The Insulin-Like Growth Factor Axis
A Review of Atherosclerosis and Restenosis
Antoni Bayes-Genis, Cheryl A. Conover, Robert S. Schwartz

Abstract—Insulin-like growth factors I and II (IGF-I and -II) and their regulatory proteins are secreted by cells of the cardiovascular system. They are growth promoters for arterial cells and mediators of cardiovascular disease. IGFs are bound to IGF binding proteins (IGFBPs), which modulate IGF ligand-receptor interaction and consequently to IGF action. IGFBPs are in turn posttranslationally modulated by specific proteases. This dynamic balance (IGFs, IGFBPs, and IGFBP proteases) constitutes the IGF axis and ultimately determines the extent of IGF-dependent cellular effects. Dysregulated actions of this axis influence coronary atherosclerosis through effects on vascular smooth muscle cell growth, migration, and extracellular matrix synthesis in the atherosclerotic plaque. IGF-I promotes macrophage chemotaxis, excess LDL cholesterol uptake, and release of proinflammatory cytokines. Endothelial cells also receive the effects of IGFs stimulating their migration and organization forming capillary networks. Neointimal hyperplasia of restenosis after coronary artery injury is also modulated by the IGF axis. IGFs stimulate vascular smooth muscle cell proliferation and migration to form the neointima and upregulate tropoelastin synthesis after disruption of the elastic layer. Understanding IGF axis regulation establishes a scientific basis for strategies directed to limit or reverse plaque growth and vulnerability in atherosclerosis and in the neointimal hyperplasia of restenosis. (Circ Res. 2000;86:125-130.)

Key Words: insulin-like growth factor ■ atherosclerosis ■ restenosis

Insulin-like growth factors I and II (IGF-I and -II) are regular constituents of human blood plasma. Over the past decade, the functions of circulating IGFs have become more clear, but the actions of locally produced IGFs are still ill defined. Accumulating evidence now indicates that IGFs and their regulatory proteins, secreted by cells of the cardiovascular system, are growth promoters for arterial cells and mediators of cardiovascular diseases.1–5 Dysregulated actions of these factors contribute to coronary atherosclerosis and restenosis. This article first reviews the basic physiology of the IGF axis and then discusses specific autocrine and paracrine actions of IGFs in atherosclerotic plaque progression and the neointimal hyperplasia of restenosis.

The IGF Axis
IGF-I and IGF-II are single-chain polypeptides (70 and 67 amino acids, respectively) that share homology with each other and with proinsulin.6 Systemic IGF-I and IGF-II levels are determined mainly by production in the liver. However, many cells of the body synthesize these growth factors.6 The IGFs have a broad range of physiological actions starting with early embryonic development and extending throughout life. Metabolic functions, particularly glucose metabolism, constitute an important aspect of IGF-I and -II activities.7 The IGFs also induce differentiated functions of cells stimulating amino acid uptake and protein synthesis,8 and promoting migration.9 Another prominent aspect of IGF-I is regulation of cell cycle progression and mitogenesis.10,11 IGFs may also function as survival factors by decreasing apoptosis in various cells.12

The actions of IGFs are mediated by specific membrane receptors.13 Type I IGF receptor, homologous to the insulin receptor, contains 2 α and 2 β subunits and has tyrosine kinase activity responsible for IGF-I and IGF-II intracellular signaling (Figure 1C). Type II IGF receptor, identical to mannose-6-phosphate receptor, has an uncertain role in growth factor signaling.14 Recently, IGF-II also showed mitogenic effects through a high-affinity interaction with isoform A of the insulin receptor.15

The ultimate cell response to IGFs depends on the context of IGF binding proteins (IGFBPs). Six different IGFBPs have been identified.16 The IGFBP that carries most circulating IGF (90% in adult serum) is IGFBP-3, existing predominantly as a 150-kDa “ternary” complex consisting of an additional protein termed the acid-labile subunit.17 This complex restricts the extravascular transit and is a circulating store for IGFs. At the cellular level, IGFBPs form a binary complex with IGFs and critically modulate local IGF actions18 (Figure 1A). A further refinement of this axis is recent evidence that IGFBPs undergo posttranslational modification.
Proteolytic activity of the IGFBP proteases results in modulation of IGFBP affinity for IGFs or complete destruction of IGF binding potential. IGFBP proteases regulate IGF half-life and play a critical role in modulating IGF availability at the cellular level (Figure 1B). This dynamic balance (IGFs, IGFBPs, and IGFBP proteases) constitutes the IGF axis and ultimately determines the extent of IGF-dependent cellular effects.

The next sections outline the relation between the IGF axis and 2 important vascular pathologies, atherosclerosis and restenosis.

Atherosclerosis
Atherosclerotic plaque develops over several decades and involves inflammatory cell infiltration, smooth muscle cell proliferation, accumulation of extracellular matrix, fibrous cap formation, and angiogenesis. Among the various growth factors involved in atherosclerotic plaque development, IGFs play a relevant role (Figure 2). The different cell types of atherosclerosis secrete IGFs, and type I IGF receptors are present on smooth muscle cells, inflammatory cells, and arterial endothelial cells within the atherosclerotic lesion.

