thematic review

Thematic Review Series: The Immune System and Atherogenesis

Cytokines affecting endothelial and smooth muscle cells in vascular disease

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Abstract **The cellular and extracellular matrix accumulations that comprise the lesions of atherosclerosis are driven by local release of cytokines at sites of predilection for lesion formation, and by the specific attraction and activation of cells expressing receptors for these cytokines. Although cytokines were originally characterized for their potent effects on immune and inflammatory cells, they also promote endothelial cell dysfunction and alter smooth muscle cell (SMC) phenotype and function, which can contribute to or retard vascular pathologies. This review summarizes in vivo studies that have characterized endothelial- and smooth muscle-specific effects of altering cytokine signaling in vascular disease. Although multiple reports have identified cytokines as pivotal players in endothelial and SMC responses in vascular disease, they also have highlighted the need to delineate the critical genes and specific cellular functions regulated by individual cytokine signaling pathways.**—Raines, E. W., and N. Ferri. **Cytokines affecting endothelial and smooth muscle cells in vascular disease.** ***J. Lipid. Res.*** **2005.** 46: **1081–1092.**

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Atherosclerosis remains the leading cause of death in Western countries and represents a specialized inflammatory process whose regulation is dependent upon an intricate network of cytokine and chemokine signaling (1–4). The slowly developing changes in the artery wall that ultimately lead to vessel blockade and clinical sequelae occur within the innermost layer (intima) of the artery. Most commonly, lesions result from the chronic inflammatory response to oxidative modification of low density lipoprotein (LDL), which leads to the subendothelial accumulation of cells. Intimal accumulation includes monocytes, lymphocytes, and some smooth muscle cell (SMC) progenitors from the blood and SMCs from the vessel wall, together with SMC-derived extracellular matrix (ECM). The cell and matrix accumulation that establishes lesions of atherosclerosis is driven by elevation and modification of lipoproteins that lead to the release of cytokines at sites of predilection for lesion formation, and by the specific attraction of cells expressing receptors for these cytokines. Local release of cytokines and limited expression of their specific receptors help explain the focal nature of lesions of atherosclerosis.

Cytokine signaling can have a multiplicity of effects on vascular cell functions and can further promote lesion expansion or, alternatively, retard progression. Cytokines and their receptors are tightly and independently controlled, and this regulation is critical to limiting the multiplicity of their effects. In our attempt to examine the cytokine effects of greatest relevance to vascular disease, we have limited this review to the cytokines and receptors that have been identified and demonstrated to have cell-specific effects in vascular pathologies in vivo. Although the definition of cytokine varies in the spectrum of cell regulatory proteins included, we have restricted our discussion to cytokines with major effects on the immune and inflammatory responses, to the exclusion of connective tissue and hematopoietic growth factors. This review focuses on cytokines acting upon the endothelium and SMCs; the accompanying review in this series by Alan

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Abbreviations: apoE, apoplipoprotein E; CCL, CC chemokine ligand; CCR, CC chemokine receptor; CD40L, CD40 ligand; GRO, growth-related oncogene; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; IL-1Ra, interleukin-1 receptor antagonist; KC, keratinocyte chemokine; MCP, monocyte chemoattractant protein; MHC, major histocompatibility complex; MIF, macrophage migration inhibitory factor; NF-κB, nuclear factor κB; SCID, severe combined immunodeficient; SDF, stromal cell-derived factor; SMC, smooth muscle cell; TGF, transforming growth factor; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule.

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Daugherty considers cytokines that modulate monocytes and lymphocytes.

UPREGULATION OF CYTOKINES AND THEIR RECEPTORS IN VASCULAR INJURY AND DISEASE

Complex networks of cytokines interact to homeostatically regulate the inflammatory and immune responses and other biological pathways. As demonstrated in **Table 1**, the array of cytokines and chemokines with known expression and actions in vivo in vascular pathologies is quite extensive and diverse. Constitutive production of these cytokines and chemokines is low or absent in normal vessels, but is significantly induced in vascular pathologies, including atherosclerosis. Cytokines such as tumor necrosis factor (TNF)-α that are transcriptionally induced by innate immune challenges, such as modified lipids associated with atherosclerosis, are potent inducers of a number of other cytokines and chemokines (5–7). This apparent autoamplification system can make it difficult to define the direct actions of a particular cytokine, yet it allows a single cytokine signaling pathway to induce a cascade of overlapping and complementary cytokines. However, cytokine production by inflammatory cells or vascular cells is usually transient, and released cytokines act mainly by binding to neighboring target cells (paracrine) or to the cell of their origin (autocrine).

Because of the multiple and potent effects of cytokines on cell adhesion, migration, proliferation, and survival, their actions are further regulated at multiple levels. Once chemokines and cytokines are secreted, their diffusion and localization can be controlled by binding to the ECM (8). Release of chemokines from the matrix is then often dependent upon specific proteolysis, such as the matrix metalloproteinase-2-mediated release that has been shown for CC chemokine ligand (CCL)11/eotaxin (9). Proteolysis is also required for the release of transmembrane-spanning cytokine precursors, such as TNF-α, and can remove necessary receptor binding domains of others, such as CCL2, that effectively transform them into antagonists (8). Specific expression of receptors is required for a cell to be responsive to individual cytokines, and can be limited to specific cell types, as shown in Table 1. For example, the chemokine fractalkine that can act as an adhesion molecule and a chemokine attractant is made by endothelial cells, SMCs, and monocytes, whereas T cells, monocytes, and SMCs express its receptor, CX3C chemokine receptor 1 (CX3CR1) (10). Within advanced human lesions, the cells expressing and responding to fractalkine appear to be even more limited, and there is a positive correlation between the number of fractalkine-expressing cells (primarily macrophages) and the number of CX3CR1-expressing cells (predominantly SMCs) (10). In addition to signaling receptors, evidence exists for decoy receptors and soluble receptors that can serve as natural ligand antagonists (8, 11, 12). Thus, the presence of the cytokine and its receptor does not mean it is active. Understanding the complexities of the cytokine regulatory network is crit-

TABLE 1. In vivo expression of cytokines and their receptors

Cytokine	Sources	Receptor	Target Cells	In Vivo Model	Reference
CC chemokines					
CCL2/MCP-1	EC, SMC, M, T	CCR2	EC, SMC, M, T	Atherosclerotic lesions in apoE$^{-/-}$ mice	(31, 81)
CCL11/eotaxin	SMC	CCR3	EC, SMC, M, B	Injured mouse femoral artery	(48)
CXC chemokines					
CXCL1/GRO/KC	EC, SMC, M	CXCR1/2	EC, SMC, M, T	ApoE$^{-/-}$ mouse lesions	(81)
CXCL8/IL-8	EC, SMC, M, T	CXCR1/2	EC, SMC, M, T	Human atherosclerosis	(38)
CXCL12/SDF-1α	EC, SMC, M	CXCR4	SMC progenitors	Carotid artery injury of apoE$^{-/-}$ mice	(47)
			T	Human atherosclerotic lesions	(82)
CX3C chemokines					
CX3CL1/FKN	EC, SMC, M	CX3CR1	SMC, M, T	Human lesions	(10)
				ApoE$^{-/-}$ mouse lesions	(83)
Interferons					
IFN-γ	M, T, SMC	IFN-γ R	EC, SMC, M, T	Human atherosclerosis	(84)
Interleukins					
IL-1	EC, SMC, M, T, B	IL-1R	EC, SMC, M, T	Human atherosclerotic lesions	(85)
				Rat carotid artery balloon injury	(86)
IL-3	T	IL-3R	EC, SMC	Human lesions	(87)
IL-4	EC, T, B	IL-4R	EC, SMC, M, T, B	Human lesions	(88)
IL-10	SMC, M, T, B	IL-10R	EC, SMC, M, T, B	Normal human tissue and balloon-injured rat carotid artery	(39, 59)
IL-11	EC, SMC	IL-11R	EC, SMC, M, T	Endothelial response in human skin tx	(27)
IL-15	EC, SMC, M	IL-15R	EC, M, T	Human lesions	(89)
IL-18	EC, SMC, M	IL-18R	EC, SMC, T	Human atherosclerosis	(62, 90)
Other					
MIF	EC, SMC, M, T	CD74	M, T	Human atherosclerosis	(91, 92)
TNF-α	EC, SMC, M, T	TNFR	EC, SMC, M, T	Human and primate lesions	(93)

Cytokines and chemokines with known expression and actions in vascular pathologies are listed in this and other tables. Chemokines are listed using their structural classification according to the position of the N-terminal cysteines. Apo, apolipoprotein; B, B lymphocyte; CCL, CC chemokine ligand; CCR, CC chemokine receptor; CX3CL1, CX3C chemokine ligand 1; CXCL, CXC chemokine ligand; CXCR, CXC chemokine receptor; EC, endothelial cell; IFN, interferon; IL, interleukin; M, monocyte/macrophage; MIF, macrophage migration inhibitory factor; TNF, tumor necrosis factor; SMC, smooth muscle cell; T, T lymphocyte; TNFR, tumor necrosis factor receptor; tx, transplant.

ical to intervening with the activities of cytokines, and may even be employed to locally control their actions.

