Interferon-inducible Protein-10 Involves Vascular Smooth Muscle Cell Migration, Proliferation, and Inflammatory Response*

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Interferon-inducible protein-10 (IP-10) is a member of the C-X-C chemokine family. Using mRNA differential display, we isolated a rat homologue to murine and human IP-10 from lipopolysaccharide-stimulated carotid arteries. Our studies demonstrated that IP-10 is a potent mitogenic and chemotactic factor for vascular smooth muscle cells, the critical features of smooth muscle cells for their contribution to the pathogenesis of atherosclerosis and restenosis. IP-10 induced a concentration-dependent stimulation of DNA synthesis, cell proliferation, and cell migration of rat aortic smooth muscle cells. A concentration- and time-dependent IP-10 mRNA induction was observed in lipopolysaccharide- or interferon-γ-stimulated, but not interleukin-1β- or tumor necrosis factor-α-stimulated smooth muscle cells. A marked synergistic effect on IP-10 mRNA expression was observed when smooth muscle cells were challenged with interferon-γ together with interleukin-1β or tumor necrosis factor-α. Furthermore, IP-10 mRNA expression was induced in the rat carotid artery after balloon angioplasty. The mitogenic and chemotactic features of IP-10 for smooth muscle cells, along with its discrete induction in cultured vascular smooth muscle cells and in carotid arteries after balloon angioplasty (neointima formation) suggest that IP-10 may play an active and distinct role in vascular remodeling processes.

Vascular smooth muscle cells (VSMCs)1 are a predominant cell type of normal mammalian arterial wall, functioning as structural support and vasomotion. In response to acute vascular injury such as balloon angioplasty or chronic pathological processes such as atherosclerosis, the normal VSMCs display phenotypic changes such as proliferation, migration, and de-differentiation. These critical changes of VSMCs may allow medial SMCs to proliferate and migrate from the media into the intima and where they contribute to neointima formation and restenosis. To date, although a number of factors that mediate SMC migration and/or proliferation have been identified, including numerous growth factors and cytokines (for review, see Ref. 1), the mechanisms responsible for the abnormal responses of VSMCs are still not fully understood.

In addition to SMC proliferation and migration, other features such as matrix production and inflammatory reaction are thought to play important roles in the development of atherosclerotic lesions and neointimal formation (for review, see Refs. 2–5). In fact, a number of growth factors and cytokines have been found to play a pleiotropic role in the process of SMC proliferation, migration, matrix production, and monocyte/macrophage recruitment in vascular injury and contribute to the vascular disease process and tissue remodeling. For example, inflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) could be directly or indirectly involved in this pathogenesis (3). IL-1β and TNF-α can also induce the expression of chemokines, such as monocyte chemotactic protein 1 (MCP-1) and IL-8, and adhesion molecules, such as intercellular adhesion molecule 1 and vascular cell adhesion molecule 1, to facilitate the recruitment of leukocytes from the circulation into the tissue (6–11) and thus to promote the inflammatory response in the injured tissue or in the atherosclerotic lesions. Similar to IL-1β, some chemokines such as IL-8 may play a role as mitogens and chemotaxants for SMCs and may be directly involved in the accumulation of SMCs in the neointimal lesions (12).

LPS is a potent mediator to induce numerous factors associated with inflammation and tissue remodeling. In an effort to identify biologically important genes associated with the inflammatory response in vascular tissue, we applied a recently developed technique of mRNA differential display (13) to isolate genes that are up-regulated with LPS stimulation in rat carotid artery. As described in this report, a rat homologue of the IFN-γ-inducible protein (IP-10) has been identified using this technique.

