An Essential Role of the Jak-2/STAT-3/Cytosolic Phospholipase A2 Axis in Platelet-derived Growth Factor BB-induced Vascular Smooth Muscle Cell Motility*

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Indira Neelit‡, Zhimin Liu‡, Nagadhara Dronadula‡, Z. Alex Ma§, and Gadiparthi N. Rao‡¶§

From the ¶Department of Physiology, University of Tennessee Health Science Center, Memphis, Tennessee 38163 and the §§Division of Experimental Diabetes and Aging, Mount Sinai School of Medicine, New York, New York 10029

Platelet-derived growth factor-BB (PDGF-BB) is a potent motogen for vascular smooth muscle cells (VSMCs). To understand its motogenic signaling events, we have studied the role of the Janus-activated kinase signal transducers and activators of transcription (Jak/STAT) pathway and cytosolic phospholipase A2 (cPLA2). PDGF-BB stimulated tyrosine phosphorylation of Jak-2 and STAT-3 in a time-dependent manner in VSMCs. In addition, AG490 and Jak-2KEpRK5, a selective pharmacological inhibitor and a dominant negative mutant, respectively, of Jak-2, attenuated PDGF-BB-induced STAT-3 tyrosine phosphorylation and its DNA binding and reporter gene activities. PDGF-BB induced VSMC motility in a dose-dependent manner with a maximum effect at 10 ng/ml. Dominant negative mutant-dependent suppression of Jak-2 and STAT-3 blocked PDGF-BB-induced VSMC motility. PDGF-BB induced the expression of cPLA2 in a Jak-2/STAT-3-dependent manner, and pharmacological inhibitors of cPLA2 prevented PDGF-BB-induced VSMC motility. Furthermore, either exogenous addition of arachidonic acid or forced expression of cPLA2 rescued PDGF-BB-induced VSMC motility from inhibition by blockade of Jak-2 and STAT-3 activation. Together, these results for the first time show that PDGF-BB-induced VSMC motility requires activation of the Jak-2/STAT-3/cPLA2 signaling axis.

Inflammation at the site of vascular injury is believed to be an initiative event in the pathogenesis of vessel wall diseases (1, 2). The dysfunctional endothelial cells and inflammatory cells at the site of vascular injury produce a large number of molecules with a broad spectrum of biological activities (1–3). A majority of these molecules are mitogenic, motogenic, or both to vascular smooth muscle cells (VSMCs) (4–8). The availability of these substances at the site of vascular injury, therefore, provides a permissive milieu for VSMC dedifferentiation (9).

The dedifferentiated VSMCs gain their embryonic non-contractile and synthetic state phenotype and contribute to the progression of lesions such as restenosis after angioplasty via their migration from media to intima and multiplication in intima (10). Several studies have reported that inhibition of expression of the molecules that are produced at the site of vascular injury or suppression of their biological activities regresses the lesion development (11, 12). As a large number of molecules are involved in the formation of arterial wall lesions, identifying the unifying mechanisms of these molecules may eventually lead to development of better therapeutic agents against these vascular lesions.