CYTOKINE INDUCTION OF LOCALIZED ENDOTHELIAL CELL DYSFUNCTION

Endothelial cells line the artery wall and are critical to the maintenance of normal homeostasis. Among the earliest changes following the administration of a hypercholesterolemic diet in experimental models of atherosclerosis is the focal adhesion of leukocytes to sites of predilection for lesion formation (1). Cytokines can significantly modify endothelial cell gene expression, and in so doing, promote this focal formation of lesions of atherosclerosis. **Table 2** highlights in vivo studies that have characterized endothelial cell-specific effects of altering cytokine signaling in vascular pathologies. Below, we discuss the significance of the different modulations for endothelial cell functions. However, the recent characterization of regional differences in endothelial gene expression profiles suggests that endothelial heterogeneity in microvessels versus macrovessels and arteries versus veins may lead to distinct cytokine responses that could contribute to divergence of disease susceptibility (13, 14).

Local release of cytokines increases endothelial cell adhesion molecule expression that promotes leukocyte recruitment

In experimental models of atherosclerosis, the initiation of a high-cholesterol diet rapidly induces expression of specific adhesion molecules at sites of predilection for lesion formation (1). Cytokines, such as TNF-α, are potent stimulants of adhesion molecule expression (5, 6, 15), and the absence of its signaling receptor can inhibit their expression and leukocyte infiltration of the vessel wall (16). Similarly, targeted deletion of a natural inhibitor of interleukin (IL)-1, the IL-1 receptor antagonist (IL-1Ra), effectively increases local concentrations of IL-1 in apolipoprotein E (apoE)$^{-/-}$ mice, increases mRNA expression of the adhesion molecules vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 in the aorta, enhances mRNA levels of the leukocyte chemokine monocyte chemoattractant protein (MCP)-1, and promotes a 1.9-fold increase in monocyte accumulation (17). Mice lacking the IL-1 receptor I have also been shown to be unable to induce E-selectin expression when injected with IL-1 (18). Thus, multiple cytokines are sufficient to induce specific adhesion molecule expression.

Transplant atherosclerosis remains the leading cause of graft failure. It is characterized by involvement of the entire wall of the artery, and results in a concentric lesion that often involves long segments of affected arteries (19). Although the exact immunologic mechanisms responsible for chronic vascular rejection are not known, evidence is consistent with involvement of alloreactive T lymphocytes and antibodies (20). Cytokine regulation of adhesion molecule expression on graft endothelial cells can contribute to lymphocyte recruitment to the graft. Analysis of rejecting murine heterotopic cardiac allografts has shown that endothelial expression of VCAM-1 can be abrogated and ICAM-1 expression reduced by treatment with either a soluble TNF-α receptor or IL-4 antagonists (monoclonal antibody and soluble IL-4 receptor) (21). Although either treatment reduced but did not eliminate leukocyte infiltration, the allografts were rejected at the same rate, potentially due to continued expression of multiple cytokines within the graft. So, although TNF-α and IL-4 have clear roles in regulating adhesion molecule expression in allografts, elimination of VCAM-1 expression is not sufficient, nor is VCAM-1 essential, for graft rejection (16). The absence of the anti-inflammatory cytokine IL-10 has also been evaluated in mice receiving cardiac allografts. Although targeted deletion of IL-10 led to enhanced leukocyte recruitment and graft rejection, molecular changes in endothelial phenotype were not evaluated (22).

Endothelial adhesion molecule function can also be altered by cytokines

For leukocytes to deposit within the intima, they must undergo a sequence of interactions with the endothelium. Initially, this includes tethering and rolling along the endothelial surface until chemokine stimuli cause the rolling leukocytes to arrest and adhere to the endothelium, followed by migration to endothelial junctions and transendothelial cell migration. Cytokines and chemokines can also significantly enhance the function of endothelial cell adhesion molecules at all stages of transendothelial cell migration. Analysis of mice with targeted deletion of macrophage migration inhibitory factor (MIF), using intravital microscopy to examine transendothelial migration in the inflamed cremaster muscle, revealed a significant reduction in P-selectin-dependent leukocyte rolling and adhesion, and reduced entry of leukocytes into the site of inflammation (23). The effect of administration of chemokines has also been examined in lesion-prone apoE$^{-/-}$ mice. Ex vivo perfusion of apoE$^{-/-}$ carotid arteries has shown a keratinocyte chemokine (KC)/growth-related oncogene (GRO)-α-dependent monocyte arrest, but no effect of inhibition of MCP-1 or its receptor, CC chemokine receptor (CCR)2 (24). The KC/GRO-α-dependent monocyte arrest could be inhibited by blockade of either integrin α4β1 or VCAM-1, and the authors have proposed that the arrest is due to chemokine regulation of integrin avidity and adhesiveness, because it is dependent upon CXCR2 signaling (24) and based upon in vitro chemokine modulation of integrin avidity (25). Perfusion of the carotid artery with KC was also able to further enhance monocyte arrest, demonstrating the ability of locally released KC to increase the extent of monocyte arrest (24), an effector mechanism also employed by TNF-α (7). IL-15 appears to act at a later step by inducing endothelial hyaluronan expression that promotes a CD44-mediated pathway, which enhances transendothelial cell migration (26). Thus, cytokines and chemokines can modulate adhesion molecule properties to further enhance leukocyte recruitment.

TABLE 2. Cytokine stimulation of endothelial cells in vivo promotes endothelial dysfunction

Cytokine	Sources	In Vivo Model	In Vivo Effects	Reference
Adhesion molecule expression and leukocyte infiltration				
IL-1	EC, SMC, M, T, B	Chow-fed apoE$^{-/-}$ mice that were also IL-1Ra$^{+/-}$ vs. $^{+/+}$ (IL-1Ra+/− results in increased IL-1)	1.9-Fold increase in monocyte accumulation at 32 weeks potentially due to increased VCAM-1, ICAM-1, and MCP-1 (cells not identified)	(50)
		IL-1 administration to IL-1R1$^{-/-}$ mice	Failed to induce E-selectin	(18)
IL-4	EC, T, B	Soluble IL-4 receptor or IL-4 antibody for murine cardiac allograft	Reduced VCAM-1, ICAM-1, and leukocyte infiltration	(18)
IL-10	M, T, B	Murine heart tx in IL-10$^{-/-}$ recipients	Enhanced leukocyte recruitment	(22)
TNF-α	EC, SMC, M, T	IV administration to rats	Induction of FKN	(5)
		Soluble TNF receptor use for murine cardiac allografts	Reduced VCAM-1, ICAM-1, and leukocyte infiltration	(21)
		TNF-α administration to porcine xenografts in SCID mice	IFN-γ induced VCAM-1 expression, but only TNF induced T cell infiltration	(6)
		TNF-α administration to WT and p55TNFR$^{-/-}$ mice	Induction of VCAM-1 and E-selectin absent in p55-null mice and blocked leukocyte infiltration	(16)
		TNF-α administration to mice	Induced P-selectin expression on endothelium	(15)
Adhesion molecule function				
CXCL1/GRO/KC	EC, SMC, M	Ex vivo perfusion of murine carotid arteries	Enhanced monocyte accumulation that is VLA-4/VCAM-1 mediated	(24)
IL-15	EC, SMC, M	IP injection of IL-15 into mice	Promotes extravasation of T cells in CD44-dependent manner; in vitro promotes HA synthesis	(26)
MIF	EC, SMC, M, T	Intravital microscopy of cremaster muscle in MIF$^{-/-}$ and $^{+/+}$ mice	Targeted deletion in EC reduces P-selectin-dependent rolling	(23)
TNF-α	EC, SMC, M, T	TNF-α-treated HUVEC with flow	GRO induced and sequestered to endothelium induces release of MCP-1	(7)
Endothelial cell survival				
IL-11	EC, SMC	Administration to SCID mice with human skin grafts	Protects endothelial cells from apoptosis by induction of survivin and no effect on inflammation	(27)
Antigen presentation				
CCL2/MCP-1	EC, SMC, M, T	Blockade of MCP-1/CCR2 signaling in apoE$^{-/-}$ mice	Decreased CD40L immunoreactivity	(31)
IL-18	EC, SMC, M	IP administration of IL-18 to apoE$^{-/-}$ mice	Increased MHCII expression	(29)
		IP adminstration of IL-18 to SCID/apoE$^{-/-}$ mice	Increased MHCII expression and VCAM-1	(30)
Endothelial proliferation and migration including angiogenesis				
CXCL1/GRO/KC	EC, SMC, M	Carotid injury of apoE$^{-/-}$ mice	Blockade of KC inhibits re-endothelialization	(33)
		KC implant in mice	Promotes angiogenesis	(94)
CXCL8/IL-8	EC, SMC, M, T	IL-8 antibody block of rat corneal angiogenesis stimulated by extract from human atherosclerotic lesion	Promotes angiogenesis	(38)
IL-3	T	Angiogenesis in murine implant model	IL-3 administration promoted angiogenesis	(35)
IL-15	EC, SMC, M	IL-15 implant in nude mice	Promotes angiogenesis	(36)
IL-18	EC, SMC, M	Matrigel IL-18 implant into mice	Promotes angiogenesis	(37)
TNF-α	EC, SMC, M, T	Injured rat carotid artery with and without soluble TNF receptor	Accelerated endothelial recovery at 1 and 2 weeks post injury	(79)
Endothelial-dependent vasorelaxation				
IL-10	M, T, B	Relaxation in IL-10$^{-/-}$ mouse vessels	Endothelial dysfunction and increased superoxide	(40)
IFN-γ	M, T, SMC	Transplanted human arteries into SCID mice	Endothelial dysfunction and reduced eNOS inhibited by anti-IFN abs	(41)
TNF-α	EC, SMC, M, T	Injured rat carotid artery with and without soluble TNF receptor	Endothelial cell recovery enhanced as measured by increase in nitric oxide production	(79)

CD40L, CD40 ligand; eNOS, endothelial nitric oxide synthase; FKN, fractalkine; GRO, growth-related oncogene; HA, hyaluronan; ICAM, intercellular adhesion molecule; IP, intraperitoneal; IV, intravenous; KC, keratinocyte chemokine; MCP, monocyte chemotactic protein; MHC, major histocompatibility complex; SCID, severe combined immunodeficient; VCAM, vascular cell adhesion molecule; WT, wild type.

