity and persistent obstruction seen in some asthmatic patients (14). Consequently, knowledge about the molecular mechanisms that contribute to ASM proliferation is important for a better understanding of this disease. The mediators of ASM proliferation in vivo are still unknown. It has been postulated that a combination of mitogens mediates this process, given that asthmatic airways contain elevated levels of enriched plasma secondary to submucosal edema and increased vascular permeability (55). In support of this hypothesis is the finding that bronchoalveolar lavage fluid from asthmatics is mitogenic for human ASM cells in culture (43). In vitro, cytokines [interleukin (IL)-1, IL-6], growth factors [platelet-derived growth factor (PDGF), epidermal growth factor (EGF), reactive oxygen species (ROS), and inflammatory mediators (histamine)] have all been shown to induce ASM mitogenesis depending on the species examined (22). In addition, contractile agonists such as thrombin have been shown to induce mitogenesis (68). In ASM, these mitogens have been shown to signal through both receptors with tyrosine kinase activity and receptors coupled to G proteins with the subsequent activation of the mitogen-activated protein kinase (MAPK) superfamily or the phosphatidylinositol 3 (PI3)-kinase pathways (32, 35, 46). One study on human ASM found that sustained rather than transient MAPK kinase activation is required for mitogenesis (44). However, in this study, the extent of MAPK activation did not always correlate with mitogenesis, suggesting that other signaling pathways might be involved in this process as well. This finding is consistent with the work of others showing that multiple signaling pathways cooperate for growth factor-induced mitogenesis (48).

The signal transducers and activators of transcription (STAT) family of transcription factors has the dual function of both transducing signals from receptors at the cell surface to the nucleus and activating transcription of target genes in the nucleus of the cell (28, 50). The STAT proteins exist in a latent form in the cytoplasm and, upon receptor activation by cytokines, become phosphorylated on tyrosine residues, in most cases by the Janus kinase (JAK) kinase family. [See Darnell (19) and Imada and Leonard for review (31)]. This phosphorylation is obligatory for STAT activation and mediates dimerization via p-Tyr-SH2 domain in-
interactions, resulting in translocation of the STATs to the nucleus, where they bind to sequence-specific DNA elements. In addition, for maximal transcriptional activity, several STATs undergo serine phosphorylation by MAPK family members (20).

STATs are also activated by growth factors, but the mechanism by which this occurs is less clear than with the cytokines. Both receptor kinases and nonreceptor tyrosine kinases, such as the JAK and Src kinase, have all been implicated in this process (D. Bhavsar and B. Cochran, unpublished observations; 66). Moreover, we have previously shown that growth factor activation of the JAK-STAT pathway requires ROS as well as the GTPase Rac1 (57, 58). In addition, after growth factor treatment, Rac1 and STAT3 directly interact with one another in COS-1 and Rat-1 fibroblasts. Downregulation of the JAK-STAT pathway is mediated by phosphatases that can directly dephosphorylate JAKs and STATs, by proteins such as suppressors of cytokine signaling (SOCs), which can directly inactivate the JAKs, and in some cases by the protein inhibitor of activated STAT, which inhibits STAT DNA binding activity (39, 59).

Most cytokines, as well as growth factors and hormones, can activate the STAT transcription factors. The STATs mediate diverse cellular processes, including proliferation, differentiation, survival, and transformation. Data from STAT knockout mice demonstrate that STATs are absolutely required for cytokine signaling and normal immune responses (21). In addition, the STAT6-/- knockout mouse highlights the importance of this pathway in mediating allergic asthma, as this mouse is protected from antigen-induced airway hyperresponsiveness and mucus production (63). In humans, STAT1 has been shown to be constitutively upregulated in the airway epithelium of patients with asthma, although the mechanism for this is not known (52).

In some but not all cellular contexts, STATs have been shown to participate in both normal and abnormal proliferative responses. For example, targeted disruption of STAT4 and STAT6 demonstrates that these STATs are essential for normal cytokine-induced proliferation of lymphocytes (62, 65). In addition, constitutive STAT signaling, particularly of STAT3 and STAT5, has been found in many human tumors (12). Furthermore, an activated form of STAT3 was shown to be necessary and sufficient for cellular transformation in vitro and in vivo (13). STATs may affect proliferation by regulating the expression of immediate-early genes, such as c-myc and c-fos, as well as cell cycle regulatory genes, such as the cyclins (10). In contrast, data from some cell types suggest that STAT1 activation may have an antiproliferative effect (17, 51).