IP-10 was originally isolated as a predominant mRNA induced by IFN-γ in monocytes, fibroblasts, and endothelial cells (14). Later, it was reported to be up-regulated in a number of other cell lines, such as keratinocytes and astrocytes, in response to either LPS, IFN-γ, IL-1β, or TNF-α (15–18). Based upon structural similarity, IP-10 has been categorized as a member of the C-X-C chemokine family (6), which includes IL-8, KC, and platelet factor-4. Although IP-10 has been discovered over a decade and both human (14) and murine (19, 20) IP-10 cDNA have been cloned for several years, the biological functions of IP-10 are largely unknown. Until recently, IP-10 has been described as a chemotaxant for monocytes and activated T lymphocytes (21), which is distinct from other C-X-C chemokine family members such as IL-8 and KC that have been characterized as chemotaxants mainly for neutrophils (6, 7, 22). Also, IP-10 have been reported to display a potent thymus-dependent antitumor effect in vivo (23), and more...
cently, IP-10 mRNA up-regulation was found in a rat model of experimental nephrosis (24). However, no biological functions of IP-10 in SMC action or in the vascular disease process have been reported.

Based upon structural and functional similarity of IP-10 to other cytokines and chemokines, such as IL-1β and IL-8 that have been demonstrated to be involved in SMC proliferation and migration (3, 12), we hypothesized a functional role for IP-10 in vascular pathogenesis and investigated the effects of IP-10 on SMC proliferation and migration. Meanwhile, since multiple cytokines are simultaneously up-regulated in response to vascular injury which may contribute to IP-10 induction as demonstrated in other cultured cellular source (15–18), we examined the effects of cytokines on IP-10 mRNA expression in cultured SMCs. Finally, we elected an animal model of balloon angioplasty, the best studied model of neointimal formation (1), to investigate the involvement of IP-10 in vascular disease process.

**EXPERIMENTAL PROCEDURES**

**Materials—** Recombinant human IL-1β and TNF-α were purchased from Genzyme, Inc. (Cambridge, MA) and IFN-γ from Life Technologies, Inc. Recombinant human IL-10 was kindly provided by R&D Systems (Minneapolis, MN). [α-32P]dATP (3000 Ci/mmol), [γ-32P]ATP (5000 Ci/mmol), and [α-32P]α-dATP (1200 Ci/mmol) were purchased from Amersham Corp. Taq polymerase was purchased from Perkin-Elmer Corp., and restriction enzymes were purchased from Life Technologies, Inc. or New England Biolabs (Beverly, MA). The RNAmap™ kit for differential display was purchased from GenHunter (Brookline, MA) and a random priming DNA labeling kit from Boehringer Mannheim.

**Oligonucleotides were synthesized by the Oligonucleotide Center of Molecular Genetics, SmithKline Beecham Pharmaceuticals (King of Prussia, PA).**

**Carotid Artery Preparation, Culture, and in Vitro Studies—** Carotid arteries were dissected from spontaneously hypertensive rats and Wistar-Kyoto rats (Taconic Farms, Germantown, NY) (250–300 g) and cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Life Technologies, Inc.) supplemented with 10% fetal bovine serum in the presence of indomethacin (5 μM/liter). The cultured vessels were challenged with 30 μg/ml LPS for 4 h or in the absence of LPS as a sham-control. The tissues were then frozen in liquid nitrogen and stored in –70 °C for RNA preparation.

**Cell Culture—** RASMCs were isolated and cultured as described in detail previously (25). Briefly, RASMCs were isolated from medial explants of the thoracic aorta of male Sprague-Dawley rats (300–350 g) (Chales River, Raleigh, NC) and cultured in DMEM (Life Technologies, Inc.) supplemented with 10% fetal bovine serum and gentamicin (50 μg/ml). Smooth muscle cells were allowed to grow out from the tissue, which was consequently removed. After confluence was reached, cells were harvested by brief trypsinization and subcultured in T-75 flasks. The purity of the SMCs was evaluated by staining the cells with monoclonal antibodies to SMC α-actin (from hybridoma cells, clone asm-1; Boehringer Mannheim). More than 96% of cells revealed immunoreactivity for SMC α-actin which is in accord with a previous report (25). RASMC under six passages was used in the present study.