Cytokines can protect endothelial cells from apoptosis

Endothelial cell apoptosis has the potential to expose the underlying basement membrane, which can lead to thrombosis and further promotion of the inflammatory response. Enhancement of endothelial cell survival and function would therefore be hypothesized to be protective, especially in transplants in which the time between donor organ removal and transfer to the recipient can lead to significant loss of endothelial cells. This possibility is supported by intradermal injection of the anti-inflammatory cytokine IL-11, which protects human microvascular endothelium in severe combined immunodeficient (SCID) mice bearing human skin grafts (27). Although IL-11 had no effect on T cell infiltration, T cell activation markers and effector molecules, or endothelial ICAM-1 expression, it was able to significantly delay the time course of graft microvessel loss because of its ability to upregulate survivin, a member of the inhibitors of apoptosis family (27). Although IL-11 did not completely prevent allograft rejection, this was the first demonstration in vivo of cytokine regulation of survivin and protection from T cell-mediated endothelial cell injury.

Cytokine-activated endothelial cells can contribute to antigen presentation and immune cell activation

Although the endothelial cell monolayer primarily serves a protective function in normal vessels, allografts place the immunologically competent endothelial cell in contact with circulating immune cells. Endothelial cells express lymphocyte costimulatory molecules, and when induced by interferon (IFN)-γ to express major histocompatibility complex (MHC) class II, they can induce proliferation of allogeneic T cells (28). Infusion of proinflammatory IL-18, a member of the IL-1 cytokine family, into apoE$^{-/-}$ mice induced a 4-fold increase in cells expressing MHC class II, including endothelial cells, and an associated increase in aortic T cells (29). MHC class II expression is enhanced by IFN-γ, and the IL-18-mediated increase in MHC class II was not seen in male IFN-γ-null/apoE$^{-/-}$ mice infused with IL-18, implying a male-specific requirement for IFN-γ. However, a T cell-independent role for IFN-γ was further supported by infusion of IL-18 into SCID/apoE$^{-/-}$ mice that lacked T cells (30). MHC class II expression was increased 3-fold following IL-18 infusion that accompanied a 2-fold induction of IFN-γ produced by macrophages, NK, and vascular cells (30). The chemokine MCP-1 can promote another proinflammatory signaling pathway, the CD40/CD40 ligand (CD40L)-coupled signaling that is required for T cell priming and other immune regulation (31). Administration of an MCP-1 antagonist to apoE$^{-/-}$ mice with established lesions decreased CD40 and CD40L expression, including endothelial expression, and reduced T cell infiltration (31). However, it is unclear whether this is a direct or indirect effect of MCP-1 blockade.

Cytokine involvement in proliferation and migration of endothelial cells, including angiogenesis

Although several cytokine and chemokine receptors are expressed on endothelial cells (see Table 1), and some of these have been shown to promote proliferation or migration of endothelial cells in vitro, only TNF-α and KC/GRO-α have been shown to alter large endothelial cell repair in vivo. Administration of a soluble TNF-α receptor following balloon injury of the rat carotid artery decreased intimal lesion formation and accelerated endothelial cell regrowth by 125–140% 1 and 2 weeks after injury by Evan's blue dye labeling of vessel not covered by endothelium (32). In contrast, blockade of KC for 3 weeks after wire injury of apoE$^{-/-}$ carotid arteries with a monoclonal antibody to KC increased neointimal plaque area and decreased endothelial cell regrowth 3-fold, as evaluated by Evan's blue dye labeling and by CD31 and VCAM-1 endothelial cell staining (33). These data are compatible with the idea that KC normally has a protective role in accelerating endothelial recovery, whereas TNF-α can inhibit regrowth, although it is unclear whether the effects are direct or indirect.

The roles of particular angiogenic cytokines have not been tested in models of atherosclerosis; however, inhibition of plaque neovascularization has been demonstrated to be sufficient to decrease macrophage accumulation and plaque progression in advanced lesions of atherosclerosis (34). IL-2, IL-8, IL-15, and IL-18 have all been shown to induce angiogenesis in vivo (35–38). Therefore, it will be important to determine the extent to which these cytokines promote angiogenesis within the context of atherosclerotic lesions.

Cytokines can regulate endothelial-dependent vasorelaxation

A product of endothelial cells that is a potent anti-inflammatory agent is nitric oxide, and therefore induction or suppression of nitric oxide by cytokines has the potential to enhance or inhibit the inflammatory response. The receptor for IL-10, an anti-inflammatory cytokine, has been shown to be upregulated under proinflammatory conditions in vivo, and subsequent infusion of IL-10 induces nitric-oxide synthase-3, which attenuates expression of proinflammatory IL-12 (39). Further, the absence of IL-10 is sufficient to impair endothelial cell-dependent vasorelaxation and is associated with increased superoxide formation, and endothelial impairment is reversed by treatment with superoxide dismutase (40). In contrast, blockade of the proinflammatory cytokine IFN-γ in human allografts is sufficient to prevent endothelial cell dysfunction and loss of endothelial nitric oxide expression (41). Thus, cytokine stimulation of endothelial cells can both positively and negatively modulate expression of endothelial gene products that control vascular tone and the ability of the vessel to respond to vasodilatory signals.

CYTOKINES PROMOTE SMC PHENOTYPIC CHANGES AND THEIR ACCUMULATION WITHIN INTIMAL LESIONS

Progression of early "fatty streak" lesions, consisting of primarily macrophages and T lymphocytes, to intermedi-

ate lesions is characterized by the emigration of medial SMCs into the intimal lesions and their deposition of ECM (1). In more advanced fibrous plaques, SMCs are a predominant cell type and their accumulation and phenotype are critical in determining the extent and characteristics of these lesions. Cytokines can alter SMC phenotype and modulate the nature of matrix synthesis and secretion. For example, cytokines can promote the uptake of modified lipoproteins that leads to SMC foam cell formation in vitro (42), but the role of cytokines in modulating SMC foam cells in vivo has not been investigated. **Table 3** provides examples of cytokine effects on SMC functions that have been characterized in vivo in different models of vascular pathologies. Cytokine effects on SMC phenotype are discussed below using these in vivo data. In considering these studies, it is important to remember that cytokines and chemokines have major effects on monocyte and lymphocyte recruitment and activation [see accompanying review by Alan Daugherty and (3, 4)]. Thus, effects of cytokine blockade on SMC functions may be indirectly mediated by changes in monocytes and lymphocytes rather than through direct signals for SMCs.

Positive cytokine signaling in vivo contributes to SMC intimal accumulation

The accumulation of SMCs within intimal lesions is the combined result of their migration from the media into the intima and their proliferation (1). Although proliferation in vivo can be evaluated with antibodies to markers such as proliferating cell nuclear antigen, it is much more difficult to evaluate a specific contribution to migration in slowly progressing diseases such as atherosclerosis. The acute injury model, in which balloon or wire injury is used to denude a normal vessel, has been useful for studying migration, because the intimal lesions that form following injury consist primarily of SMCs, and kinetics of proliferation and migration have been characterized (43). Using this approach, the proinflammatory cytokines MCP-1, stromal cell-derived factor (SDF)1α, and CCL11 (eotaxin) have been implicated in the promotion of SMC migration and proliferation.

Femoral artery injury in mice lacking the MCP-1 receptor CCR2 results in smaller intimal SMC-rich lesions and less SMC proliferation (44). The same injury in MCP-1-null mice also resulted in a reduction in intimal lesion size, as compared with MCP-$1^{+/+}$ littermates, but there was no decrease in the SMC proliferative index (45). These data have been interpreted to suggest that MCP-1 may have a more important role in mediating SMC migration, whereas CCR2 may regulate cell proliferation (45). Differences have also been noted between vascular repair in normolipidemic versus hyperlipidemic models, raising the possibility of functional alterations in the MCP-1/CCR2 axis with differing levels of hypercholesterolemia (4). These effects may also be explained by the recent description of a second MCP-1 receptor (46). Blockade of SDF-1α in apo$E^{-/-}$ mice with a blocking antibody also strongly inhibits the accumulation of SMC in the neointima after vascular injury without any significant change in neointimal macrophage content, an effect mediated to a large degree by recruitment of hematopoietic SMC precursors (47). The role of CCL11 (eotaxin) on SMC accumulation following injury has not been directly addressed in vivo. However, CCL11 and its receptor, CCR3, are not expressed in normal artery but are abundant in medial and neointimal SMCs after injury (48). In vitro, CCL11 promotes SMC migration (48), so it will be interesting to determine whether CCR3 or CCL11 antagonists can inhibit SMC accumulation in injury models. Thus, at least the proinflammatory cytokines MCP-1 and SDF-1α promote SMC intimal accumulation following arterial injury.

