Upregulation and Activation of Stat6 Precede Vascular Smooth Muscle Cell Proliferation in Carotid Artery Injury Model

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Abstract—The role of signal transducers and activators of transcription (STAT) proteins in modulating proliferation and differentiation of various cell types in the hematopoietic system and the central nervous system has been well established. In contrast, the pathophysiological role of these proteins in vascular proliferative diseases has remained unproven, despite in vitro observations emphasizing the involvement of the STAT system in mediating vascular smooth muscle cell (VSMC) proliferation. On the basis of our previous observations demonstrating the occurrence of a specific modulation of Stat6 protein during the proliferative, migratory, and differentiation phases of the developing brain, we investigated whether Stat6 protein is present and modulated in arterial tissue challenged by perivascular injury. The time course of expression and localization of Stat6 after arterial injury was analyzed by immunohistochemistry, Western blot analysis, and confocal microscopy. Six hours after injury, the expression of Stat6 was markedly increased. This overexpression preceded the onset of VSMC proliferation and was downregulated starting from 7 days after injury, coincident with the decline of VSMC proliferation. Moreover, early after injury, Stat6 was predominantly localized at the nuclear level, denoting its functional activation. Conversely, Stat6 staining at later time points was largely cytosolic, suggesting silencing effects of this signaling pathway. These data indicate that Stat6 signaling may contribute to the modifications of gene expression underlying VSMC activation in the context of acute vascular proliferative diseases. (Arterioscler Thromb Vasc Biol. 2000;20:931-939.)

Key Words: Stat6 ■ vascular smooth muscle ■ proliferation ■ intima hyperplasia ■ vascular injury

Intimal thickening in response to vascular injury results from excessive multiplication of vascular smooth muscle cells (VSMCs) and deposition of extracellular matrix in the intimal layer of the vessel wall.1– 6 In normal arteries, VSMCs are in a fully differentiated, low proliferative rate phenotype; however, a number of growth factors or inflammatory cytokines elaborated by infiltrating leukocytes and activated intrinsic vascular cells can promote VSMCs to dedifferentiate and begin to replicate within the media, to migrate from the media into the intima, and to massively proliferate within the intima.5,7,8

Changes in VSMC behavior induced by extracellular stimuli require the execution of a rapid and complex program of transcriptional events, which results in the emergence of VSMCs from quiescence (for a review see Reference 9). The nature of the molecular pathways specifically coupling the vascular injury stimuli to the VSMC dedifferentiation process is currently under intense investigation in an attempt to identify critical targets for interventional therapies.10–19

The quantitative and qualitative changes observed in VSMC gene expression during this transition involve, among others, the activation of distinct cytosolic signaling pathways.9 A signaling cascade that has been correlated with mitogenic and pleiotropic functional responses induced by a variety of growth factors and cytokines involves the members of the Janus kinase (JAK) family of cytoplasmic tyrosine kinases (Jak1, Jak2, Jak3, and Tyk2) and the signal transducers and activators of transcription (STAT) proteins (Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b, and Stat6).20–23 The STAT proteins, once phosphorylated on tyrosine by the JAKs, translocate to the nucleus, where they bind to DNA elements, eliciting the transcription of specific genes. Studies performed in various cellular systems have identified genes associated with the regulation of cell proliferation and differentiation.20–23

Extensive molecular and biochemical analysis of the JAKs and STATs has been performed in the hematopoietic system, where they were found to transduce signals of proliferation

Received May 18, 1999; revision accepted September 29, 1999.
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Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org
and differentiation. However, similar regulatory activities of the JAK/STAT system have been recently proposed also for cells of different origin, such as developing neurons, fibroblasts, and vascular cells. In particular, we found a specific modulation of Stat6 expression during the phases of proliferation, migration, and differentiation in the developing brain; the other STATs remained unchanged or only mildly modified.