Janus-activated kinases (Jaks) are a group of non-receptor tyrosine kinases that via tyrosine phosphorylation modulate the activities of a group of transcriptional factors, namely signal transducers and activators of transcription (STATs) (13, 14). A large body of data indicates that STATs play an important role in the regulation of cell proliferation and differentiation (15–17). Arachidonic acid, a polyunsaturated fatty acid, is an important component of membrane phospholipids and is released acutely in response to a variety of agents, including growth factors, cytokines, hormones, and oxidants (18–22). Upon release, it is either metabolized via the cyclooxygenase, lipoxigenase, or cytochrome P450 monooxygenase pathways producing prostaglandins, hydroperoxyeicosatetraenoic acids, and epoxyeicosatrienoic acids, respectively, or is reincorporated into membrane phospholipids via esterification involving arachidonoyl-CoA synthase and arachidonoyl-lysophospholipid transferase (18, 23). Arachidonic acid and its metabolites are involved in the regulation of a variety of physiological processes including vascular tone (23, 24). In addition, these lipid molecules have been reported to mediate intracellular signaling events in response to a number of stimulants (25–30). A large body of data also suggests that arachidonic acid and its metabolites play an important role in cell survival and proliferation (31–35). Among the large family of phospholipase A2s characterized thus far, cPLA2 appears to be one of the major sources of arachidonic acid release and therefore one of the major sources of production of the eicosanoid metabolites of arachidonic acid in response to various agonists in different cell types (36–38). Recently, we have reported that activation of the Jak/STAT pathway is required for PDGF-BB-induced cPLA2 expression and proliferation in VSMCs (39). PDGF-BB is also a potent motogen for VSMCs and is involved in the migration of VSMCs from media to intima leading to neointima formation (4, 5). To understand the molecular events of PDGF-BB-induced motility in VSMCs, we now have studied the role of Jak-2/STAT-3 and cPLA2. Here, we report for the first time that PDGF-BB induces VSMC motility via activation of Jak-2/STAT-3, which targets the induction of expression of cPLA2.
cPLA₂ Mediates Cell Migration

MATERIALS AND METHODS

Reagents—Aprotinin, dithiothreitol, phenylmethylsulfonyl fluoride, sodium orthovanadate, sodium deoxycholate, leupeptin, and HEPES were purchased from Sigma. AG490 was obtained from Calbiochem. Arachidonic acid was purchased from Cayman Chemicals (Ann Arbor, MI). Anti-cPLA₂ antibodies (2832) and phospho-specific anti-STAT-3 (9131S) antibodies were procured from Cell Signaling Technology (Beverly, MA). Phospho-specific anti-Jak-2 (44-4262) antibodies were from Upstate Biotechnology, Inc. (Lake Placid, NY). Anti-Jak-2 (SC-294) and anti-STAT-3 (SC-482) antibodies and consensus STAT-3 oligonucleotide (5'-GATCCCTTGGGAATTCCTAGATC-3', 3'-GAGGAGACCCTTGAGATCTTAG-5') (SC-2571) were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). T4 polynucleotide kinase was purchased from Promega (Madison, WI). [γ-32P]ATP (3000 Ci/mmol) was obtained from PerkinElmer Life Sciences.

Cell Culture—VSMCs were isolated from the thoracic aortae of male Sprague-Dawley rats by enzymatic dissociation as described earlier (40). Cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin. Cultures were maintained at 37 °C in a humidified 95% air and 5% CO₂ atmosphere. Cells were growth-arrested by incubating in DMEM containing 0.1% calf serum for 72 h and were used to perform the experiments unless otherwise stated.

Cell Motility—VSMC motility was measured by cell-wounding assay (41). Quiescent confluent monolayers of VSMCs were wounded with a sterile pipette tip to generate a cell-free gap of ~1 mm in width, and the wound location in the culture dish was marked. Cells were washed, and fresh serum-free DMEM was added and photographed to record the wound width at 0 h. To prevent replicative DNA synthesis, hydroxyurea was added to the medium to a final concentration of 5 mM just before the addition of agonist. Twenty-four h after the appropriate treatments, the cell extracts were prepared. An equal amount of protein from the control and each treatment was analyzed by Western blotting for pJak-2 and pSTAT-3 using their phospho-specific antibodies. As a loading control, the blots were reprobed with anti-STAT-3 antibodies.