**DNA Synthesis—** DNA synthesis in RASMCs was assayed by measurement of the incorporation of [3H]thymidine into the cells as reported previously (26). Briefly, RASMCs (1–2 × 10^6/cm^2) were grown in 24-well plates to subconfluence (3 days) in DMEM containing 10% fetal bovine serum. Cells were made quiescent by incubation with serum-free DMEM for 48 h. After replenishing with fresh DMEM, cells were incubated with IP-10, PDGF, or ET-1 for 24 h in 1 μM of [3H]thymidine was added to each well, and cells were incubated for another 4 h. Cells were washed with DMEM and treated with 0.2 ml of 0.2 N NaOH for 30 min followed by incubation in 1 ml of trichloroacetic acid (15%) and stored at room temperature for minimum of 2 h. The total sample was transferred from each well and filtered under vacuum. Filters were washed three times with 2 ml each of 5% trichloroacetic acid, and the radioactivity in the insoluble fraction of cells was determined microscopically, and four high power fields (×100) were counted per filter. Experiments were performed in triplicate.

**Cells Challenged with Cytokines—** RASMCs were cultured in T-150 flasks, grown to confluency, and serum-deprived for 48 h, then stimulated with different amount of LPS, TNF-α, IL-1β, and/or IFN-γ for various times as indicated in the figure legend.

**Left Common Carotid Artery Balloon Angioplasty—** Left common carotid artery balloon angioplasty was performed on male Sprague-Dawley rats as described previously (28). Vessels were removed at the following time points: 0 (control) and 6 h, and 1, 3, 7, and 14 days after surgery. Once isolated, vessels were immediately frozen in liquid N2 and stored at −70 °C for RNA preparation. Each of the six individual time points consisted of vessels pooled from three rats, and five separate pooled samples were analyzed.

**Isolation of RNA—** For RNA preparation, carotid arteries were homogenized, whereas cultured SMCs were directly lysed in an acid guanidinium thiocyanate solution and extracted with phenol and chloroform as described previously (29).

**mRNA Differential Display—** Differential display experiments were carried out using an RNAmap™ kit (GenHunter) as described in detail previously (30).

**Northern Blot Analysis—** RNA samples (10–20 μg/lane) extracted from LPS-stimulated or unstimulated carotid artery or from cultured RASMCs were resolved by electrophoresis on agarose gels and transferred to a GeneScreen Plus membrane (DuPont NEN), and subjected to Northern hybridization as described in detail previously (25, 31). For Northern analysis, cDNA fragments of rat IP-10, MCP-1 (32), and ribosomal protein L32 (rpL32; generated by reverse transcription-polymerase chain reaction (RT-PCR) as described below) were isolated from electrophoresis and uniformly labeled with [α-32P]dATP (3000 Ci/mmol, Amersham Corp.) using a random priming DNA labeling kit (Boehringer Mannheim). The rpL32 gene is relatively constantly expressed in the experimental conditions (25) and therefore was used to normalize the differences of the samples loaded in each lane.

**cDNA Library Screening and DNA Sequence Analysis—** A rat kidney cDNA library in λZAP II vector (purchased from Stratagene, La Jolla, CA) was screened using the LPS-7 DNA probe isolated from differential display as described in detail previously (31). Positive clones were isolated, and the corresponding phagemids were generated by *in vivo* excision according to the manufacturer’s specifications. DNA sequencing was performed by the dyeodeoxy nucleotide sequencing method using both a cycle-sequencing kit (Life Technologies, Inc.) and automated DNA sequencing on a DNA sequencer model 373A (Applied Biosystems). The complete cDNA sequence was determined from both strands using universal primers (T7 and T3 primers) and specific primers synthesized according to the rat IP-10 cDNA sequence. DNA sequence analysis and computer data base searches were performed using the Genetics Computer Group program.