Although other investigators have extensively studied the MAPK and PI3-kinase pathways and their role in ASM cell proliferation, no published data exist regarding the role of the STAT pathway in proliferation of this cell type. It is well known that both STAT regulation and function vary depending on the stimulus and the cell type examined. For example, tissue-specific STAT3 knockout mice reveal that STAT3 is proapoptotic in mammary epithelium, whereas it is antiapoptotic in T cells (1). Given that the STAT pathway plays a pivotal role in mediating inflammatory and immune responses, it is likely that this signaling pathway also participates in the chronic inflammation and subsequent airway remodeling seen in some asthmatics. In this paper we characterize the regulation of the STAT pathway by PDGF in human airway smooth muscle cells (HASMC) and find that elements of this signaling pathway contribute to growth factor-induced HASMC proliferation, one important feature of airway remodeling.

**METHODS**

**Reagents.** Ham’s F-12 medium and Dulbecco’s PBS were purchased from GIBCO Laboratories (Grand Island, NY). For stimulations, recombinant human PDGF-AB, EGF, and IL-6 were obtained from Research and Diagnostic Systems (Minneapolis, MN). AG-490, PP2, SU-6656, and PD-98059 were from Calbiochem (La Jolla, CA). All STAT antibodies were purchased from New England Biolabs (Beverly, MA). JAK2 antibody was from Upstate Biotechnology (Lake Placid, NY), and phosphospecific JAK2 antibody was from Affinity BioReagents (Golden, CO). All other reagents were purchased from Sigma (St. Louis, MO).

**ASM cell culture.** HASMC were kindly provided by Dr. Reynold Panettieri (University of Pennsylvania, Philadelphia, PA). These cells were derived by enzymatic dissociation of the trachea of lung transplant donors and characterized for the presence of smooth muscle actin and agonist-induced calcium changes as previously described (47). Cells were plated at a density of $8 \times 10^5$ cells per 100-mm plate (3rd–8th passage) in Ham’s F-10 media supplemented with 10% fetal bovine serum (FBS), 25 mM HEPES, 100 U/ml penicillin, and 100 µg/ml t-glutamine. Cells were growth arrested with Ham’s F-10 medium without FBS but supplemented with 5 µg/ml each of insulin and transferrin for 48 to 72 h.

**Adenoviruses.** The replication-deficient adenovirus suppressor of cytokine signaling-1 (SOCS-1) was kindly provided by Dr. Daniel Frantz and Steve Shoelson (Harvard University, Boston, MA). Titering experiments were performed in growing HASMC, and, depending on the virus, 1,000–10,000 particles/cell were used to infect growing cells for ~16 h. Protein overexpression was confirmed by Western blot analysis. As a control, cells not infected with the above adenoviruses were infected with adenoviruses lacking the cDNA insert.

**[3H]Thymidine incorporation and cell proliferation assays.** HASMC were plated at a density of 45,000 cells in 12-well plates in DMEM. After 3 days, cells were growth arrested for 72 h in 0.5% FBS-DMEM. The following mitogens were then added at the noted concentrations: PDGF-AB (25 ng/ml), EGF (50 ng/ml), and serum (15% FCS). After the addition of mitogen, cells were labeled with [methyl-3H]thymidine (1 µCi/well, 20 Ci/mmol) for 36 h. AG-490, PP2, SU-6656, or PD-98059 was added 1 h before mitogens. The experiment was terminated by washing the cells with ice-cold PBS and then with cold 6% TCA. Cells were then lysed in 0.2 N NaOH, and radioactivity was counted in a liquid scintillation counter. The results represent at least two separate experiments done in duplicate for each condition. Cell proliferation assays were performed in parallel with [3H]thymidine uptake experiments. Cell number was determined by removing...
the cells from the wells using 0.5% trypsin-1 mM EDTA solution. Cell counts were then obtained from each well using a Coulter counter.

**Western blot analysis.** Quiescent HASMC were treated for 10 min with 50 ng/ml of PDGF and lysed in lysis buffer as previously described (57). Lysates (40 μg of protein) were run on an 8% SDS polyacrylamide gel and transferred to nitrocellulose. The membrane was then blotted with the indicated antibody and processed via chemiluminescence (Pierce). All Western blotting experiments were performed at least three times, and representative examples are shown in Figs. 2–4, and 6.