Two other models of vascular injury that have been used to examine cytokine effects on lesion development involve either the placement of a silastic cuff around the femoral artery or ligation of the carotid artery. These models are less well characterized and both have a more significant involvement of inflammatory cells in the injury response. Analysis of mice lacking the natural IL-1 inhibitor IL-1Ra that had significantly increased IL-1 concentrations in the cuff model, showed a 2.5-fold increase in intimal thickness comprised primarily of SMCs and a 110% increase in intimal proliferation (17). Carotid artery ligation was used to investigate the role of TNF-α, and TNF-$\alpha^{-/-}$ mice showed reduced SMC accumulation (49). Therefore, the proinflammatory cytokines IL-1 and TNF-α both appear to promote SMC accumulation after injury, although the relative contribution of migration versus proliferation is not clear.

Analyses of models of murine atherosclerosis have provided further support for the involvement of the proinflammatory cytokines IL-1β, TNF-α, IFN-γ, and MIF in SMC accumulation. However, the relative effects are not always the same as those seen following injury in the models discussed above. Advanced lesions in apoE-deficient mice (32 weeks) with reduced expression of IL-1Ra (IL-$1Ra^{+/-}$ mice) showed a small but significant reduction (15%) in α-SMC actin-positive area (50), in contrast to enhanced SMC proliferation and accumulation following wire injury in mice on a C57BL/6 background (17). The reduction in SMC in lesions of IL-$1Ra^{+/-}$/$APO^{-/-}$ mice was likely due to the different cellular composition in these advanced lesions in which there was an 86% increase in lesion macrophages and expression of other inflammatory cytokines (50). Expression of a noncleavable mutant of TNF-α that effectively lowers TNF-α levels because it remains cell-associated seems to alter SMC phenotype in the medial SMCs adjacent to the lesion by reducing α-actin expression, with no marked effect on SMC proliferation (51). Thus, the accumulation of SMCs in lesions of atherosclerosis does not appear to be strongly dependent upon the action of IL-1β and TNF-α.

In IFN-γ receptor-null mice, atherosclerotic lesions appear strikingly less cellular, with increased accumulation of extracellular collagen, suggesting that signaling from IFN-γ positively contributes to SMC proliferation (52). This possibility is further supported by studies of LDLR-null mice crossed with IFN-γ-deficient mice (53). Finally, in a model of transplant atherosclerosis that utilized SCID/

TABLE 3. Cytokines alter smooth muscle gene expression and cellular function in vivo

Cytokine	Sources	In Vivo Model	In Vivo Effects	Reference
Promote smooth muscle cell migration and proliferation				
CCL/MCP-1	EC, SMC, M, T	Femoral artery injury in mice with targeted deletion of MCP-1	Reduced SMC proliferation and accumulation	(44, 45)
		MCP-1/CCR2 block in apoE$^{-/-}$ mice	Increase in lesion SMC and collagen	(31)
CCL11/eotaxin	SMC	Balloon injury mouse femoral artery	Increase in CCL11 and CCR3 with intimal migration of SMC in vivo and stimulation of migration in vitro	(48)
CX3CL1/FKN	EC, SMC, M	Human atherosclerosis	Positive correlation between SMC-expressing CX3CR1 and fractalkine-expressing cells	(10)
		ApoE$^{-/-}$ and LDLR$^{-/-}$ mice with targeted deletion of FKN	Reduction in SMC less complex lesions with fewer macrophages	(56)
IFN-γ	M, T, SMC	Infusion of artery tx in SCID mice	Enhanced SMC accumulation	(54)
IL-1	EC, SMC, M, T, B	Chow-fed apoE$^{-/-}$ mice that were also IL-1Ra$^{+/-}$ vs. $^{+/+}$ (IL-1Ra$^{+/-}$ results in increased IL-1)	15% Decrease in SMC accumulation at 32 weeks	(50)
		Cuff injury of femoral artery of IL-1Ra$^{+/-}$ vs. $^{+/+}$ mice with effective increase in IL-1 (IL-1Ra$^{+/-}$)	2.5-Fold increase in SMC accumulation at 21 days and 110% increase in PCNA^{+} intimal SMC	(17)
MIF	EC, SMC, M, T	MIF$^{-/-}$ vs. $^{+/+}$ in LDLR$^{-/-}$ mice	Reduction in SMC proliferation	(55)
		MIF block with injury in LDLR$^{-/-}$ mice	Inhibition of SMC proliferation	(80)
SDF-1α	EC, SMC, M	Carotid artery injury in mice	Antibody to SDF-1 inhibited neointima formation and recruitment of SMC progenitors inhibited	(47)
		Murine transplant atherosclerosis	Ab to SDF-1 inhibited neointima and progenitors	(95)
TGF-β	EC, SMC, M, T	Chow-fed apoE$^{-/-}$ mice with TGF-β signaling blockade	50% Decrease in fibrosis	(66, 96)
TNF-α	EC, SMC, M, T	Carotid artery ligation in TNFα$^{-/-}$ mice	Reduced intimal SMC accumulation	(49)
		Transmembrane TNF-α transgenic mice and atherogenic diet	Increased α-actin expression in intimal SMC	(51)
Inhibit smooth muscle accumulation				
IL-10	M, T, B	IV injection IL-10 following rat carotid artery injury	Reduced intima and SMC proliferation	(59)
		Heart transplant in IL-10$^{-/-}$ mice	Increase in SMC partially reversed by IFN antibody	(22)
IL-18	EC, SMC, M	Adenoviral expression IL-18 in cuff injury in apoE$^{-/-}$ mice	No change SMC number	(69)
		IM IL-18 binding protein expression plasmid into apoE$^{-/-}$ mice	2-Fold increase in SMC	(62)
		ApoE$^{-/-}$ mice and IL-18$^{+/+}$ vs. $^{-/-}$	3-Fold increase in SMC lesion area	(61)
Matrix remodeling and synthesis				
CCL2/MCP-1	EC, SMC, M, T	MCP-1/CCR2 block in apoE$^{-/-}$ mice	Increase in lesion SMC and collagen	(31)
IFN-γ	M, T, SMC	IFN-γ-null and apoE$^{-/-}$ mice	Decreased collagen content	(52)
IL-1	EC, SMC, M, T, B	Chow-fed apoE$^{-/-}$ and IL-1Ra$^{+/-}$ vs. $^{+/+}$	Enhanced medial elastic lamina destruction	(50)
IL-18	EC, SMC, M	Adenoviral expression of IL-18 in cuff injury in apoE$^{-/-}$ mice	No change SMC number but decrease in collagen and increase in MMP-13	(69)
		IM IL-18 binding protein expression plasmid into apoE$^{-/-}$ mice	80% Increase in collagen content	(62)
MIF	EC, SMC, M, T	MIF$^{-/-}$ vs. +/+ in LDLR$^{-/-}$ mice	Reduction in cysteine proteases and elastinogenic and collagenolytic activity	(55)
TGF-β	EC, SMC, M, T	Chow-fed apoE$^{-/-}$ mice with TGF-β signaling blockade	50% Decrease in fibrosis, including reduced collagen	(66, 96)
Anti-inflammatory signaling				
IL-10	M, T, B	IV injection IL-10 following rat carotid artery injury	Inhibits SMC NF-κB activation	(59)
		IL-10 for mouse carotid injury	Inhibits MCP-1 and NF-κB	(72)
TGF-β	EC, SMC, M, T	Chow-fed apoE$^{-/-}$ mice with TGF-β signaling blockade	Increase in NF-κB and IFN-γ	(66, 73)
Adhesion molecule expression				
IL-10	M, T, B	Adenoviral expression of IL-10 in rat venous injury model	Decreased vessel wall ICAM-1 mRNA levels	(78)
TNF-α	EC, SMC, M, T	Vein graft from TNFRp55$^{-/-}$ mice into p55$^{-/-}$ and $^{+/+}$ mice	50–60% Decrease in ICAM-1 and VCAM-1 in SMC of the vein graft wall	(77)
TGF-β	EC, SMC, M, T	Chow-fed apoE$^{-/-}$ mice with TGF-β signaling blockade	No change in VCAM-1 expression	(66, 73)
Smooth muscle cell antigen presentation				
CCL2/MCP-1	EC, SMC, M, T	MCP-1/CCR2 block in apoE$^{-/-}$ mice	Decreased CD40 and CD40L expression	(31)
IFN-γ	M, T, SMC	Infusion of artery transplants in SCID mice	Restored MHC class I in graft SMC	(54)
IL-18	EC, SMC, M	IL-18 administration to apoE$^{-/-}$ mice	Increase in MHC class II in lesions	(29)
TGF-β	EC, SMC, M, T	Chow-fed apoE$^{-/-}$ mice with TGF-β signaling blockade	Increase in CD40/CD40L and IA expression	(66, 73)

Ab, antibody; NF-κB, nuclear factor κB; PCNA, proliferating cell nuclear antigen.

beige mice as hosts, deficient in T and B lymphocytes and natural killer cell function, IFN-γ has been shown to induce SMC proliferation, but synergistically with the action of platelet-derived growth factor (54). The proinflammatory cytokine MIF appears to be one of the more potent cytokines in promoting SMC proliferation. MIF deletion leads to an ~80% reduction of SMC proliferation in atherosclerotic lesions of LDLR-null mice (55). CX3C chemokine ligand 1 (fractalkine) may also play a role in SMC accumulation, because it promotes SMC migration in vitro and is found in intimal SMCs in human atherosclerotic lesions (10). A positive correlation was observed between SMCs expressing the fractalkine receptor CX3CR1 and fractalkine-expressing cells in human lesions (10), and targeted deletion of fractalkine on either the LDLR$^{-/-}$ or apoE$^{-/-}$ background resulted in decreased SMC accumulation (56). However, because both lesion size and macrophage accumulation were also reduced, it is unclear whether the effect on SMCs is direct or indirect.