Electrophoretic Mobility Shift Assay (EMSA)—Nuclear extracts were prepared from treated or untreated VSMCs as described previously (40). The protein content of the nuclear extracts was determined using a Micro BCA™ protein assay reagent kit (Ference). Protein-DNA complexes were formed by incubating 5 µg of nuclear protein in a total volume of 20 µl consisting of 15 mM HEPES, pH 7.9, 5 mM Tris-HCl, pH 7.9, 80 mM KCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 4.5 µg of bovine serum albumin, 2 µg of poly(dI-dC), 15% glycerol, and 10,000 cpm of 32P-labeled oligonucleotide probe for 30 min on ice. The protein-DNA complexes were resolved by electrophoresis on a 4% polyacrylamide gel using 1× Tris-glycine-EDTA buffer (25 mM Tris-HCl, pH 8.5, 200 mM glycine, 0.1 mM EDTA). Double-stranded oligonucleotides were labeled with [γ-32P]ATP using the T4 polynucleotide kinase kit (Promega) following the supplier’s protocol.

Western Blot Analysis—After appropriate treatments, VSMCs were rinsed with cold phosphate-buffered saline and frozen immediately in liquid nitrogen. Cells were lysed by thawing in 250 µl of lysis buffer (phosphate-buffered saline, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 100 µg/ml phenylmethylsulfonyl fluoride, 100 µg/ml aprotinin, 1 µg/ml leupeptin, and 1 mM sodium orthovanadate) and scraped into 1.5-ml Eppendorf tubes. After standing on ice for 20 min, the cell extracts were cleared by centrifugation at 12,000 rpm for 20 min at 4 °C. Cell extracts containing an equal amount of protein were resolved by electrophoresis on a 1% SDS and 10% polyacrylamide gels. The proteins were transferred electrophoretically to a nitrocellulose membrane (Hybond, Amersham Biosciences). After blocking in 10 mM Tris-HCl buffer, pH 8.0, containing 150 mM sodium chloride, 0.1% Tween 20, and 5% (w/v) nonfat dry milk, the membrane was treated with appropriate primary antibodies followed by incubation with horse-radish peroxidase-conjugated secondary antibodies. The antigen-antibody complexes were detected using a chemiluminescence reagent kit.

FIG. 1. PDGF-BB stimulates tyrosine phosphorylation of Jak-2 and STAT-3 in a time-dependent manner in VSMCs. Quiescent VSMCs were treated with and without PDGF-BB (20 ng/ml) for the indicated times, and cell extracts were prepared. An equal amount of protein from the control and each treatment was analyzed by Western blotting for pJak-2 and pSTAT-3 using their phospho-specific antibodies.

FIG. 2. AG490 and Jak-2KeprK5, a specific pharmacological inhibitor and a dominant negative mutant, respectively, of Jak-2, attenuate PDGF-BB-induced STAT-3 tyrosine phosphorylation. Quiescent VSMCs were treated with and without PDGF-BB (20 ng/ml) in the presence and absence of AG490 (25 µM) for the indicated times, and cell extracts were prepared. An equal amount of protein from the control and each treatment was analyzed by Western blotting for pSTAT-3 using its phospho-specific antibodies. To test the effect of Jak-2KeprK5 on STAT-3 tyrosine phosphorylation, VSMCs were transfected first with and without Jak-2KeprK5 and quiesced. Cells were then treated with and without PDGF-BB (20 ng/ml) for 10 min, and cell extracts were prepared. An equal amount of protein from the control and each treatment was analyzed for pSTAT-3 as described above. As a loading control, the blots were reprobed with anti-STAT-3 antibodies.
with DMEM containing 0.1% calf serum, and cells were transfected with pSIE-CAT plasmid using LipofectAMINE Plus reagent according to the manufacturer's instructions (Invitrogen). Thirty h after transfection, VSMCs were treated with and without PDGF-BB (20 ng/ml) in the presence and absence of AG490 (25 μM) for 2 h, and nuclear extracts were prepared. An equal amount of nuclear protein from the control and each treatment was incubated with 100,000 cpm of 32P-labeled consensus STAT-3 oligonucleotide probe, and the protein-DNA complexes were separated by EMSA and subjected to autoradiography. In the case of the use of Jak-2KEpRK5 and FS3DM, dominant negative mutants of Jak-2 and STAT-3, respectively, cells were transfected first with these plasmid DNAs and quiesced. Cells were then treated with and without PDGF-BB (20 ng/ml) for 2 h, and nuclear extracts were prepared and analyzed by EMSA as described above. B, VSMCs that were transfected with a STAT-3-dependent reporter plasmid, pSIE-CAT, were quiesced and treated with and without PDGF-BB (20 ng/ml) in the presence and absence of AG490 (25 μM) for 4 h, and cell extracts were prepared. Cell extracts containing an equal amount of protein from the control and each treatment were analyzed for CAT activity using [14C]chloramphenicol and acetyl coenzyme A as substrates. The substrate and products were extracted with ethyl acetate, separated by thin layer chromatography, and subjected to autoradiography. Wherever the effect of FS3DM, a dominant negative mutant of STAT-3, was tested, cells were co-transfected with pSIE-CAT and FS3DM and quiesced before they were subjected to the indicated treatments.