**Northern blot analysis.** Total RNA from HASMC was extracted using TRIzol reagent according to the manufacturer’s instructions (GIBCO). Equal RNA loading in the gel wells was assessed by ethidium bromide staining of 28S and 18S rRNA. Denatured RNA (10–20 μg) samples were separated by gel electrophoresis through a 1% agarose gel containing formaldehyde and transferred to a nylon membrane (GeneScreen). Radioactively labeled full-length human cyclin D1 or c-myc was used as a probe. After baking and ultraviolet cross-linking, the membrane was hybridized using ExpressHyb (Clontech) with radiolabeled probe (1–2 million counts·min⁻¹·μl⁻¹) and salmon sperm DNA (100 μg/ml) for 3 h. After hybridization, blots were rinsed according to the manufacturer’s directions (Clontech). The membrane was exposed to X-ray film at −80°C overnight. In addition, the blots were stripped and reprobed with radiolabeled glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to ensure equal RNA loading. All results were quantitated by densitometry on the phosphorimager. Each Northern blot experiment was repeated at least twice.

**Nuclear extracts and electrophoretic mobility shift assay.** After the indicated treatments, nuclear extracts were obtained as previously described (57). Nuclear extracts (12 μg) were added to 32P-end-labeled oligonucleotide m67-SIE probe (−30,000 counts·min⁻¹·μl⁻¹, −5 fmol/reaction), and the mixture was incubated in binding buffer containing 5 μg/ml poly(dI-dC) for 30 min at 30°C. The reaction mixture was electrophoresed through 5% polyacrylamide gels containing 2% glycerol in 0.5× Tris-borate-EDTA buffer at room temperature. The gel was then dried and subjected to autoradiography. Each electrophoretic mobility shift assay (EMSA) experiment was repeated at least three times.

**RESULTS**

**STATs are activated by PDGF in HASMC.** To investigate whether members of the STAT family of transcription factors are activated in HASMC by growth factors, quiescent HASMC were stimulated with PDGF for 10 min. Nuclear extracts were prepared from the treated cells and analyzed by EMSA using the high-affinity c-fos SIE as the probe (Fig. 1). This probe binds with high affinity to most STATs (53, 67). PDGF treatment resulted in robust STAT DNA binding activity. STAT DNA binding was also induced by EGF (data not shown). To confirm the identity of these PDGF-induced SIE binding complexes, we performed antibody supershift analysis. Previous work with fibroblasts indicates that there are three DNA binding complexes induced after PDGF stimulation: the upper complex is composed of STAT3 homodimers, the lower complex of STAT1 homodimers, and the middle complex of STAT1-STAT3 heterodimers (57, 66). Addition of anti-STAT3 antiserum to the binding reactions supershifted the upper and middle complexes but left the lower complex unaltered. Addition of anti-STAT1 antibody, made against the unique COOH-terminal peptide of STAT1, supershifted the lower complex and diminished the middle complex. Antibody to STAT5 and STAT6 did not shift the DNA binding complexes. Thus PDGF treatment induces STAT1 and STAT3 in HASMC.

To further confirm the activation of STATs by PDGF in HASMC, we examined the phosphorylation state of STAT3 with an anti-STAT3 (Y705) phosphospecific (anti-STAT3pTyr) antibody. The phosphorylation of this conserved COOH-terminal tyrosine is required for STAT dimerization and translocation to the nucleus (56, 74). Western blotting of cellular extracts showed an increase in STAT3 phosphorylation after PDGF treatment (Fig. 2) consistent with the induction of STAT DNA binding activity. The same blot was stripped and rebotted with an anti-STAT3 antibody to confirm that the increase in tyrosine phosphorylation was not due to increased amounts of STAT3 protein.