In the present study, we were interested in extending our observation to the potential role of Stat6 in the arterial wall, a tissue district different from the developing central nervous system but in which similar cellular events are known to occur in response to activating stimuli. Therefore, we analyzed the time-dependent expression and activation of Stat6 in a rabbit model of perivascular injury in which a reproducible intimal lesion, primarily composed of smooth muscle cells, is generated by placing a nonocclusive, biologically inert, soft, and hollow silicone collar around 1 of the common carotid arteries. We observed that Stat6 protein is upregulated and activated within hours after arterial injury. Stat6 levels remained elevated throughout the entire thickness of the smooth muscle tissue during the first 5 days after perivascular manipulation, a time period during which smooth muscle cells are actively proliferating and intimal thickening is forming. These observations set Stat6 activation among the early events preceding VSMC proliferation in response to acute vascular injury.

Methods

Animal Model

The study was performed according to the Guidelines for Animal Care and Treatment of the European Community. Twenty-one male New Zealand White rabbits (2.3 to 2.5 kg, Charles River, Calco, Italy) were anesthetized by an intramuscular injection of xylazine (5 mg/kg, Rompun, Bayer AG) and ketamine (35 mg/kg, Inoketam, Virbac). A nonocclusive, biologically inert, soft, hollow Silastic collar (Silicoll, MediGene Oy) was positioned around the right carotid artery, as previously described. In each animal, the contralateral carotid artery was sham-operated by placing the collar around the vessel but removing it just before both carotid arteries were carefully removed and divided into 2 equal parts. The proximal half was directly processed for frozen sections; the distal half was immersion-fixed in 4% paraformaldehyde and embedded in paraffin. For Western blot analyses, arteries were immediately frozen in liquid nitrogen and homogenized in 50 mmol/L Tris, pH 7.5, 150 mmol/L NaCl, 5 mmol/L EDTA, 1 mmol/L EGTA, 1% Triton X-100, 100 mg/mL aprotinin, 1 mmol/L NaVO₃, and 10 mmol/L NaF) in the presence of 1 mmol/L phenylmethylsulfonyl fluoride, 10 mg/mL aprotinin, and 5 mg/mL leupeptin at 4°C.

Cell Cultures

Carotid Artery Segments

Tissue samples were pulverized in liquid nitrogen and homogenized in lysis buffer (10 mL per milligram of tissue, containing 50 mmol/L Tris, pH 7.5, 150 mmol/L NaCl, 5 mmol/L EDTA, 1 mmol/L EGTA, 1% Triton X-100, 100 mg/mL aprotinin, 1 mmol/L NaVO₃, and 10 mmol/L NaF) in the presence of 1 mmol/L phenylmethylsulfonyl fluoride, 10 mg/mL aprotinin, and 5 mg/mL leupeptin at 4°C.

Cell Cultures

Smooth muscle cells were cultured, according to the method of Ross, from the intimal-medial layers of the common carotid artery of the male New Zealand White rabbits. Cells were maintained in monolayer culture at 37°C in a humidified atmosphere containing 5% CO₂ in DMEM (GIBCO Laboratories) supplemented with 10% FCS, penicillin-streptomycin, and sodium pyruvate (GIBCO). At confluence, cultures were replaced by enzymatic dissociation with trypsin/EDTA (GIBCO). Cells were seeded onto 13-mm-diameter round glass coverslips at a density of 50,000 cells per milliliter, grown over 2 days, and serum-starved for an additional 2 days in DMEM containing 0.4% FCS before use. All experiments were performed within the 10th subculture.