**Fig. 3.** AG490, dominant negative Jak-2 (Jak-2KEpRK5), and dominant negative STAT-3 (FS3DM) reduce PDGF-BB-induced DNA binding and reporter gene activities of STAT-3. A, quiescent VSMCs were treated with and without PDGF-BB (20 ng/ml) in the presence and absence of AG490 (25 μM) for 2 h, and nuclear extracts were prepared. An equal amount of nuclear protein from the control and each treatment was incubated with 100,000 cpm of 32P-labeled consensus STAT-3 oligonucleotide probe, and the protein-DNA complexes were separated by EMSA and subjected to autoradiography. In the case of the use of Jak-2KEpRK5 and FS3DM, dominant negative mutants of Jak-2 and STAT-3, respectively, cells were transfected first with these plasmid DNAs and quiesced. Cells were then treated with and without PDGF-BB (20 ng/ml) for 2 h, and nuclear extracts were prepared and analyzed by EMSA as described above. B, VSMCs that were transfected with a STAT-3-dependent reporter plasmid, pSIE-CAT, were quiesced and treated with and without PDGF-BB (20 ng/ml) in the presence and absence of AG490 (25 μM) for 4 h, and cell extracts were prepared. Cell extracts containing an equal amount of protein from the control and each treatment were analyzed for CAT activity using [14C]chloramphenicol and acetyl coenzyme A as substrates. The substrate and products were extracted with ethyl acetate, separated by thin layer chromatography, and subjected to autoradiography. Wherever the effect of FS3DM, a dominant negative mutant of STAT-3, was tested, cells were co-transfected with pSIE-CAT and FS3DM and quiesced before they were subjected to the indicated treatments.

**Fig. 4.** PDGF-BB induces VSMC motility in a Jak-2/STAT-3-dependent manner. A, a cell-free gap was made in a monolayer of quiescent VSMCs and treated with and without various doses of PDGF-BB for 24 h, and cell motility was measured using the NIH Image 1.62 program. B, VSMCs were transfected with and without Jak-2KEpRK5 (DnJAK-2) or FS3DM, the dominant negative mutants of Jak-2 and STAT-3, respectively, and quiesced prior to testing their responsiveness to PDGF-BB-induced motility. *, p < 0.01 versus control; **, p < 0.01 versus PDGF-BB treatment alone.
Results
To understand the signaling events underlying PDGF-BB-induced motility in VSMCs, we have studied the role of the Jak/STAT pathway. Quiescent VSMCs were treated with and without PDGF-BB (20 ng/ml) for various times, and cell extracts were prepared. An equal amount of protein from the control and each treatment was analyzed by Western blotting using its specific antibodies. The bar graph represents the quantitative data of three independent experiments on the time course effect of PDGF-BB on cPLA₂ expression. VSMCs were treated with and without PDGF-BB (20 ng/ml) for the indicated times, and cell extracts were prepared. An equal amount of protein from the control and each treatment was analyzed by Western blotting for cPLA₂ using its specific antibodies. The bar graph represents the quantitative data of three independent experiments on the time course effect of PDGF-BB on cPLA₂ expression. VSMCs were treated with and without PDGF-BB (20 ng/ml) for 16 h, and cell extracts were prepared and analyzed for cPLA₂ levels as described above. *, p < 0.01 versus control.