**JAK and Src kinases mediate PDGF activation of STATs.** Although cytokine activation of STAT tyrosine phosphorylation is largely mediated by the JAK family, the mechanism of growth factor activation of STAT tyrosine phosphorylation is less clear: JAK, Src, and receptor tyrosine kinases have all been implicated (D. Bhavsar and B. Cochran, unpublished observations; 66). To determine which kinase(s) mediates PDGF activation of STAT tyrosine phosphorylation in HASMC, we initially employed chemical inhibitors of the STAT kinases JAK and Src. Pretreatment of HASMC with AG-490, a specific JAK2 kinase inhibitor, blocked STAT3 tyrosine phosphorylation by PDGF (Fig. 2A) (3, 42).
To confirm the importance of the JAK kinases in STAT activation by PDGF, we overexpressed a naturally occurring JAK kinase inhibitor, SOCS-1 (60). As primary cells, HASMC are not readily transfectable by conventional techniques; therefore, we used adenoviral-mediated gene transfer to introduce the SOCS-1 gene into these cells. To this end, HASMC were infected with adenovirus (5,000 particles/cell) containing the cDNA insert for SOCS-1 (AdSOCS-1). Overexpression of AdSOCS-1 inhibited STAT activation in response to PDGF as evaluated by Western blot (Fig. 2B). Thus STAT activation by PDGF appears to require both JAK and Src kinases, and it is likely that the two kinases reside in a common pathway as has been previously suggested (15, 72).

To confirm that JAK2 is activated in HASMC in response to PDGF, we employed two methods: 1) Western blot analysis using a phosphospecific antibody to JAK2 that recognizes the tyrosine phosphorylation sites required for kinase activation and 2) immunoprecipitation with phosphotyrosine antibody (4G10) followed by Western blot with JAK2 antibody. As judged by both of these methods, JAK2 was activated by PDGF in HASMC (Fig. 3). In addition, both AG-490 and PP2 blocked JAK2 activation. This indicates that

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Pretreatment of HASMC with specific Src family kinase inhibitors PP2 or SU-6656 (3, 7, 26) also prevented STAT3 phosphorylation by PDGF (Fig. 2C). The inhibition of STAT activation by PP2 was not seen with IL-11 stimulation of HASMC (data not shown), confirming that PP2 does not inhibit JAK kinases, since IL-11 receptor signaling requires JAK kinases. Neither AG-490 nor PP2 inhibited autophosphorylation of the PDGF receptor, and therefore both appear to be specifically inhibiting STAT3 activation by PDGF (Fig. 3B). Thus STAT activation by PDGF appears to require both JAK and Src kinases, and it is likely that the two kinases reside in a common pathway as has been previously suggested (15, 72).

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Fig. 2. PDGF activates STAT3 in HASMC (HA). A: Q HASMC were stimulated with PDGF-AB (25 ng/ml) for 10 min. Where indicated, cells were incubated in the presence of AG-490 (50 μM) for 30 min before PDGF stimulation. Cell extracts were subsequently obtained as previously described (57) and then processed for Western blotting with phosphospecific STAT3 antibody (STAT3pTyr). The blot was stripped and reprobed with STAT3 antibody. SOCS-1, suppressor of cytokine signaling-1. B: growing HASMC were infected with adenoviruses containing SOCS-1 (AdSOCS) at 5,000 particles/cell overnight or empty adenovirus. Cells were then made quiescent before stimulation with 25 ng/ml PDGF for 10 min. Cellular extracts were then processed for Western blotting with the indicated antisera. C: Q HASMC were stimulated with PDGF-AB as above. Where indicated, cells were pretreated with SU-6656 (2 μM) or PP2, 10 μM for 30 min before PDGF stimulation. Cell extracts were analyzed by Western blot analysis using anti-STAT3pTyr and STAT3 antibody.

Fig. 3. PDGF activation of the Janus kinase 2 (JAK2) kinase is inhibited by the Src inhibitor PP2. A: Q HASMC were stimulated with PDGF-AB (25 ng/ml) for 5 min before total lysates were obtained and analyzed by protein immunoblotting with anti-Jak2 phosphotyrosine (Y1007/Y1008) antibody. Where indicated, cells were pretreated with AG-490 (50 μM) or PP2 (10 μM) before stimulation. The blot was stripped and reprobed with antibody to JAK2, phosphomitogen-activated protein kinase (MAPK), and MAPK. B: protein lysates from above were immunoprecipitated (IP) on protein A-G plus beads using antiphosphotyrosine antibody as previously described (57, 58). Immune complexes were subsequently analyzed by Western blot analysis with antibody to JAK2. The blot was stripped and reprobed with antibody to the PDGF receptor (αPDGF-R).
Src kinase is required for JAK2 activation and suggests that Src is in a pathway upstream of JAK for PDGF signaling in HASMC. AG-490 did not affect PDGF activation of the MAPK pathway as determined by Western blot analysis using a phosphospecific MAPK antibody (Fig. 3B). PP2, however, did inhibit PDGF activation of MAPK. This result is consistent with the results of others that demonstrate that Src kinase in some cell types is upstream of Erk activation and can participate in Raf activation (25).