Primary Antibodies

Two different murine monoclonal antibodies directed against Stat6 were used in the present study: IL4-Stat (clone 38), purchased from Transduction Laboratories, and Stat6(C-9), purchased from Santa Cruz Biotechnology Inc. The monoclonal mouse IgG2b antibody generated from a 30.5-kDa protein fragment corresponding to amino acids 1 to 272 of the human Stat6. This antibody is reported by the manufacturer to display its reactivity against Stat6 of mouse, rat, human, and chick origin. Stat6(C-9) is a monoclonal mouse IgG1 antibody raised against a recombinant protein corresponding to amino acids 280 to 480 of Stat6 of mouse origin. According to the manufacturer’s instructions, this antibody recognizes Stat6 of mouse, rat, and human origin and does not cross-react with Stat1α p91, Stat1β p84, Stat2 p113, Stat3, Stat4, or Stat5. Both antibodies gave the same results in our assays.

Mouse monoclonal antibody against proliferating cell nuclear antigen (PCNA, clone PC10, IgG2a) was from Dako. Mouse monoclonal antibody against the human interleukin (IL)-4 receptor (CD132, IgG1) was from Genzyme Diagnostics. Mouse monoclonal anti–platelet-derived growth factor (anti-PDGF)-β receptor (clone PDGFR-B2, IgG2b) was from Sigma Chemical Co.

Immunoblot Analysis

Cells were rinsed 3 times in PBS and then lysed in lysis buffer (600 µL/cm²). The collected material was passed several times through a 1-mL (26-gauge) insulin syringe needle, incubated for 30 minutes on ice, and then centrifuged. Protein concentrations were determined by the method of Lowry et al. Aliquots of extracts were diluted with SDS-sample buffer and boiled for 5 minutes. Equal amounts of proteins (50 µg) were loaded onto 7.5% SDS-polyacrylamide gel. After transfer to nitrocellulose, the blots were blocked in 5% nonfat dry milk in TBS-T (20 mL/mL/L Tris, pH 7.5, 500 mL/mL/L NaCl, and 0.01% Tween 20) overnight at 4°C. Blots were then incubated with individual mouse monoclonal Stat6 antibodies [IL4-Stat, working dilution 1:1000; Stat6(C-9), working dilution 1:200] for at least 1 hour at room temperature. After they were washed with TBS-T, membranes were exposed to goat anti-mouse horseradish peroxidase–conjugated secondary antibody (1:10,000 dilution; 1 hour, room temperature; Kirkegaard and Perry Laboratories). Immunoreactivitiess were detected by the enhanced chemiluminescence method (ECL, Amersham) according to manufacturer’s instructions.

Immunocytochemistry

Cells were fixed for 5 minutes in –20°C methanol, air-dried, and permeabilized by incubation in TBS-Tween (0.1%) for 10 minutes at room temperature. Coverslips were then incubated under humidified conditions for 45 minutes with the primary antibody (200 µL of a 1:100 dilution in PBS/BSA at 3% for both the anti-Stat6 antibodies used; see Primary Antibodies). Primary antibodies were detected by a 45-minute incubation with an FITC-conjugated goat anti-mouse IgG antibody (1:64 working dilution, Sigma). Finally, the coverslips were rinsed twice in PBS and mounted on slides by inversion over 10 µL Vectashield mounting medium (Vector Laboratories) as an antibleaching agent. Cells were examined with a Zeiss Axioskop.
secondary antibody as described in Methods. B, Western blot analysis of total cell lysates from cultured VSMCs, showing a strongly immunoreactive 100-kDa band. Cells were lysed as described in Methods, electrophoresed, and blotted with anti-Stat6 antibody (IL4-Stat). Jurkat total cell lysate is the positive control for Stat6 expression.

Immunohistochemistry
All immunohistochemical analyses were performed on PFA-fixed paraffin-embedded 5-μm sections, with the exception of IL-4 receptor staining, which was performed on OCT-embedded 10-μm cryosections. Sections were incubated for 20 minutes in 10% BSA (Sigma), followed by enzymatic digestion for antigen retrieval, when required (see later details).