Statistics—All of the experiments were repeated three times with similar results. Data are presented as mean ± S.D. The treatment effects were analyzed by Student’s t test. p values of <0.05 were considered to be statistically significant. In the case of CAT activity, EMSA, and Western blot analysis, one representative set of data is shown.

FIG. 5. AG490, Jak-2KEpRK5, and FS3DM inhibit PDGF-BB-induced expression of cPLA₂. A, quiescent VSMCs were treated with and without PDGF-BB (20 ng/ml) for the indicated times, and cell extracts were prepared. An equal amount of protein from the control and each treatment was analyzed by Western blotting for cPLA₂ using its specific antibodies. The bar graph represents the quantitative data of three independent experiments on the time course effect of PDGF-BB on cPLA₂ expression. B, quiescent VSMCs were treated with and without PDGF-BB (20 ng/ml) in the presence and absence of AG490 (25 μM) for the indicated times, and cell extracts were prepared. An equal amount of protein from the control and each treatment was analyzed by Western blotting for cPLA₂ using its specific antibodies. In testing the effect of dominant negative Jak-2 and STAT-3 mutants on cPLA₂ expression, VSMCs were transfected first with and without Jak-2KEpRK5 or FS3DM and quiescent. Cells were then treated with and without PDGF-BB (20 ng/ml) for 16 h, and cell extracts were prepared and analyzed for cPLA₂ levels as described above. *, p < 0.01 versus control.

For protein and assayed for CAT activity using [14C]chloramphenicol and acetyl coenzyme A as substrates. The substrate and products were extracted with ethyl acetate, separated by thin layer chromatography, and subjected to autoradiography.

To understand the signaling events underlying PDGF-BB-induced motility in VSMCs, we have studied the role of the Jak/STAT pathway. Quiescent VSMCs were treated with and without PDGF-BB (20 ng/ml) for the indicated times, and cell extracts were prepared. An equal amount of protein from the control and each treatment was analyzed by Western blotting for tyrosine phosphorylation of Jak-2 and STAT-3 using their phospho-specific antibodies. PDGF-BB stimulated tyrosine phosphorylation of both Jak-2 and STAT-3 in a time-dependent manner with a maximum effect of about 20-fold at 10 min and reaching basal levels by 4 h (Fig. 1). Jaks phosphorylate STATs on tyrosine residues and activate them, although other mechanisms were also reported to be involved in the activation of these transcriptional factors (42, 43). To find whether PDGF-BB-stimulated STAT-3 tyrosine phosphorylation is mediated by Jak-2, we tested the effect of AG490, a potent and specific inhibitor of Jak-2 (44). AG490 (25 μM) significantly inhibited PDGF-BB-stimulated tyrosine phosphorylation of STAT-3 (Fig. 2, upper left panel). To confirm the pharmacological effect of AG490 on PDGF-BB-induced STAT-3 phosphorylation, we next tested the effect of dominant negative Jak-2 (Jak-2KEpRK5). Forced expression of Jak-2KEpRK5 also reduced PDGF-BB-induced STAT-3 phosphorylation by about 50% (Fig. 2, upper right and lower panels).
Upon tyrosine phosphorylation, STATs undergo either homo- or heterodimerization and translocate to the nucleus, where they bind (in this case STAT-3) to their consensus DNA binding sequence present in the promoter regions of genes and induce transcription (13, 45). To find whether tyrosine phosphorylation of STAT-3 correlates with an increase in its transcriptional activation, its DNA binding activity was measured. Quiescent VSMCs were treated with and without PDGF-BB (20 ng/ml) for 2 h, and nuclear extracts were prepared. Equal amounts of nuclear protein from the control and each treatment were analyzed by EMSA for STAT-3-DNA binding activity using 32P-labeled consensus STAT-3 oligonucleotide as a probe. PDGF-BB increased STAT-3-DNA binding activity by 4-fold, and it was inhibited by AG490 (Fig. 3A, left panel). Forced expression of either dominant negative Jak-2 (Jak-2KEpRK5) or STAT-3 (FS3DM) also substantially reduced PDGF-BB-induced STAT-3-DNA binding activity (Fig. 3A, right panel). To confirm that increased STAT-3-DNA binding activity leads to an increase in its transactivation activity, VSMCs were transiently transfected with a STAT-3-dependent reporter plasmid, pSIE-CAT, quiesced, and treated with and without PDGF-BB (20 ng/ml) for 4 h, and cell extracts were prepared. Cell extracts normalized for protein were assayed for CAT activity. PDGF-BB induced STAT-3-dependent CAT activity by 3-fold, and AG490 substantially inhibited this response (Fig. 3B, left panel). FS3DM also blocked PDGF-BB-induced STAT-3-dependent CAT activity (Fig. 3B, right panel). These findings are consistent with our previous observations on PDGF-BB activation of the Jak/STAT pathway (39). To understand the role of the Jak/STAT pathway in PDGF-BB-induced VSMC motility, we first studied a dose-response effect of PDGF-BB. A cell-free gap was generated in a monolayer of quiescent VSMCs and treated with and without PDGF-BB (20 ng/ml) in the presence and absence of MAFP (10 μM) or PACOCF3 (10 μM) for 24 h, and cell motility was measured using the NIH Image 1.62 program. *, p < 0.01 versus control; **, p < 0.01 versus PDGF-BB treatment alone.