STAT activation by PDGF requires ROS. Our prior work with Rat-1 fibroblasts and COS-1 cells showed that growth factor activation of the STAT pathway requires ROS (57). To determine whether STATs are similarly regulated in HASMC, we pretreated cells with diphenylene iodonium (DPI), a NADPH oxidase inhibitor, before stimulation with PDGF. Total lysates were obtained and analyzed by Western blot analysis as described above. STAT3 tyrosine phosphorylation in response to PDGF treatment was inhibited by treatment with DPI, suggesting that ROS are important in growth factor-mediated STAT activation in HASMC (Fig. 4A). In addition, overexpression of the antioxidant catalase via adenoviral-mediated gene transfer decreased PDGF activation of STATs, as seen on Western blot analysis (Fig. 4B). The blot was subsequently stripped and reprobed with an antibody to catalase to confirm protein overexpression. Control cells were infected with adenovirus without the cDNA insert to control for nonspecific effects of viral infection.

JAK and Src kinase activity is required for PDGF-induced thymidine incorporation. PDGF has been shown to be mitogenic in multiple cell types, including HASMC. The STAT pathway has been shown to participate in cellular proliferation depending on the cell type and the specific STAT transcription factor. To examine whether PDGF activation of the STAT pathway participates in HASMC proliferation, we assessed thymidine incorporation in the presence or absence of STAT kinase inhibitors. Growth-arrested HASMC responded to a 24-h incubation with PDGF by a 18-fold elevation of [3H]thymidine incorporation (Fig. 5). This increase in thymidine uptake was inhibited by AG-490, SU-6656, and PP2, suggesting that JAK and Src kinases are required for proliferation. Similar to what others have found in airway SMC, pretreatment with the MAPK kinase inhibitor PD-98059 significantly decreased thymidine uptake (data not shown) (44). The inhibition of thymidine uptake by treatment with the STAT kinase inhibitors was not due to cytotoxicity, as cell viability determined by trypan blue exclusion was unchanged. In addition, AG-490 does not generally inhibit the cell cycle machinery as it did not prevent PDGF-induced thymidine uptake in NIH 3T3 cells (unpublished data). Proliferation assays were performed in parallel with [3H]thymidine uptake experiments by determining the change in cell number using a Coulter counter. PDGF treatment resulted in a 90% increase in cell number, which was inhibited by both AG-490 and PP2 (data not shown).

Cyclin D1 and c-myc gene expression requires JAK and Src kinases. A potential target gene of PDGF activation of the STAT pathway is cyclin D1, which has been shown by others to be upregulated by STAT3 and STAT5, depending on the cell type (13, 40). Cyclin D1 is a protein that is induced by mitogenic growth factors and regulates progression through the G1 phase of the cell cycle (41). Northern blot analysis of HASMC stimulated with PDGF for various time points, from 1 to 8 h, showed a significant increase in cyclin D1 mRNA expression beginning at 4 h (2.5-fold), with a further increase at 8 h (threefold) (Fig. 6A), as determined by densitometry. Pretreatment with AG-490 or PP2 decreased the elevation of cyclin D1 mRNA seen at 8 h to
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DISCUSSION

Although the STAT pathway has been shown to be involved in proliferative responses in some cell types, only limited data exist regarding the signaling or function of the STAT pathway in any type of ASMC (36, 38). Detailing STAT signaling in HASMC is important because these cells participate in the pathogenesis of asthma, and STAT regulation and function differ greatly depending on the cell type. Here we have shown that the STAT pathway is activated in HASMC by growth factors. Our data indicate that JAK and Src kinases are required for STAT3 activation by PDGF in these cells. Furthermore, our data suggest that the Src kinases are upstream of the JAK kinases in HASMC. This is consistent with data from other laboratories showing that overexpression of v-Src or c-Src kinase inhibitor SOCS-1 was introduced into these cells. Furthermore, our data suggest that the Src kinases are required for STAT3 activation by PDGF in these cells. Furthermore, our data suggest that the Src kinases are upstream of the JAK kinases in HASMC. This is consistent with data from other laboratories showing that overexpression of v-Src or c-Src kinase activity resulted in constitutive JAK kinase activation (15, 69).