Stat6
Sections were treated with 0.05% pepsin (Sigma) in 20 mmol/L HCl, pH 1.5, at 37°C for 20 minutes. Slides were then incubated for 1 hour at room temperature with individual primary antibodies: IL4-Stat, working dilution 1:100, or Stat6(C-9), working dilution 1:10. Labeling was performed with an FITC-conjugated goat anti-mouse IgG antibody (working dilution 1:64, Sigma). The average intensity of Stat6 staining over the entire thickness of the smooth muscle tissue was quantitatively evaluated by using computer-assisted image quantification (Optimas 6.2, Media Cybernetics) and standard image-processing methods. Measurements have been performed on TIFF images obtained from representative microphotographs (original magnification ×400) from independent sets of experiments (one section per time point per each experiment). An identical time of exposure was selected for taking all photographs. TIFF images were collected by using a high-resolution scanner (Agfa DuoScan Color Scanner, Agfa Corp) in conjunction with Agfa FotoLook 3.0, which was used as scanner control software that allows an automatic mode of operation. To minimize artifactual electronic noise and to remove the intensity contribution due to autofluorescence of elastin fibers and internal elastic lamina, the intensity value obtained for each specimen image (luminance) has been adjusted by the arithmetic subtraction of the corresponding fluorescein-conjugated antibody control (ie, an adjacent section in which primary antibody was omitted and incubation was performed in the presence of normal horse serum), processed under the same conditions of image acquisition and analysis.

Confocal microscopic analysis of Stat6 staining was performed by use of a Nikon apparatus with optical sections of 0.5 μm.

Proliferating Cell Nuclear Antigen
Nonpredigested sections were incubated with primary antibody (working dilution 1:50) for 1 hour at room temperature. Primary antibody was detected by use of an avidin-biotin-peroxidase kit (Vectastain ABC Elite, Vector Laboratories Inc) followed by 3,3-diaminobenzidine. Sections were counterstained with hematoxylin according to standard procedures, and analysis of PCNA-positive nuclei count per area unit was performed by the aid of Optimas 6.2.

Results
Expression of Stat6 Protein in Cultured Rabbit Carotid Artery Smooth Muscle Cells
As the first step in determining the potential involvement of Stat6 in the signaling pathways leading to VSMC activation, we investigated whether Stat6 is expressed by cultured VSMCs isolated from rabbit carotid arteries. Immunocytochemical analysis performed on methanol-fixed VSMC cultures that made use of the anti-Stat6 antibody IL4-Stat disclosed a strong cytosolic Stat6 staining (Figure 1A). The expression of Stat6 by rabbit VSMCs was confirmed by Western blot analysis with IL4-Stat antibody on total carotid lysates obtained at different time points after vascular injury (1, 3, and 5 days). For each displayed time point (Figure 2), immunoreactivities of the sham-operated uninjured artery (left blots) and the corresponding collared artery (right blots) are reported. In sham-operated uninjured arteries, Stat6 expression is very low or undetectable at all time points, whereas an intense immunoreactive 100-kDa band (arrow) is detectable in lysates from collared carotid arteries. Similar results were obtained in 3 additional independent sets of experiments.

PDGF-β Receptor
Sections were predigested with pepsin, as described for Stat6 staining. Slides were then incubated overnight at 4°C with primary antibody (working dilution 1:50), followed by incubation with FITC-conjugated goat anti-mouse IgG antibody (working dilution 1:64) for 45 minutes at room temperature.

IL-4 Receptor
Cryosections were incubated overnight at 4°C with primary antibody (working dilution 1:20). Labeling was performed with FITC-conjugated goat anti-mouse IgG antibody (working dilution 1:64) for 45 minutes at room temperature.

All immunofluorescence analyses were performed on both collared and sham-operated rabbit carotid arteries (n = 3 per time point). In all experiments, negative controls were included in which the primary antibody was omitted and sections were incubated with normal horse serum.
blot analysis of total cell lysates, in which a strongly immunoreactive 100-kDa band could be detected (Figure 1B). Identical results were obtained by immunocytochemistry and Western blot analysis that made use of the anti-Stat6 antibody Stat6(C-9) (data not shown), which recognizes epitopes in the fragment corresponding to amino acids 280 to 480 of Stat6 of mouse origin.