It was reported that arachidonic acid and its eicosanoid metabolites play an important role in cell migration (46–49). In addition, arachidonic acid, the precursor for eicosanoids, and other mitogenically active lipids such as phosphatidic acid have been shown to activate GTPases via inhibition of GTPase-activating proteins (29, 30, 50, 51). GTPases play an essential role in cell proliferation and migration (52). We have shown previously that STATs are involved in PDGF-BB-induced cPLA2 expression (39). Because cPLA2 plays a predominant role in arachidonic acid release in response to a variety of bioactive agents including growth factors (27, 36–38), we envisioned that Jak-STAT signaling may be mediating cell migration via induction of expression of cPLA2. To test this possibility, quiescent VSMCs were treated with and without PDGF-BB (20 ng/ml) for various times, and cell extracts were prepared. An equal amount of protein from control and PDGF-BB-treated cells was analyzed by Western blotting for cPLA2 expression. PDGF-BB induced cPLA2 expression in a time-dependent manner with 2- and 3-fold increases at 8 and 16 h of treatment, respectively (Fig. 5A). PDGF-BB also induced the expression of cPLA2 mRNA by 4- and 5-fold at 8 and 16 h of treatment, respectively, as measured by Northern blot analysis using 32P-labeled rat cPLA2 cDNA probe. To understand whether the Jak/STAT pathway plays a role in PDGF-BB-induced expression of cPLA2, we next studied the effect of AG490. AG490 completely inhibited PDGF-BB-induced cPLA2 expression (Fig. 5B). To obtain additional evidence on the role of the Jak/STAT pathway in PDGF-BB-induced cPLA2 expression, we tested the effect of dominant negative Jak-2 and STAT-3 mutants, Jak-2KEpRK5 and FS3DM, respectively (17, 53). As shown in Fig. 5B, forced expression of Jak-2KEpRK5 or FS3DM also