Cytokine signaling in vivo can prevent SMC intimal accumulation

Negative regulators of SMC accumulation have also been identified in vivo. Among cytokines with anti-inflammatory properties, as defined by their actions on lymphocytes and monocytes, IL-10 is the best characterized. IL-10 directly inhibits mitogen-induced SMC proliferation in vitro (57), and its role in vivo has recently been evaluated following injury. In a rabbit model of balloon injury, IL-10 infusion reduced SMC proliferation by 81% (58), and a similar reduction was seen with IL-10 administration following balloon injury of the rat carotid artery (59). IL-10 also inhibited intimal and medial SMC accumulation in a murine heart transplant model (22). Therefore, IL-10 shows inhibitory activity for SMCs in vivo under conditions in which SMCs are the primary intimal cell, and its action may counteract the proatherogenic activity of other cytokines that accumulate in lesions. Lesions in mice overexpressing IL-10 also appear less advanced than those in mice transplanted with wild-type bone marrow, with larger necrotic core area and reduced accumulation of SMCs and ECM, suggesting an antiproliferative SMC activity of IL-10 in murine atherosclerosis as well (60). Surprisingly, the proinflammatory cytokine, IL-18, also inhibits SMC accumulation in apoE-deficient mice, and its absence leads to a 2- to 3-fold increase in the proportion of α-SM-actin-positive cells (61, 62). Thus, although the majority of cytokines and chemokines promote SMC accumulation either directly or indirectly, two cytokines, IL-10 and IL-18, appear to negatively regulate SMC accumulation in lesions.

Matrix synthesis and remodeling by SMCs alters structural properties of lesions

The pathogenesis of atherosclerosis and restenosis following angioplasty or stent placement includes the abnormal production of ECM proteins by "synthetic" SMCs as well as remodeling of existing ECM components. Disruption and/or modification of SMC interactions with matrix components can significantly influence their responses to locally expressed cytokines and growth factors (63), and will further alter the structural properties of the vessel. Several in vivo studies have demonstrated direct or indirect roles for cytokines, including transforming growth factor (TGF)-β, MCP-1, MIF, IL-18, and IFN-γ, on ECM remodeling.

TGF-β has a well-established profibrotic activity that has been confirmed in vivo using different approaches. Injection of neutralizing anti-TGF-β_1 antibody, or a soluble TGF-β receptor that acts as an antagonist, into apoE$^{-/-}$ mice has demonstrated a significant reduction in lesion collagen content (~50%), with no apparent effect on SMC accumulation (64, 65). However, transgenic expression of a dominant negative TGF-β receptor II in T cells led to the development of thicker and more advanced lesions compared with apoE$^{-/-}$ with intact TGF-β signaling, although disrupted TGF-β signaling was associated with reduced collagen staining (66). Reduced accumulation of collagen and other ECM components has also been observed in lesions of MIF-deficient mice, suggesting that MIF, similar to TGF-β, positively contributes to matrix deposition (55). However, reduced collagen deposition in MIF-null mice has been partially attributed to their increased expression of matrix proteolytic enzymes, such as cathepsin S and l (55). Other in vivo evidence supports a profibrotic effect of both IL-6 and IL-10 (67, 68).

In contrast, blockade of MCP-1 signaling in apoE$^{-/-}$ mice, through the expression of an N-terminal-deleted mutant of MCP-1, leads to the development of more stable atherosclerotic plaques with increased SMC content and collagen deposition (31). Thus, MCP-1 may be a central mediator in the progression and destabilization of established atherosclerotic plaques, but a direct versus indirect effect has not been determined (31). Destruction of elastin lamina within the media has also been observed in IL-1Ra-deficient mice, suggesting that IL-1β signaling may promote the progression of unstable atherosclerotic plaques (50). Moreover, at least two in vivo studies indicate that a loss of IFN-γ signaling leads to substantial changes in lesion composition, supporting the notion that IFN-γ antagonists may serve to stabilize atherosclerotic plaques (52, 53). Similarly, overexpression of IL-18 decreases intimal collagen content in apoE-deficient mice (69), whereas overexpression of its endogenous binding protein increases collagen content (62).

Thus, TGF-β and MIF promote collagen deposition, whereas MCP-1, IL-1, IL-18, and IFN-γ enhance ECM remodeling. Collagen and elastin degradation, mediated by specific proteolytic enzymes, may facilitate the response of SMCs to the proliferative signaling of different cytokines, and consequently the enlargement of atherosclerotic lesions. Uncontrolled accumulation of SMCs, expressing proinflammatory cytokines, may also perpetuate the local inflammatory response in the arterial wall, leading to the progression and destabilization of advanced atherosclerotic plaques.

Anti-inflammatory cytokine signaling may limit SMC activation

Two cytokines, IL-10 and TGF-β, are notable in their ability to significantly inhibit the nuclear factor κB (NF- κB) proinflammatory signaling pathway. NF-κB is a pleiotropic

transcription factor that has been linked to atherosclerosis (70) and has the ability to modulate a wide array of SMC functions (71). Activation of NF-κB in neointimal SMCs lining the vessel wall is observed after balloon injury of rat carotid arteries, and this response is significantly inhibited in mice and rats treated with IL-10 (59, 72). In vivo administration of blocking anti-TGF-β antibody for 9 weeks in atherosclerotic mice is sufficient to induce expression of activated NF-κB in the myocardium (73). Thus, both IL-10 and TGF-β are potent inhibitors of the pleotropic NF-κB signaling pathway.

Adhesion molecule expression by SMCs following cytokine stimulation may contribute to retention of cells within lesions

Although cell adhesion molecules expressed on endothelial cells directly mediate leukocyte emigration into the vessel wall, increased expression of ICAM-1, VCAM-1, and P-selectin has also been observed in SMCs after vascular injury (74–76). More significantly, targeted deletion of the TNF-α receptor 1 decreased VCAM-1 and ICAM-1 expression by 50–60% in murine vein graft SMCs and decreased graft neointimal formation (77). Cytokines can also decrease adhesion molecule expression in vivo, as shown by the ability of adenoviral expression of IL-10 to inhibit ICAM-1 induction following rat venous injury (78). In contrast, in vivo administration of anti-TGF-β antibody for 9 weeks or disruption of TGF-β signaling in T cells did not alter VCAM-1 expression in atherosclerotic lesions at the aortic sinus or in SMCs (66, 73). Thus, TGF-β does not appear to alter adhesion molecule expression in SMCs during atherogenesis. Because blockade of cytokines such as TNF-α inhibits multiple effects, including the levels of NF-κB and other cytokines, it is not possible to evaluate their specific role in decreasing adhesion molecule expression. However, it is tempting to speculate that SMC adhesion molecules may contribute to retention of inflammatory cells within the vessel wall, and consequently further promote the inflammatory response within lesions.

Cytokine signaling may promote antigen presentation and processing

The importance of the adaptive immune response in atherosclerosis remains controversial, with several studies demonstrating that immunization with specific antigens can protect against disease and others showing that disease-related antigens may be responsible for increased atherosclerosis (3). However, atherosclerosis is dramatically enhanced in apoE$^{-/-}$ mice with loss of the potent immune inhibitor TGF-β resulting from transgenic expression of a dominant negative TGF-β receptor II in T cells (66). Inhibition of TGF-β led to an increase in the number of cells, including SMCs, expressing I-A^{b} region of the MHC in lesions of atherosclerosis (66). In contrast, in post-transplant graft atherosclerosis, administration of immune-promoting cytokine IFN-γ restored the weak basal expression of MHC class I antigen by graft SMCs (54). T cells recognize SMC MHC antigens, and their dependence on IFN-γ for basal expression indicates that IFN-γ has a physiological role in noninflammatory states. Another proinflammatory cytokine, IL-18, has also been shown to increase the mean number of SMCs expressing MHC II in atherosclerotic lesions of apoE-deficient mice, and appears to act upstream of IFN-γ, inasmuch as administration of IL-18 in IFN-γ-null mice did not alter MHC class II expression (29). MCP-1 also promotes antigen presentation and the immune response, as has been demonstrated in apoE-null mice treated with an inactive MCP-1 mutant that showed decreased expression of two crucial regulators of antigen presentation, CD40 and CD40L (31).

SUMMARY

During the last five years, transgenic and gene knockout studies in murine models of vascular disease have established cytokines and chemokines as pivotal players in the regulation of endothelial and SMC functions. Although genetic differences between mouse and man preclude direct translation of these findings to human disease, these studies have identified several pathways whose perturbation has the potential to significantly shift the balance between disease progression and retardation. Among the cytokines that promote disease progression, TNF-α plays a major role in the induction of endothelial and SMC adhesion molecule expression and blockade of endothelial regrowth after injury (5, 6, 15, 16, 21, 77, 79). MIF also induces disease progression as a potent stimulant of SMC accumulation and matrix deposition following vascular injury and in atherosclerosis (55, 80). In contrast, IL-10 retards lesion progression through its reduction of SMC accumulation (59) and inhibition of both endothelial (22, 40, 78) and SMC (59, 72) activation.

An important goal of future studies will be more-detailed investigation of the particular genes and pro- and anti-inflammatory pathways regulated by different cytokines in atherogenesis. A better understanding of the responses of specific vascular cells, as well as of the implications of the ability of a single cytokine to induce an amplification cascade of multiple additional downstream cytokines and chemokines, is also needed. The function of cytokines and chemokines within advanced lesions of atherosclerosis merits particular attention, because this represents the clinically relevant lesion. This challenge could lead to promising novel therapeutic targets for anti-inflammatory therapies, potentially even harnessing some of the sophisticated regulatory systems designed to normally limit the inflammatory response.