**Reverse Transcription and Polymerase Chain Reaction—** For RT, total cellular RNA (5 μg/sample) isolated from 0 h and 6, 1, 3, 7, and 14 days following carotid artery balloon angioplasty was reverse transcribed in the presence of 200 units of RNase H−SuperScript II reverse transcriptase (Life Technologies, Inc.) and 1 μg of oligo(dT) 12–18 primer at 37 °C for 60 min according to the manufacturer’s specifications. The resultant cDNA products were phenol-chloroform-extracted and ethanol-precipitated. Total cDNA pellets were then dried under speed vacuum, resuspended in 120 μl of TE (10 mM Tris-HCl and 1 mM EDTA, pH 7.5), and stored at −20 °C until required for PCR amplification.

**PCR primers (Table I) were synthesized according to rat IP-10 cDNA sequence and published sequences for rat MCP-1 (32, 33) and rpL32 cDNAs (34). For quantitative purpose, IP-10 or MCP-1 cDNA was...
The positions of bases are indicated in Fig. 4 for rat IP-10 or according to the published cDNA sequences for MCP-1 (33) and rpL32 (34). The letters S and A represent sense and antisense oligonucleotides, respectively. The predicted size for IP-10, MCP-1, and rpL32 PCR products are 357, 527, and 413 base pairs, respectively.

| Gene   | Sequence                          | S/A | Position |
|--------|-----------------------------------|-----|----------|
| IP-10  | 5'-AAGGCTCTTGCTTCTCTCG-3'          | +/- | 489–508  |
| MCP-1  | 5'-CAGGCTCTTGACTGCTTCCT-3'         | +/- | 826–845  |
| rpL32  | 5'-GATACGTAGAGAATGAG-3'            | +/- | 4–23     |

![Figure 1](http://www.jbc.org/)

**FIG. 1.** Identification of induced gene expression for LPS-7 in rat carotid artery stimulated with LPS using mRNA differential display. mRNA differential display was carried out using an upstream primer (5'-GACCGCCTTGT-3') and a downstream primer (T<sub>12</sub>MA). PCR products were resolved in a sequencing gel in the following order: lane 1, unstimulated; lane 2, LPS-stimulated carotid artery from spontaneously hypertensive rats; lane 3, unstimulated; and lane 4, stimulated carotid artery from Wistar-Kyoto rats. The band indicated with an arrowhead (designated as LPS-7) shows a marked induction in response to LPS stimulation on the differential display gel.

![Figure 2](http://www.jbc.org/)

**FIG. 2.** Northern hybridization analysis of LPS-7 mRNA in multiple tissues. The multiple tissue RNA blot, 10 µg of poly(A) RNA/lane, was purchased from Invitrogen (San Diego, CA). LPS-7 cDNA amplified from an mRNA differential display was used as a probe for Northern hybridization. The mRNA size of the LPS-7 is indicated on the right.
a base deletion in the sequence reported by Liang et al. (38), respectively. These three individual base replacements occurred in the coding region, of which the base change at 206 (C instead of A) affects the deduced amino acid sequence. Therefore, the 39th amino acid in our clone is proline, whereas it is threonine in the clone of Liang et al. (38). The encoding sequence of proline appears to be conserved among different species, i.e. human (14), mouse (19, 20), and rat (this report), supporting the possibility that our sequence is the correct one.

Since the rat IP-10 was identified in the LPS-stimulated vessels, we speculated whether the induced expression of IP-10 in vessels might be associated with vascular pathogenesis. To test this possibility, we evaluated the effects of IP-10 on smooth muscle cell proliferation and migration, two key elements of the vascular disease process.

**IP-10 Stimulates DNA Synthesis and Cell Proliferation in VSMCs—**

As shown in Fig. 4, bacterially expressed recombinant human IP-10 induced a concentration-dependent [3H]thymidine incorporation in RASMCs. A 2.0- or 4.4-fold \( (p < 0.001) \) increase over the control level in [3H]thymidine incorporation was observed after 1 day of incubation in the presence of 0.3 and 1 \( \mu M \) IP-10, respectively. Similarly, IP-10 induced a concentration-dependent stimulation of cell proliferation after 5 (data not shown) and 7 days of incubation (Fig. 5). A significant increase in SMC number was observed, with a 1.5-, 1.6-, and 1.8-fold increase \( (p < 0.001) \) over the control value after 7 days of incubation in the presence of 0.1, 0.3, and 1 \( \mu M \) IP-10, respectively.