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2 N. Dronadula et al., unpublished observations.
The important finding of the present study is that PDGF-BB, a receptor tyrosine kinase agonist, induces VSMC motility via a pharmacological approach. Use of methyl arachidonoyl fluorophosphonate (MAFP) and palmityl trifluoromethyl ketone (PACOCF3), two structurally different and specific inhibitors of cPLA2 (38, 54), significantly blocked PDGF-BB-induced VSMC motility (Fig. 6). If Jak-2/STAT-3 signaling mediates PDGF-BB-induced VSMC motility via induction of expression of cPLA2, then one would expect that exogenous addition of arachidonic acid would rescue PDGF-BB-induced VSMC motility from inhibition by blockade of Jak-2/STAT-3 activation. To test this, a cell-free gap was made in a monolayer of quiescent VSMCs that were forced to express FS3DM and treated with and without PDGF-BB (20 ng/ml) in the presence and absence of exogenously added arachidonic acid (5 μM) for 24 h, and cell motility was measured. As shown in Fig. 7, arachidonic acid rescued PDGF-BB-induced VSMC motility from inhibition by FS3DM. Exogenous arachidonic acid (5 μM) also surpassed the inhibitory effect of AG490 on PDGF-BB-induced VSMC motility (Fig. 7). To confirm this result further, VSMCs were co-transfected with dominant negative Jak-2 (Jak-2KEpRK5) or STAT-3 (FS3DM) along with and without an expression plasmid for rat cPLA2 (pcDNA3-cPLA2) and were quiesced. These cells then were treated with and without PDGF-BB (20 ng/ml) for 24 h, and cell motility was measured using the NIH Image 1.62 program as described above in the legend of Fig. 7. *, p < 0.05 versus control; **, p < 0.05 versus PDGF-BB treatment alone; ***, p < 0.05 versus Jak-2KEpRK5 + PDGF-BB or FS3DM + PDGF-BB.

**DISCUSSION**

The important finding of the present study is that PDGF-BB, a receptor tyrosine kinase agonist, induces VSMC motility via Jak-2/STAT-3-dependent induction of expression of cPLA2. This conclusion is supported by the following observations. 1) PDGF-BB stimulated tyrosine phosphorylation of Jak-2 and STAT-3 in a time-dependent manner. 2) The tyrosine phosphorylation of STAT-3 and its DNA binding and reporter gene activities induced by PDGF-BB are mediated by Jak-2 as its inhibition by pharmacological and dominant negative mutant approaches suppressed the activation of STAT-3. 3) Jak-2-dependent STAT-3 activation is required for VSMC motility induced by PDGF-BB as dominant negative mutants of these signaling molecules prevented the VSMC motility in response to this agonist. 4) PDGF-BB-induced cPLA2 expression is sensitive to inhibition by dominant negative mutants of Jak-2 and STAT-3. 5) Pharmacological inhibition of cPLA2 blocked PDGF-BB-induced VSMC motility. 6) Exogenous addition of arachidonic acid rescued PDGF-BB-induced VSMC motility from inhibition by blockade of Jak-2 and STAT-3 activation. 7) Forced expression of rat cPLA2 also overcame the inhibitory effect of dominant negative Jak-2 and STAT-3 on PDGF-BB-induced VSMC motility. In addition to its role in cell proliferation and differentiation (15–17), emerging evidence indicates the involvement of the Jak/STAT pathway in the regulation of cell migration (55, 56). Although the structure and function relationship of these signaling molecules was well studied (13, 14), the mechanisms of their action in the regulation of cell migration and proliferation are largely unclear. In this regard, the present study provides evidence for a role of cPLA2 as one of the distal molecules in Jak-2/STAT-3-mediated cell migration. Analysis of both human and rat cPLA2 promoters for regulatory elements also revealed the presence of several binding sequences for STATs (57, 58). The finding that AG490, a specific and potent inhibitor of Jak-2 (44), and dominant negative mutants of both Jak-2 and STAT-3 suppress PDGF-BB-induced cPLA2 protein levels as well as the presence of STAT regulatory elements in its promoter further support a role for cPLA2 in cellular responses mediated by Jak/STAT signaling.