Induction of Stat6 Protein Expression After Perivascular Collaring of Rabbit Carotid Arteries

To elucidate whether Stat6 is involved in intimal thickening in vivo, we examined its pattern of expression after perivascular injury of rabbit carotid arteries. Western blot analysis from total carotid lysates with IL4-Stat antibody indicated that in sham-operated uninjured artery (B). Detectable levels of Stat6 immunofluorescence are also evident in the media and in the intima of carotid sections obtained at 1 day (G) and 5 days (H) after collar positioning. At later time points (L, 7 days; M, 14 days), Stat6 expression is downregulated, as shown by the marked decline in the immunofluorescence staining, in both the medial and, to a larger extent, the intimal tissue. In some of the samples, the incubation with fluorescein-conjugated secondary antibody was followed by nuclear staining with Vectashield mounting medium containing propidium iodide (Vector Laboratories). Results are shown in the 4 right panels: C, sham-operated uninjured artery; F, 12-hour collared artery; I, 5-day collared artery; and N, 14-day collared artery. Arrows indicate internal elastic lamina; L, lumen. Arrowheads in panels L to N indicate the luminal edge of the intimal tissue. All panels are representative microphotographs (original magnification ×400) of at least 3 independent sets of experiments.

Figure 3. Time course of Stat6 expression after perivascular collaring of rabbit carotid arteries. Stat6 expression was detected by immunohistochemical staining on PFA-fixed paraffin-embedded carotid sections as described in Methods. At 6 hours (D) and 12 hours (E) after perivascular collaring, Stat6 is already abundantly present in medial tissue, whereas no immunostaining is evident in either the negative control (A, primary antibody omitted) or the sham-operated uninjured artery (B).
operated uninjured arteries, Stat6 is expressed at very low or undetectable levels (basal); however, analysis of lysates from carotid arteries subjected to periadventitial collaring revealed an intense band at 100 kDa corresponding to the correct molecular mass of Stat6 (Figure 2). As shown, Stat6 expression in collared arteries was found to be elevated over basal levels at day 1, and the expression remained higher at days 3 and 5 after injury. At later time points, Stat6 was returning to basal levels (see below).

**Upregulation of Stat6 Precedes VSMC Proliferation Induced by Arterial Injury**

We also analyzed Stat6 immunoreactivity of carotid sections retrieved from animals euthanized at different time points after perivascular collaring (Figure 3). As already shown by Western blot analysis (Figure 2), Stat6 protein is not detected in uninjured sham-operated arteries (Figure 3B and 3C) at any of the time points analyzed. However, after arterial collaring, Stat6 immunostaining was found to be dramatically increased throughout the entire thickness of the smooth muscle tissue (Figure 3D to 3I). Even in this case, both the antibodies used gave identical results. Staining of adjacent serial sections with a specific anti-smooth muscle actin monoclonal antibody (clone 1A4, 1:50 dilution, Sigma) established that VSMCs were the predominant cell type responsible for Stat6 expression (data not shown). Immunohistochemistry also revealed that Stat6 was already abundantly expressed at 6 hours after collar positioning and suggested nuclear translocation of this transcription factor early after injury (Figure 3D). Moreover, Stat6 overexpression preceded the appearance of detectable levels of VSMC proliferation, as assessed by PCNA immunostaining (Figure 4), indicating that intimal thickening in collared arteries follows a distinct wave of cellular proliferation occurring throughout the smooth muscle tissue.