**IP-10 Stimulates VSMC Migration—**

As shown in Fig. 7, IP-10 caused a concentration-dependent stimulation of SMC migration after 24 h of incubation. A marked induction in SMC migration was observed in the presence of IP-10, with a 9.3-fold \( (p = 0.01) \) and 34.9-fold \( (p < 0.001) \) increase over the basal level for 37.5 nM and 1250 nM, respectively.

**IP-10 mRNA Expression in Cultured RASMCs in Response to LPS, IFN-\( \gamma \), IL-1\( \beta \), and TNF-\( \alpha \) Stimulation—**

Unstimulated, serum-deprived cultured RASMC expressed only a very low or undetectable level of IP-10 mRNA (Figs. 8 and 9). The concentration of PDGF (1 nM) and ET-1 (1 nM) produced maximal mitogenic effects on RASMCs as determined previously (26), whereas the same concentration of IP-10 failed to show any effects on SMC proliferation. At a high concentration \( (1 \mu M) \), however, IP-10 appeared to be more potent than ET-1 but less than PDGF (Fig. 6). No synergistic mitogenic effect was found when SMCs were co-stimulated with IP-10 and PDGF.

**IP-10 Stimulates VSMC Migration—**

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almost no effect on IP-10 mRNA expression in RASMCs. Fig. 9 illustrates the time course of increase in the IP-10 mRNA expression after incubating RASMCs with 200 ng/ml LPS (Fig. 9A), 100 units/ml IFN-γ (Fig. 9B), 1 nM IL-1β (Fig. 9C) or 5 ng/ml TNF-α (Fig. 9D). A considerable increase in IP-10 mRNA was observed at 8 h after LPS stimulation. IP-10 mRNA up-regulation in response to IFN-γ was earlier than LPS, i.e. at 3 h, reached peak levels at 8 h, and maintained high levels for at least 24 h after stimulation. The cytokine-induced expression of MCP-1 mRNA was used as a control to demonstrate a differential temporal induction profile to LPS, IFN-γ, IL-1β, or TNF-α stimulation in the cultured RASMCs compared to that of IP-10. In contrast to IP-10 mRNA expression, a concentration- and time-dependent induction in MCP-1 mRNA expression was observed in response to each factor-stimulated RASMC (Figs. 8 and 9).

Although IL-1β or TNF-α hardly show any effect on IP-10 mRNA expression in cultured SMCs, the combination of IFN-γ with IL-1β, or IFN-γ with TNF-α, revealed a remarkable synergistic induction (Fig. 10). Quantitative data (not shown) indicated that more than 550-fold increase in either of these synergistic effects could be reached compared to IP-10 mRNA.

**Fig. 6.** Comparison of the effects of IP-10, PDGF, and ET-1 on the [3H]thymidine incorporation (A) and the increase in cell number (B) of RASMCs. The assays were performed as described in Figs. 4 and 5. The data are illustrated as percentage increase in mitogen-stimulated SMCs compared to vehicle. The concentration of mitogen used was predetermined to give maximal mitogenic effect on RASMCs: IP-10 (1 μM; n = 5), PDGF A/B (1 nM; n = 4), and ET-1 (1 nM; n = 11). Only an additive effect was found when the cells were treated with IP-10 (1 μM) and PDGF A/B (1 nM).

**Fig. 7.** IP-10-induced SMC migration. The migration assay was performed in the Transwell cell culture chamber as described under "Experimental Procedures." The indicated concentration of IP-10 was coated on the lower surface of the membrane. The cell suspension was added in the upper compartment and incubated for 24 h. The number of migrated RASMCs (on the lower surface of the filters) was determined microscopically by counting four high power field per filter. The data are the mean ± S.E. of five experiments performed in duplicate. **p < 0.01, ***p < 0.001, compared to the control.