It was reported that arachidonic acid and its eicosanoid metabolites play a role in cell migration (46–49). Arachidonic acid and its eicosanoid metabolites have also been shown to activate GTPases via inhibition of GTPase-activating proteins (29, 30, 50, 51). GTPases play an essential role in cell migration (52). Based on these observations it is plausible that Jak-2/STAT-3-mediated cPLA2 expression may be involved in a sustained arachidonic acid release producing eicosanoids, which in turn promote VSMC chemotaxis. A large body of evidence indicates that cPLA2 mediates the release of arachidonic acid and, thereby, eicosanoid production in response to a variety of bioactive agents (18–22). In earlier studies, we have reported that Jak-2/STAT-3 mediates PDGF-BB-induced arachidonic acid and its eicosanoid metabolites play a role in cell migration (46–49). Arachidonic acid and its eicosanoid metabolites have also been shown to activate GTPases via inhibition of GTPase-activating proteins (29, 30, 50, 51). GTPases play an essential role in cell migration (52). Based on these observations it is plausible that Jak-2/STAT-3-mediated cPLA2 expression may be involved in a sustained arachidonic acid release producing eicosanoids, which in turn promote VSMC chemotaxis. A large body of evidence indicates that cPLA2 mediates the release of arachidonic acid and, thereby, eicosanoid production in response to a variety of bioactive agents (18–22). In earlier studies, we have reported that Jak-2/STAT-3 mediates PDGF-BB-induced arachidonic acid-etichon.
acid release in VSMCs (39). Arachidonic acid, while serving as a precursor for the production of biologically important lipid mediators, is also a crucial component for membrane phospholipid remodeling. Phospholipase A₂ via production of arachidonic acid and lipid mediators have been reported to play a role in membrane phospholipid remodeling (59, 60). Plasma membrane protrusion, retraction, and contraction forces that are essential for cell motility involve membrane phospholipid remodeling. In this regard, it is tempting to speculate that Jak-2/STAT-3 mediates PDGF-BB-induced motility. Future studies are required to test the role of the Jak/STAT-3-dependent cPLA₂ expression may be involved in membrane phospholipid remodeling during cell motility. In VSMCs via targeting the induction of expression of cPLA₂.

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REFERENCES

1. Ross, R. (1999) *N. Engl. J. Med.* 340, 115–126
2. Hansson, G. K., Libby, P., Schonbeck, U., and Yan, Z. Q. (2002) *Curr. Res. 91*, 281–291
3. Berk, B. C. (2001) *Physiol. Rev.* 81, 999–1030
4. Jawien, A., Bowen-Pope, D. F., Lindner, V., Schwartz, S. M., and Clowes, A. W. (1999) *J. Clin. Investig.* 103, 357–364
5. Kalies, A., Vesti, B. R., Duann, G., Abraham, J. A., and Clowes, A. W. (2000) *Circ. Res.* 87, 92–98
6. Goetzke, S., Xi, X. P., Kawano, Y., Kawano, H., Fleck, E., Hsueh, W. A., and Law, R. E. (2000) *Hypertension* 36, 739–745
7. Duan, G., Baas, A. S., Glasgow, W. C., Eling, T. E., Runge, M. S., and Alexander, R. W. (2000) *Biochim. Biophys. Acta* 149, 345–347
8. Piomelli, D. (1993) *Curr. Opin. Cell Biol.* 5, 278–284
9. Dethlefsen, S. M., Shepro, D., and D’Amore, P. A. (1994) *Exp. Cell Res.* 212, 262–273
10. Gronich, J., Konieczkowski, M., Gelb, M. H., Nemenoff, R. A., and Seader, J. R. (1994) *J. Clin. Investig.* 93, 1224–1233
11. Rao, G. N., Runge, M. S., and Alexander, R. W. (1995) *Biochim. Biophys. Acta* 1263, 67–72
12. Smith, W. L. (1989) *Biochem. J.* 259, 315–324
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