Proliferation starts at 1 day after perivascular manipulation (Figure 4D), increases at 3 days after injury (Figure 4E), and peaks at 5 days (Figure 4F). The proliferation rate decreases by 7 days (Figure 4G) and returns to control levels at 14 days after injury (Figure 4H). Notably, our immunohistochemical analysis revealed downregulation of Stat6 protein starting from 7 days after injury (Figure 3L to 3N), coincident with the decline of VSMC proliferation in this experimental system (Figure 4G and 4H). These data suggest the possible existence of a correlation between Stat6 expression and cell proliferation induced by arterial damage, as schematically represented in Figure 5, which shows the temporal pattern of

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**Figure 4.** Immunohistochemical assessment of VSMC proliferation in medial and intimal carotid tissue after perivascular collaring. PCNA staining (brown color) is shown for sham-operated uninjured arteries (A) and for collared carotid arteries at 6 hours (B), 12 hours (C), 1 day (D), 3 days (E), 5 days (F), 7 days (G), and 14 days (H). Intimal thickening in collared arteries is preceded by a distinct wave of cellular proliferation throughout the arterial wall. Proliferation starts at 1 day after perivascular manipulation (D), increases at 3 days after injury (E), and peaks at 5 days (F). Proliferation rate decreases by 7 days (G) and returns to control levels at 14 days after injury (H). Uninjured sham-operated arteries do not show PCNA immunoreactivity (A). Arrows indicate internal elastic lamina. All panels are representative microphotographs (original magnification ×400) of at least 3 independent sets of experiments.
quantitative Stat6 expression and PCNA staining during intimal thickening in injured carotid arteries.

**Nuclear Translocation of Stat6 at Earlier Time Points After Arterial Injury**

To better assess the intracellular localization of Stat6 protein, we performed confocal microscopic analysis of immunofluorescence staining (Figure 6). This approach demonstrated that during the initial stages of lesion formation, Stat6 was predominantly localized at the nuclear level (Figure 6A); starting from day 1, Stat6 localization was found to be mainly cytosolic (Figure 6B).

**IL-4/IL-13 Receptor α-Chain and PDGF-β Receptor Are Found In Vivo in Injured Rabbit Carotid Arteries.**

To identify potential ligand/receptor complexes involved in Stat6 activation after periarterial injury, we investigated the expression of IL-4 receptor α-chain (equivalent to IL-13 receptor α-chain) and PDGF-β receptor, because signaling from either of these 2 receptors may trigger Stat6 phosphorylation. Immunohistochemical analysis of collared rabbit carotid arteries showed that injury results in the induction of smooth muscle–associated immunoreactivity to both the 130-kDa IL-4/IL-13 receptor α-chain and the PDGF-β receptor (Figure 7). Immunoreactivity to these antigens became evident at 6 hours, coincident with the appearance of nuclear Stat6 staining (Figures 3D and 6A), whereas it was absent at earlier time points (2 hours), when Stat6 expression could not be detected (data not shown).

These data indicate a close temporal correlation between upregulation of the IL-4 α-receptor and PDGF-β receptor and Stat6 expression after arterial collaring. However, the existence of a mechanistic linkage between IL-4 and/or PDGF receptor stimulation and Stat6 activation in response to vascular injury remains to be proven.

**Discussion**

In their basal or resting state, VSMCs do not generally exhibit proatherogenic properties. However, when exposed to act...
vating stimuli, they can shift from the contractile (quiescent) to the synthetic (proliferative) phenotype and become responsive to growth factors. In disease states such as atherosclerosis and restenosis, VSMCs are under the influence of a large variety of factors, including mediators from the circulation and substances released by endothelial as well as inflammatory cells. In addition, VSMCs may produce and release agents that affect their own function. Although the contribution of several mitogenic factors and growth-stimulatory genes to VSMC proliferation has been well established, the signaling pathways underlying VSMC dedifferentiation are relatively unknown. Despite that, this area of research holds great promise for the development of therapeutic strategies influencing vascular proliferative diseases, such as primary atherosclerosis, postangioplasty restenosis, vein graft disease, and transplant vasculopathy.