**Fig. 8.** Dose-dependent induction of IP-10 mRNA in LPS-, IFN-γ-, IL-1β-, and TNF-α-stimulated RASMCs. RASMCs were deprived of serum for 48 h and stimulated with various stimuli for the indicated doses for 3 and 8 h, and analyzed by Northern blot. Total cellular RNA was isolated, resolved (10 μg/lane) by electrophoresis, transferred to a nylon membrane, and hybridized to a rat IP-10, MCP-1, or rpL32 cDNA probe sequentially as described under "Experimental Procedures." The mRNA size is indicated on the right.
induction in RASMCs treated with IFN-γ alone. No synergistic effect was observed when RASMCs were treated with IL-1β and TNF-α.

**IP-10 Expression in Rat Carotid Artery following Balloon Angioplasty**—Since IP-10 is mitogenic and chemotactic for cultured SMCs and its mRNA expression could be significantly up-regulated in SMCs in response to cytokine stimulation, it is of interest to explore the potential role of IP-10 in vivo. To this end, the rat carotid artery balloon angioplasty model has been employed to study the temporal relationship of IP-10 mRNA expression in injured vessels, since balloon angioplasty involves not only an inflammatory response but also SMC migration and proliferation. As only a limited amount of tissue is available for RNA extraction from the rat carotid artery, we elected to use a quantitative RT-PCR method in this study. Fig. 11A illustrates a representative autoradiograph of IP-10 mRNA expression in rat carotid artery following balloon angioplasty using the quantitative RT-PCR technique. Since MCP-1 is a chemokine closely related to IP-10 and its mRNA expression after balloon angioplasty has been previously demonstrated (39), we examined the temporal expression profiles of IP-10 mRNA in parallel with MCP-1 mRNA. The data, after normalizing to rpL32 mRNA, are depicted in Fig. 11B. A very low level of IP-10 mRNA expression was observed in the normal carotid artery. The level of IP-10 mRNA was markedly increased at 6 h (5.1-fold increase of the mean value over control, \(p < 0.001\)) following balloon angioplasty. The increase in IP-10 mRNA diminished significantly at 24 h (2.5-fold increase over control), but then progressively increased to 3.1-fold at day 3 (\(p < 0.05\)) and 4.6-fold at day 14 (\(p < 0.001\)) following balloon angioplasty (Fig. 11). Comparatively, MCP-1 mRNA increased rapidly at 6 h (16.1-fold increase \(p < 0.001\)), but then rapidly returned to basal levels, and no secondary elevation of MCP-1 mRNA was noted (Fig. 11).

**DISCUSSION**

In the present report we described the molecular cloning of the rat homologue of human IP-10 from vascular tissue in response to LPS stimulation and demonstrated the novel bio-
controls (time points) are presented as the mean ± S.E. of five separate experiments (*p < 0.05, **p < 0.01, ***p < 0.001, compared to the controls (time = 0)).

logical functions of IP-10 induction of SMC proliferation and migration, two critical features in neointima formation following acute vascular injury such as restenosis. In this report, IP-10 was found to be one of the most potent chemotactic agents for SMCs. Comparatively, IP-10 is more effective than IL-8, angiotensin II, basic fibroblast growth factor, ET-1, and transforming growth factor-β on SMC migration; only PDGF is more potent than IP-10, as previously reported (12, 40). The chemotactic feature of IP-10 for SMCs is in accordance with previous studies. This early up-regulation of IP-10 supports the possibility of its involvement in the initiation of SMCs proliferation, as also suggested by previous work on MCP-1 induction after balloon angioplasty in rabbits (39). The secondary increase in IP-10 mRNA from days 3 to 14 after angioplasty may bear on neointimal formation following balloon angioplasty in rats. Nevertheless, SMC proliferation plays a critical role in neointima formation after acute vascular injury. Together with other factors, e.g. fibroblast growth factor, PDGF, transforming growth factor-β, and angiotensin II, as being recognized previously (1), IP-10 may also play an important role in SMC proliferation in neointima formation.