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REFERENCES

1. Ross, R. 1999. Atherosclerosis—an inflammatory disease. *N. Engl. J. Med.* **340:** 115–126.

2. Libby, P. 2002. Inflammation in atherosclerosis. *Nature.* **420:** 868–874.
3. Hansson, G. K., P. Libby, U. Schonbeck, and Z. Q. Yan. 2002. Innate and adaptive immunity in the pathogenesis of atherosclerosis. *Circ. Res.* **91:** 281–291.
4. Weber, C., A. Schober, and A. Zernecke. 2004. Chemokines: key regulators of mononuclear cell recruitment in atherosclerotic vascular disease. *Arterioscler. Thromb. Vasc. Biol.* **24:** 1997–2008.
5. Ahn, S. Y., C. H. Cho, K. G. Park, H. J. Lee, S. Lee, S. K. Park, I. K. Lee, and G. Y. Koh. 2004. Tumor necrosis factor-alpha induces fractalkine expression preferentially in arterial endothelial cells and mithramycin A suppresses TNF-alpha-induced fractalkine expression. *Am. J. Pathol.* **164:** 1663–1672.
6. Tereb, D. A., N. C. Kirkiles-Smith, R. W. Kim, Y. Wang, R. D. Rudic, J. S. Schechner, M. I. Lorber, A. L. Bothwell, J. S. Pober, and G. Tellides. 2001. Human T cells infiltrate and injure pig coronary artery grafts with activated but not quiescent endothelium in immunodeficient mouse hosts. *Transplantation.* **71:** 1622–1630.
7. Weber, K. S., P. von Hundelshausen, I. Clark-Lewis, P. C. Weber, and C. Weber. 1999. Differential immobilization and hierarchical involvement of chemokines in monocyte arrest and transmigration on inflamed endothelium in shear flow. *Eur. J. Immunol.* **29:** 700–712.
8. Comerford, I., and R. Nibbs. 2005. Post-translational control of chemokines: a role for decoy receptors? *Immunol. Lett.* **96:** 163–174.
9. Corry, D., K. Rishi, J. Kanellis, A. Kiss, L. L. Song, J. Xu, L. Feng, Z. Werb, and F. Kheradmand. 2002. Decreased allergic lung inflammatory cell egression and increased susceptibility to asphyxiation in MMP2-deficiency. *Nat. Immunol.* **3:** 347–353.
10. Lucas, A. D., C. Bursill, T. J. Guzik, J. Sadowski, K. M. Channon, and D. R. Greaves. 2003. Smooth muscle cells in human atherosclerotic plaques express the fractalkine receptor CX3CR1 and undergo chemotaxis to the CX3C chemokine fractalkine (CX3CL1). *Circulation.* **108:** 2498–2504.
11. Mantovani, A., M. Locati, A. Vecchi, S. Sozzani, and P. Allavena. 2001. Decoy receptors: a strategy to regulate inflammatory cytokines and chemokines. *Trends Immunol.* **22:** 328–336.
12. Levine, S. 2004. Mechanisms of soluble cytokine receptor generation. *J. Immunol.* **173:** 5343–5348.
13. Passerini, A., D. Polacek, C. Shi, N. Francesco, E. Manduchi, G. Grant, W. Pritchard, S. Powell, G. Chang, C. J. Stoeckert, et al. 2004. Coexisting proinflammatory and antioxidative endothelial transcription profiles in a disturbed flow region of the adult porcine aorta. *Proc. Natl. Acad. Sci. USA.* **100:** 10623–10628.
14. Simmons, C., G. Grant, E. Manduchi, and P. Davies. 2005. Spatial heterogeneity of endothelial phenotypes correlates with side-specific vulnerability to calcification in normal porcine aortic valves. *Circ. Res.* In press.
15. Gotsch, U., U. Jager, M. Dominis, and D. Vestweber. 1994. Expression of P-selectin on endothelial cells is upregulated by LPS and TNF-alpha in vivo. *Cell Adhes. Commun.* **2:** 7–14.
16. Neumann, B., T. Machleidt, A. Lifka, K. Pfeffer, D. Vestweber, T. W. Mak, B. Holzmann, and M. Kronke. 1996. Crucial role of 55-kilodalton TNF receptor in TNF-induced adhesion molecule expression and leukocyte organ infiltration. *J. Immunol.* **156:** 1587–1593.
17. Isoda, K., M. Shiigai, N. Ishigami, T. Matsuki, R. Horai, K. Nishikawa, M. Kusuhara, Y. Nishida, Y. Iwakura, and F. Ohsuzu. 2003. Deficiency of interleukin-1 receptor antagonist promotes neointimal formation after injury. *Circulation.* **108:** 516–518.
18. Labow, M., D. Shuster, M. Zetterstrom, P. Nunes, R. Terry, E. Cullinan, T. Bartfai, C. Solorzano, L. Moldawer, R. Chizzonite, et al. 1997. Absence of IL-1 signaling and reduced inflammatory response in IL-1 type I receptor-deficient mice. *J. Immunol.* **159:** 2452–2461.
19. Billingham, M. 1987. Cardiac transplant atherosclerosis. *Transplant. Proc.* **19:** 19–25.
20. Libby, P., and J. S. Pober. 2001. Chronic rejection. *Immunity.* **14:** 387–397.
21. Bergese, S. D., E. H. Huang, R. P. Pelletier, M. B. Widmer, R. M. Ferguson, and C. G. Orosz. 1995. Regulation of endothelial VCAM-1 expression in murine cardiac grafts. Expression of allograft endothelial VCAM-1 can be manipulated with antagonist of IFN-alpha or IL-4 and is not required for allograft rejection. *Am. J. Pathol.* **147:** 166–175.
22. Raisanen-Sokolowski, A., T. Glysing-Jensen, and M. E. Russell. 1998. Leukocyte-suppressing influences of interleukin (IL)-10 in cardiac allografts: insights from IL-10 knockout mice. *Am. J. Pathol.* **153:** 1491–1500.
23. Gregory, J. L., M. T. Leech, J. R. David, Y. H. Yang, A. Dacumos, and M. J. Hickey. 2004. Reduced leukocyte-endothelial cell interactions in the inflamed microcirculation of macrophage migration inhibitory factor-deficient mice. *Arthritis Rheum.* **50:** 3023–3034.
24. Huo, Y., C. Weber, S. B. Forlow, M. Sperandio, J. Thatte, M. Mack, S. Jung, D. R. Littman, and K. Ley. 2001. The chemokine KC, but not monocyte chemoattractant protein-1, triggers monocyte arrest on early atherosclerotic endothelium. *J. Clin. Invest.* **108:** 1307–1314.
25. Weber, C., R. Alon, B. Moser, and T. Springer. 1996. Sequential regulation of alpha 4 beta 1 and alpha 5 beta 1 integrin avidity by CC chemokines in monocytes: implications for transendothelial chemotaxis. *J. Cell Biol.* **134:** 1063–1073.
26. Estess, P., A. Nandi, M. Mohamadzadeh, and M. H. Siegelman. 1999. Interleukin 15 induces endothelial hyaluronan expression in vitro and promotes activated T cell extravasation through a CD44-dependent pathway in vivo. *J. Exp. Med.* **190:** 9–19.
27. Kirkiles-Smith, N. C., K. Mahboubi, J. Plescia, J. M. McNiff, J. Karras, J. S. Schechner, D. C. Altieri, and J. S. Pober. 2004. IL-11 protects human microvascular endothelium from alloinjury in vivo by induction of survivin expression. *J. Immunol.* **172:** 1391–1396.
28. Pober, J., T. Collins, M. J. Gimbrone, R. Cotran, J. Gitlin, W. Fiers, C. Clayberger, A. Krensky, F. S. Burakof, and C. Reiss. 1983. Lymphocytes recognize human vascular endothelial and dermal fibroblast Ia antigens induced by recombinant immune interferon. *Nature.* **305:** 726–729.
29. Whitman, S. C., P. Ravisankar, and A. Daugherty. 2002. Interleukin-18 enhances atherosclerosis in apolipoprotein E(−/−) mice through release of interferon-gamma. *Circ. Res.* **90:** E34–E38.
30. Tenger, C., A. Sundborger, J. Jawien, and X. Zhou. 2005. IL-18 accelerates atherosclerosis accompanied by elevation of IFN-{gamma} and CXCL16 expression independently of T cells. *Arterioscler. Thromb. Vasc. Biol.* **25:** 1–6.
31. Inoue, S., K. Egashira, W. Ni, S. Kitamoto, M. Usui, K. Otani, M. Ishibashi, K. Hiasa, K. Nishida, and A. Takeshita. 2002. Anti-monocyte chemoattractant protein-1 gene therapy limits progression and destabilization of established atherosclerosis in apolipoprotein E-knockout mice. *Circulation.* **106:** 2700–2706.
32. Krasinski, K., I. Spyridopoulos, M. Kearney, and D. Losordo. 2001. In vivo blockade of tumor necrosis factor-alpha accelerates functional endothelial recovery after balloon angioplasty. *Circulation.* **104:** 1754–1756.
33. Liehn, E. A., A. Schober, and C. Weber. 2004. Blockade of keratinocyte-derived chemokine inhibits endothelial recovery and enhances plaque formation after arterial injury in apoE-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **24:** 1891–1896.
34. Moulton, K., K. Vakili, D. Zurakowski, M. Soliman, C. Butterfield, E. Ylvin, K. Lo, S. Gillies, K. Javaherian, and J. Folkman. 2003. Inhibition of plaque neovascularization reduces macrophage accumulation and progression of advanced atherosclerosis. *Proc. Natl. Acad. Sci. USA.* **100:** 4736–4741.
35. Dentelli, P., L. Del Sorbo, A. Rosso, A. Molinar, G. Garbarino, G. Camussi, L. Pegoraro, and M. F. Brizzi. 1999. Human IL-3 stimulates endothelial cell motility and promotes in vivo new vessel formation. *J. Immunol.* **163:** 2151–2159.
36. Angiolillo, A. L., H. Kanegane, C. Sgadari, G. H. Reaman, and G. Tosato. 1997. Interleukin-15 promotes angiogenesis in vivo. *Biochem. Biophys. Res. Commun.* **233:** 231–237.
37. Park, C. C., J. C. Morel, M. A. Amin, M. A. Connors, L. A. Harlow, and A. E. Koch. 2001. Evidence of IL-18 as a novel angiogenic mediator. *J. Immunol.* **167:** 1644–1653.
38. Simonini, A., M. Moscucci, D. W. Muller, E. R. Bates, F. D. Pagani, M. D. Burdick, and R. M. Strieter. 2000. IL-8 is an angiogenic factor in human coronary atherectomy tissue. *Circulation.* **101:** 1519–1526.
39. Cattaruzza, M., W. Slodowski, M. Stojakovic, R. Krzesz, and M. Hecker. 2003. Interleukin-10 induction of nitric-oxide synthase expression attenuates CD40-mediated interleukin-12 synthesis in human endothelial cells. *J. Biol. Chem.* **278:** 37874–37880.
40. Gunnett, C. A., D. D. Heistad, D. J. Berg, and F. M. Faraci. 2000. IL-10 deficiency increases superoxide and endothelial dysfunction during inflammation. *Am. J. Physiol. Heart Circ. Physiol.* **279:** H1555–H1562.
41. Koh, K. P., Y. Wang, T. Yi, S. L. Shiao, M. I. Lorber, W. C. Sessa, G. Tellides, and J. S. Pober. 2004. T cell-mediated vascular dysfunction of human allografts results from IFN-gamma dysregulation of NO synthase. *J. Clin. Invest.* **114:** 846–856.