Recently, there has been an appreciation that ROS are not just by-products of cellular respiration but also are important second messengers that participate in vital signaling processes (64). We previously demonstrated that growth factor activation of STATs requires the generation of ROS by an NADPH-like oxidase in Rat-1 fibroblasts and COS-1 cells (57). Similarly, we now have shown that PDGF activation of STATs in HASMC is also redox dependent. Inhibition of PDGF activation of STAT3 by DPI suggests that generation of ROS in these cells is also likely through an NADPH-like oxidase. Although the precise identity of the nonphagocytic NADPH oxidase is unknown, components or homologs of the oxidase have been isolated in multiple nonphagocytic cells, including vascular smooth muscle cells (16, 24, 37). In addition, work in vascular smooth muscle and bovine ASM suggests that the generation of ROS by PDGF is via an NADPH-like oxidase (11, 45, 61).

The STAT pathway participates in cell proliferation, apoptosis, and transformation (30). Work from other laboratories has shown that activation of the MAPK and PI3-kinase pathways is important for ASM cell proliferation.
proliferation in vitro (32, 35). The present studies indicate that the STAT pathway is also required for proliferation in HASMC, because inhibition of the STAT kinases Src and JAK prevented PDGF-induced proliferation as determined by thymidine incorporation. Because inhibition of JAK2 with AG-490 inhibits proliferation, but not MAPK activation, multiple pathways must be involved in the proliferative response. Given that PDGF activates multiple signaling cascades, including PI3-kinases and the MAPK pathway, it is likely that STATs cooperate with these other signaling pathways in mediating the proliferative response in HASMC. The STAT pathway has already been shown to interact with both the MAPK and PI3-kinase pathways in different cellular contexts (2, 20).

The STAT transcription factors are a potential convergence site for the MAPK and JAK kinase pathways, since JAKs phosphorylate STATs on tyrosine, leading to dimerization and nuclear localization, whereas MAPK phosphorylates STAT1 and STAT3 on serine, leading to maximal transcriptional activation (23, 31).

In addition, PDGF is known to activate PI3-kinase, which in some cell types is upstream of Rac1 GTPase (5, 27, 73). Previously, we showed that Rac1 is required for PDGF activation of STAT3 in fibroblasts (58). Therefore, the PI3-kinase pathway could play an indirect role in STAT activation by PDGF in HASMC. Although it has been shown previously by others in some cellular contexts that JAK kinases are upstream of both MAPK and PI3-kinase activation, this does not fit entirely with our data (54, 71). The fact that AG–490 could completely inhibit PDGF-induced mitogenesis in HASMC, while having no effect on MAPK activation, suggests that the JAK kinases are essential for proliferation, independent of MAPK activation, in this cell type.

Potential STAT target genes for mediating PDGF-induced proliferation of HASMC include the immediate-early gene c-myc, the cell cycle regulatory gene cyclin D1, and the antiapoptotic gene Bcl-x (10, 70). All of these genes have been shown to be STAT regulated and important in cellular proliferation. We have shown in the present studies that mitogen activation of cyclin D1 gene expression in HASMC likely requires the STAT pathway as inhibition of STAT kinases prevented PDGF-induced cyclin D1 expression. It has been shown in other cell types that STATs are important in cyclin D1 gene expression (13, 40). In addition we have found that inhibitors of the Src and JAK kinases inhibit c-myc induction. This finding is consistent with recent work that shows that Src and JAK kinases are necessary for c-myc induction in fibroblasts (9).

Induction of c-myc gene expression by itself may contribute to the upregulation of cyclin D (8, 18, 49). Thus STATs may contribute to cyclin D upregulation directly through STAT binding sites in the cyclin D promoter and indirectly by inducing c-myc.

The STAT pathway plays a pivotal role in both inflammatory and immune responses. As such, it is likely that this pathway contributes to the pathogenesis of asthma and airway remodeling. We have now demonstrated that growth factors regulate the STAT pathway in HASMC. Specifically, we have established that both JAK and Src kinases are important for PDGF activation of STATs as well as for the proliferative response in HASMC. Future studies will examine the interaction between the Src-JAK pathway and the MAPK and PI3-kinase pathways in regulating cellular proliferation in response to growth factors in HASMC. A better understanding of the molecular mechanisms of ASM proliferation may provide more rational targets for the treatment of patients with persistent airflow hyperreactivity or fixed obstruction associated with asthma.

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