Since IP-10 was discovered in vascular tissue stimulated in vitro by LPS and SMCs are known to play an important role in neointimal formation following percutaneous transluminal coronary angioplasty, the regulation of IP-10 expression in RASMCS was investigated thoroughly. Notably, our studies demonstrated that LPS, IFN-γ, IL-1β, and TNF-α have very different effects on IP-10 mRNA up-regulation in SMCs compared to all other cell types reported, including monocytes, endothelial cells, fibroblasts, keratinocytes, and astrocytes (14–18). Our study demonstrated that IP-10 mRNA expression was induced in LPS- and IFN-γ-stimulated RASMCS in a concentration- and time-dependent manner, but not in response to IL-1β or TNF-α stimulation. In contrast, IL-1β and TNF-α induced a high level of IP-10 mRNA expression in fibroblasts and osteoblasts (16, 18). Surprisingly, IL-1β and TNF-α revealed a remarkable synergistic effect with IFN-γ on IP-10 mRNA expression in RASMCS. This synergistic effect may have important biological significance since all these factors are simultaneously up-regulated during pathogenesis of atherosclerosis and restenosis (1, 3). Therefore, in the presence of a certain cytokine combination, IP-10 can be produced in SMCs and may in turn function in an autocrine fashion on SMC proliferation and migration.

It also should be pointed out that, since inflammation has been associated with vascular disease processes that involve leukocyte infiltration and accumulation in the lesions, the previous demonstration of chemotactic effects of IP-10 for monocytes and T lymphocytes, two cellular components in the atherosclerotic lesions (2, 4), may suggest that IP-10 produced by SMCs may also serve as a paracrine mediator that amplifies the recruitment of monocytes into the lesions and thereby escalates the process. Similarly, IP-10 can be produced by monocytes, endothelial cells, fibroblasts, and other cellular source in the lesions and serve as a paracrine to be chemotactic for SMCs and leukocytes during vascular disease processes.

In an effort to further explore the potential role of IP-10 in vascular injury in vivo, we examined the expression of IP-10 throughout the entire course of restenosis in a rat model of carotid artery balloon angioplasty. In this model, the procedure similar to restenosis after percutaneous transluminal coronary angioplasty elicits VSMC transformation from a quiescent/contractile cell to a proliferative/activated cell characterized by cell replication, migration, and activation. At the same time, a wound-healing response develops, which includes an inflammatory reaction characterized by recruitment and accumulation of leukocytes. In this model, IP-10 mRNA expression was found to be significantly up-regulated following balloon angioplasty with a unique pattern that has not as yet been reported for other mediators such as MCP-1. Specifically, IP-10 mRNA expression after angioplasty exhibited a biphasic induction pattern, with an early response that is in accord with the in vitro studies. This early up-regulation of IP-10 supports the possibility of its involvement in the initiation of SMCs proliferation, as also suggested by previous work on MCP-1 induction after balloon angioplasty in rabbits (39). The secondary increase in IP-10 mRNA from days 3 to 14 after angioplasty may bear on SMC proliferation, migration, and the recruitment of leukocytes into the lesions. However, the exact role of IP-10 in neointima formation and the cellular source of IP-10 expression in the neointima after balloon angioplasty remain to be investigated when appropriate tools are available.

In summary, the demonstration of IP-10 as a mitogen and chemotactrant for SMCs and the demonstration of IP-10 up-regulation by cytokines such as IL-1β/TNF-α in conjunction with IFN-γ and its expression at critical time points of SMCs present a novel potential role of IP-10 as a mitogenic and chemotactrant for SMCs.
response to vascular injury support a role of IP-10 in pathomechanism of vascular response to injury. This hypothesis may be further supported once the tools such as neutralizing antibodies against IP-10 or specific antagonist to IP-10 receptor are developed.

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