42. Li, H., M. Freeman, and P. Libby. 1995. Regulation of smooth muscle cell scavenger receptor expression in vivo by atherogenic diets and in vitro by cytokines. *J. Clin. Invest.* **95:** 122–133.
43. Jawien, A., D. F. Bowen-Pope, V. Lindner, S. M. Schwartz, and A. W. Clowes. 1992. Platelet-derived growth factor promotes smooth muscle migration and intimal thickening in a rat model of balloon angioplasty. *J. Clin. Invest.* **89:** 507–511.
44. Roque, M., W. J. Kim, M. Gazdoin, A. Malik, E. D. Reis, J. T. Fallon, J. J. Badimon, I. F. Charo, and M. B. Taubman. 2002. CCR2 deficiency decreases intimal hyperplasia after arterial injury. *Arterioscler. Thromb. Vasc. Biol.* **22:** 554–559.
45. Kim, W. J., I. Chereshnev, M. Gazdoiu, J. T. Fallon, B. J. Rollins, and M. B. Taubman. 2003. MCP-1 deficiency is associated with reduced intimal hyperplasia after arterial injury. *Biochem. Biophys. Res. Commun.* **310:** 936–942.
46. Schecter, A., A. Berman, L. Yi, H. Ma, C. Daly, K. Soejima, B. Rollins, I. Charo, and M. Taubman. 2004. MCP-1-dependent signaling in CCR2(−/−) aortic smooth muscle cells. *J. Leukoc. Biol.* **75:** 1079–1085.
47. Schober, A., S. Knarren, M. Lietz, E. A. Lin, and C. Weber. 2003. Crucial role of stromal cell-derived factor-1alpha in neointima formation after vascular injury in apolipoprotein E-deficient mice. *Circulation.* **108:** 2491–2497.
48. Kodali, R. B., W. J. Kim, I. I. Galaria, C. Miller, A. D. Schecter, S. A. Lira, and M. B. Taubman. 2004. CCL11 (eotaxin) induces CCR3-dependent smooth muscle cell migration. *Arterioscler. Thromb. Vasc. Biol.* **24:** 1211–1216.
49. Rectenwald, J. E., L. L. Moldawer, T. S. Huber, J. M. Seeger, and C. K. Ozaki. 2000. Direct evidence for cytokine involvement in neointimal hyperplasia. *Circulation.* **102:** 1697–1702.
50. Isoda, K., S. Sawada, N. Ishigami, T. Matsuki, K. Miyazaki, M. Kusuhara, Y. Iwakura, and F. Ohsuzu. 2004. Lack of interleukin-1 receptor antagonist modulates plaque composition in apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **24:** 1068–1073.
51. Canault, M., F. Peiretti, C. Mueller, F. Kopp, P. Morange, S. Rihs, H. Portugal, I. Juhan-Vague, and G. Nalbone. 2004. Exclusive expression of transmembrane TNF-alpha in mice reduces the inflammatory response in early lipid lesions of aortic sinus. *Atherosclerosis.* **172:** 211–218.
52. Gupta, S., A. M. Pablo, X. Jiang, N. Wang, A. R. Tall, and C. Schindler. 1997. IFN-gamma potentiates atherosclerosis in apoE knockout mice. *J. Clin. Invest.* **99:** 2752–2761.
53. Buono, C., C. E. Come, G. Stavrakis, G. F. Maguire, P. W. Connelly, and A. H. Lichtman. 2003. Influence of interferon-gamma on the extent and phenotype of diet-induced atherosclerosis in the LDLR-deficient mouse. *Arterioscler. Thromb. Vasc. Biol.* **23:** 454–460.
54. Tellides, G., D. A. Tereb, N. C. Kirkiles-Smith, R. W. Kim, J. H. Wilson, J. S. Schechner, M. I. Lorber, and J. S. Pober. 2000. Interferon-gamma elicits arteriosclerosis in the absence of leukocytes. *Nature.* **403:** 207–211.
55. Pan, J. H., G. K. Sukhova, J. T. Yang, B. Wang, T. Xie, H. Fu, Y. Zhang, A. R. Satoskar, J. R. David, C. N. Metz, et al. 2004. Macrophage migration inhibitory factor deficiency impairs atherosclerosis in low-density lipoprotein receptor-deficient mice. *Circulation.* **109:** 3149–3153.
56. Teupser, D., S. Pavlides, M. Tan, J. C. Gutierrez-Ramos, R. Kolbeck, and J. L. Breslow. 2004. Major reduction of atherosclerosis in fractalkine (CX3CL1)-deficient mice is at the brachiocephalic artery, not the aortic root. *Proc. Natl. Acad. Sci. USA.* **101:** 17795–17800.
57. Selzman, C. H., R. C. McIntyre, Jr., B. D. Shames, T. A. Whitehill, A. Banerjee, and A. H. Harken. 1998. Interleukin-10 inhibits human vascular smooth muscle proliferation. *J. Mol. Cell. Cardiol.* **30:** 889–896.
58. Feldman, L. J., L. Aguirre, M. Ziol, J. P. Bridou, N. Nevo, J. B. Michel, and P. G. Steg. 2000. Interleukin-10 inhibits intimal hyperplasia after angioplasty or stent implantation in hypercholesterolemic rabbits. *Circulation.* **101:** 908–916.
59. Mazighi, M., A. Pelle, W. Gonzalez, el M. Mtairag, M. Philippe, D. Henin, J. B. Michel, and L. J. Feldman. 2004. IL-10 inhibits vascular smooth muscle cell activation in vitro and in vivo. *Am. J. Physiol. Heart Circ. Physiol.* **287:** H866–H871.
60. Pinderski, L. J., M. P. Fischbein, G. Subbanagounder, M. C. Fishbein, N. Kubo, H. Cheroutre, L. K. Curtiss, J. A. Berliner, and W. A. Boisvert. 2002. Overexpression of interleukin-10 by activated T lymphocytes inhibits atherosclerosis in LDL receptor-deficient mice by altering lymphocyte and macrophage phenotypes. *Circ. Res.* **90:** 1064–1071.
61. Elhage, R., J. Jawien, M. Rudling, H. G. Ljunggren, K. Takeda, S. Akira, F. Bayard, and G. K. Hansson. 2003. Reduced atherosclerosis in interleukin-18 deficient apolipoprotein E-knockout mice. *Cardiovasc. Res.* **59:** 234–240.
62. Mallat, Z., A. Corbaz, A. Scoazec, S. Besnard, G. Leseche, Y. Chvatchko, and A. Tedgui. 2001. Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. *Circulation.* **104:** 1598–1603.
63. Koyama, H., E. W. Raines, K. E. Bornfeldt, J. M. Roberts, and R. Ross. 1996. Fibrillar collagen inhibits arterial smooth muscle proliferation through regulation of Cdk2 inhibitors. *Cell.* **87:** 1069–1078.
64. Lutgens, E., M. Gijbels, M. Smook, P. Heeringa, P. Gotwals, V. E. Koteliansky, and M. J. Daemen. 2002. Transforming growth factor-beta mediates balance between inflammation and fibrosis during plaque progression. *Arterioscler. Thromb. Vasc. Biol.* **22:** 975–982.
65. Mallat, Z., A. Gojova, C. Marchiol-Fournigault, B. Esposito, C. Kamate, R. Merval, D. Fradelizi, and A. Tedgui. 2001. Inhibition of transforming growth factor-beta signaling accelerates atherosclerosis and induces an unstable plaque phenotype in mice. *Circ. Res.* **89:** 930–934.
66. Robertson, A. K., M. Rudling, X. Zhou, L. Gorelik, R. A. Flavell, and G. K. Hansson. 2003. Disruption of TGF-beta signaling in T cells accelerates atherosclerosis. *J. Clin. Invest.* **112:** 1342–1350.
67. Mallat, Z., S. Besnard, M. Duriez, V. Deleuze, F. Emmanuel, M. F. Bureau, F. Soubrier, B. Esposito, H. Duez, C. Fievet, et al. 1999. Protective role of interleukin-10 in atherosclerosis. *Circ. Res.* **85:** e17–e24.
68. Schieffer, B., T. Selle, A. Hilfiker, D. Hilfiker-Kleiner, K. Grote, U. J. Tietge, C. Trautwein, M. Luchtefeld, C. Schmittkamp, S. Heeneman, et al. 2004. Impact of interleukin-6 on plaque development and morphology in experimental atherosclerosis. *Circulation.* **110:** 3493–3500.
69. de Nooijer, R., J. H. von der Thusen, C. J. Verkleij, J. Kuiper, J. W. Jukema, E. E. van der Wall, J. C. van Berkel, and E. A. Biessen. 2004. Overexpression of IL-18 decreases intimal collagen content and promotes a vulnerable plaque phenotype in apolipoprotein-E-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **24:** 2313–2319.
70. Collins, T., and M. I. Cybulsky. 2001. NF-kappaB: pivotal mediator or innocent bystander in atherogenesis? *J. Clin. Invest.* **107:** 3–10.
71. Raines, E., K. Garton, and N. Ferri. 2004. Beyond the endothelium: NF-kappaB regulation of smooth muscle function. *Circ. Res.* **94:** 706–708.
72. Zimmerman, M. A., L. L. Reznikov, C. D. Raeburn, and C. H. Selzman. 2004. Interleukin-10 attenuates the response to vascular injury. *J. Surg. Res.* **121:** 206–213.
73. Mallat Z., A. Gojova, C. Marchiol-Fournigault, B. Esposito, C. Kamate, R. Merval, D. Fradelizi, and A. Tedgui. 2001. Inhibition of transforming growth factor-beta signaling accelerates atherosclerosis and induces an unstable plaque phenotype in mice. *Circ. Res.* **89:** 930–934.
74. Landry, D. B., L. L. Couper, S. R. Bryant, and V. Lindner. 1997. Activation of the NF-kappa B and I kappa B system in smooth muscle cells after rat arterial injury. Induction of vascular cell adhesion molecule-1 and monocyte chemoattractant protein-1. *Am. J. Pathol.* **151:** 1085–1095.
75. Breuss, J. M., M. Cejna, H. Bergmeister, A. Kadl, G. Baumgartl, S. Steurer, Z. Xu, Y. Koshelnick, J. Lipp, R. De Martin, et al. 2002. Abstract activation of nuclear factor-kappa B significantly contributes to lumen loss in a rabbit iliac artery balloon angioplasty model. *Circulation.* **105:** 633–638.
76. Zeiffer, U., A. Schober, M. Lietz, E. Liehn, W. Erl, N. Emans, Z. Yan, and C. Weber. 2004. Neointimal smooth muscle cells display a proinflammatory phenotype resulting in increased leukocyte recruitment mediated by P-selectin and chemokines. *Circ. Res.* **94:** 776–784.
77. Zhang, L., K. Peppel, L. Brian, L. Chien, and N. Freedman. 2004. Vein graft neointimal hyperplasia is exacerbated by tumor necrosis factor receptor-1 signaling in graft-intrinsic cells. *Arterioscler. Thromb. Vasc. Biol.* **24:** 2277–2283.
78. Henke, P. K., L. A. DeBrunye, R. M. Strieter, J. S. Bromberg, M. Prince, A. M. Kadell, M. Sarkar, F. Londy, and T. W. Wakefield. 2000. Viral IL-10 gene transfer decreases inflammation and cell adhesion molecule expression in a rat model of venous thrombosis. *J. Immunol.* **164:** 2131–2141.
79. Krasinski, K., I. Spyridopoulos, M. Kearney, and D. W. Losordo. 2001. In vivo blockade of tumor necrosis factor-alpha accelerates functional endothelial recovery after balloon angioplasty. *Circulation.* **104:** 1754–1756.