The purpose of the present study was to examine the possible role of Stat6 in diseased arterial tissue by using a rabbit carotid artery injury model in which a hyperplastic intimal lesion, primarily composed of smooth muscle cells, arises in the presence of an intact, albeit morphologically altered, endothelium. The rationale for the choice of Stat6 in this analysis was based on previous findings from our group showing that this transcription factor is largely present and activated in the germinal epithelium of the embryonic brain, a stage at which massive proliferation and migration of brain neuroblasts are known to occur. In the present study, we evaluated whether changes in Stat6 levels were occurring in VSMCs in response to arterial injury. By analyzing the in vivo immunoreactivity to Stat6, we observed a rapid upregulation of this transcription factor, with high levels of expression throughout the entire thickness of the smooth muscle tissue during the first 5 days after perivascular manipulation (Figures 2 and 3), a time period during which VSMCs leave the quiescent phenotype to undergo active proliferation, as shown by PCNA staining (Figure 4A to 4F). Between 7 and 14 days, when in this model the quiescent phenotype of VSMCs is being reestablished (Figure 4G and 4H), a decline in Stat6 expression was clearly detectable (Figure 3L to 3N). Importantly, at the earlier time points (6 and 12 hours), Stat6 was found mostly localized at the nuclear level (Figures 3D, 3E, and 6A), suggesting functional activation of this transcription factor. This activation precedes the onset of cell proliferation (≥1 day, Figure 4), and it may represent a triggering step. On the contrary, between 1 and 5 days, Stat6 localization was found to be mainly cytosolic (Figures 3G, 3H, and 6B), despite sustained proliferation of VSMCs. Thus, long-term activation of Stat6 does not seem to be involved in protracted changes in the genomic programming of VSMCs in this experimental system.

In the hematopoietic system, Stat6 is mainly activated in response to IL-4 and IL-13 (which share the same transducing subunit), regulating T-cell proliferation and differentiation. In nonhematopoietic cells, however, Stat6 phosphorylation may also be triggered by other ligands, including PDGF, a known mitogen and chemoattractant factor for VSMCs. In the present study, we found that the expression of the IL-4/IL-13 receptor α-chain and the PDGF-β receptor is rapidly upregulated after perivascular collaring. Although our results do not elucidate the identity of the signals that evoke the activation of Stat6 after perivascular collaring, they are suggestive of possible candidates. At this regard, it is interesting to note that IL-4 has been recently proposed to synergize with PDGF to enhance PDGF-mediated biological responses.

Finally, it was shown that homodimerization of the 140-kDa IL-4 receptor α-chain alone can induce intracellular signaling. Because Stat6 is involved in the induction of transcription of the IL-4 and IL-4 receptor α-chain genes, our data point at the possible existence of an autocrine loop...
occurring in VSMCs after arterial injury, in which IL-4/IL-13 and/or PDGF induce Stat6 phosphorylation, which then stimulates IL-4 and IL-4 receptor transcription.

In conclusion, our results suggest that Stat6 signaling may contribute to the modifications of gene expression underlying VSMC activation in the context of hyperplastic lesion formation. This hypothesis is particularly attractive because a recent study with Stat6-deficient lymphocytes has suggested that Stat6 may play a “permissive” role in cell proliferation in response to mitogenic stimuli by downregulating the expression of p27\(^{Kip1}\), a major inhibitor of cell cycle progression.\(^{57}\)

Acknowledgments

This study was supported by grants from BIOMED 2 PL-950329 to Dr Somor, Associazione Italiana per la Ricerca sul Cancro, and the Italian Ministry of University and Scientific and Technological Research (National Program for the development of new drugs, second phase, Programma Nazionale di Ricerca sui Farmaci). This article is dedicated to the memory of Dr Maurizio Soma.

References


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doi: 10.1161/01.ATV.20.4.931
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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