80. Chen, Z., M. Sakuma, A. C. Zago, X. Zhang, C. Shi, L. Leng, Y. Mizue, R. Bucala, and D. Simon. 2004. Evidence for a role of macrophage migration inhibitory factor in vascular disease. *Arterioscler. Thromb. Vasc. Biol.* **24:** 709–714.
81. Martin, G., F. Dol, A. M. Mares, V. Berezowski, B. Staels, D. W. Hum, P. Schaeffer, and J. M. Herbert. 2004. Lesion progression in apoE-deficient mice: implication of chemokines and effect of the AT1 angiotensin II receptor antagonist irbesartan. *J. Cardiovasc. Pharmacol.* **43:** 191–199.
82. Abi-Younes, S., A. Sauty, F. Mach, G. K. Sukhova, P. Libby, and A. D. Luster. 2000. The stromal cell-derived factor-1 chemokine is a potent platelet agonist highly expressed in atherosclerotic plaques. *Circ. Res.* **86:** 131–138.
83. Lesnik, P., C. A. Haskell, and I. F. Charo. 2003. Decreased atherosclerosis in CX3CR1−/− mice reveals a role for fractalkine in atherogenesis. *J. Clin. Invest.* **111:** 333–340.
84. Hansson, G. K., J. Holm, and L. Jonasson. 1989. Detection of activated T lymphocytes in the human atherosclerotic plaque. *Am. J. Pathol.* **135:** 169–175.
85. Moyer, C. F., D. Sajuthi, H. Tulli, and J. K. Williams. 1991. Synthesis of IL-1 alpha and IL-1 beta by arterial cells in atherosclerosis. *Am. J. Pathol.* **138:** 951–960.
86. Wang, X., A. M. Romanic, T. L. Yue, G. Z. Feuerstein, and E. H. Ohlstein. 2000. Expression of interleukin-1beta, interleukin-1 receptor, and interleukin-1 receptor antagonist mRNA in rat carotid artery after balloon angioplasty. *Biochem. Biophys. Res. Commun.* **271:** 138–143.
87. Brizzi, M. F., L. Formato, P. Dentelli, A. Rosso, M. Pavan, G. Garbarino, M. Pegoraro, G. Camussi, and L. Pegoraro. 2001. Interleukin-3 stimulates migration and proliferation of vascular smooth muscle cells: a potential role in atherogenesis. *Circulation.* **103:** 549–554.
88. Frostegard, J., A. K. Ulfgren, P. Nyberg, U. Hedin, J. Swedenborg, U. Andersson, and G. K. Hansson. 1999. Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines. *Atherosclerosis.* **145:** 33–43.
89. Wuttge, D. M., P. Eriksson, A. Sirsjo, G. K. Hansson, and S. Stemme. 2001. Expression of interleukin-15 in mouse and human atherosclerotic lesions. *Am. J. Pathol.* **159:** 417–423.
90. Gerdes, N., G. K. Sukhova, P. Libby, R. S. Reynolds, J. L. Young, and U. Schonbeck. 2002. Expression of interleukin (IL)-18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for atherogenesis. *J. Exp. Med.* **195:** 245–257.
91. Leng, L., C. N. Metz, Y. Fang, J. Xu, S. Donnelly, J. Baugh, T. Delohery, Y. Chen, R. A. Mitchell, and R. Bucala. 2003. MIF signal transduction initiated by binding to CD74. *J. Exp. Med.* **197:** 1467–1476.
92. Burger-Kentischer, A., H. Goebel, R. Seiler, G. Fraedrich, H. E. Schaefer, S. Dimmeler, R. Kleemann, J. Bernhagen, and C. Ihling. 2002. Expression of macrophage migration inhibitory factor in different stages of human atherosclerosis. *Circulation.* **105:** 1561–1566.
93. Barath, P., M. C. Fishbein, J. Cao, J. Berenson, R. H. Helfant, and J. S. Forrester. 1990. Detection and localization of tumor necrosis factor in human atheroma. *Am. J. Cardiol.* **65:** 297–302.
94. Barcelos, L. S., A. Talvani, A. S. Teixeira, G. D. Cassali, S. P. Andrade, and M. M. Teixeira. 2004. Production and in vivo effects of chemokines CXCL1–3/KC and CCL2/JE in a model of inflammatory angiogenesis in mice. *Inflamm. Res.* **53:** 576–584.
95. Sakihama, H., T. Masunaga, K. Yamashita, T. Hashimoto, M. Inobe, S. Todo, and T. Uede. 2004. Stromal cell-derived factor-1 and CXCR4 interaction is critical for development of transplant arteriosclerosis. *Circulation.* **110:** 2924–2930.
96. Lutgens, E., M. Gijbels, M. Smook, P. Heeringa, P. Gotwals, V. Koteliansky, and M. Daemen. 2002. Transforming growth factor-beta mediates balance between inflammation and fibrosis during plaque progression. *Arterioscler. Thromb. Vasc. Biol.* **22:** 975–982.