 Activation of Vascular Smooth Muscle Cells (VSMCs)
VSMC dysregulation at atherosclerotic sites is associated with a shift from the so-called contractile-to-synthetic phenotype and displays many features of growth factor activation. Several in vitro studies of both animal and human VSMCs show that IGF-I induces cell cycle changes resulting in VSMC proliferation and migration. IGF-I and platelet-derived growth factor (PDGF) act synergistically to stimulate VSMC proliferation. IGF growth-promoting effects are also interactive with the effects of angiotensin II and basic fibroblast growth factor.

Studies of ischemic patients reinforce the IGF effects seen in vitro. Coronary atherectomy specimens show markedly increased IGF-I, type I IGF receptor, and IGFBPs 1 to 5 in VSMCs. Intense localization of these proteins occurs primarily in the cytoplasm of VSMCs exhibiting the synthetic phenotype. Pfeifle and Ditschuneit showed that type I IGF receptor expression varies with cellular growth status. These observations suggest that IGF axis protein synthesis and secretion depend on the phenotypic state of plaque-derived VSMCs.

A recent study of severely atherosclerotic patients showed higher IGF-I mRNA expression in regions containing densely packed VSMCs within the active plaque, compared with lower levels found in stable plaques. Morbidity and mortality of atherosclerotic patients is significantly reduced with the use of statins. The statins inhibit 3-hydroxy-3-methylglutharyl-coenzyme A reductase and thus reduce endogenous cholesterol synthesis. Some statin effects also are mediated by IGF-I, because lovastatin efficiently blocks intracellular signaling pathways activated by IGF-I and limits VSMC proliferation.

Migration of VSMCs from the media is a major pathologic vascular response leading to the development and progression of the lesions of atherosclerosis. IGFs are potent stimuli of VSMC migration, and the effect appears to be mediated through type I IGF receptor. Interestingly, one of the IGFBPs, IGFBP-1, stimulates migration independent of IGF-I via the integrin.

VSMC apoptosis occurs in the evolutionary process of atherosclerotic plaques. This programmed cell death can be suppressed by high IGF-I and PDGF concentrations, a finding consistent with recent studies of IGF-I and PDGF as potent survival factors for rat VSMCs. Likewise, constitutive overexpression of IGF-I prevents cell death of viable myocardium after infarction, limiting ventricular dilation, myocardial loading, and cardiac hypertrophy. Current information on IGF cardioprotective activity can be found in recent reviews. Yet the IGF-I requirement for cell survival shown both in vitro and in vivo is uncertain in the vessel wall in the presence of IGFBP and IGFBP proteases.

Little is known about IGFBPs and proteases in atherosclerosis. IGFBP-2 and -4 are the more prevalent binding proteins secreted by VSMCs. IGFBP-2 stimulates IGF-dependent mitosis in porcine VSMCs in the presence of platelet-poor...
plasma and inhibits DNA synthesis in serum-free medium, suggesting that the presence of a factor in plasma is required for IGFBP-2 to potentiate IGF effects. Interestingly, IGFBP-2 inhibits IGF-stimulated VSMC migration by preventing type I IGF receptor interaction. IGFBP-4 consistently inhibits IGF-mediated actions in all cell types studied. Specific proteases have been identified in VSMCs for these binding proteins, which modify IGFBPs, and hence, IGF biological activity (Figure 1). We recently isolated a novel IGF-dependent IGFBP-4–specific protease and identified it as pregnancy-associated plasma protein-A (PAPP-A), a member of the metzincin family of metalloproteinases. By cleaving IGFBP-4 and releasing free IGF-I, PAPP-A appears to modulate growth in local proliferative responses.

Independent of its growth-promoting properties, IGF-I induces coronary vasorelaxation acting primarily on VSMCs. Sowers found increased VSMC production of inducible NO under IGF-I stimulation, whereas others suggest that coronary vasorelaxation is due to activation of the potassium channels independently of NO. Whichever pathway prevails, IGF-I maintains coronary epicardial vasomotor tone in pathophysiologic states.

Macrophage Activation
Macrophage accumulation is an early event in atherosclerosis. Macrophages are crucial in inflammatory processes associated with tissue injury through the ability to induce phagocytosis and to release proteases and cytokines.

High-affinity type I IGF receptors on the macrophage surface allow IGFs to modulate macrophage concentrations at injury sites. Human macrophages also synthesize and secrete IGF-1 and some of the binding proteins. IGF-I secreted within the atherosclerotic lesion is important for monocyte chemotaxis, activation, and cytokine release (ie, tumor necrosis factor-α). It is likely that macrophage-derived IGF enhances cellular LDL uptake and degradation and also the macrophage cholesterol esterification rate.

Angiogenesis
Normal coronary arteries have no vessels within the inner media or intima. Angiogenesis occurs as part of the normal wound-healing process and also in atherosclerosis. Many growth factors regulate angiogenesis, stimulating migration, proliferation, proteolytic activity, and organizational behavior of endothelial cells. These angiogenic factors include basic fibroblast growth factor, vascular endothelial growth factor, transforming growth factor-β, and IGF-I.

Endothelial cells from both capillaries and arteries possess specific receptors for IGF-I and IGFBPs (specifically IGFBP-2, -3, and -4). Locally synthesized IGF-I at sites of lesion formation stimulates vascular injury repair promoting endothelial cell migration. IGF-I has a chemotactic action on vascular endothelial cells and induces endothelial tube–forming activity in vitro. These endothelial conduits receive the influence of other factors in the process of maturation to become fully organized capillaries.

Figure 2. IGF effects in atherogenesis. IGF axis elements are synthesized by the different cells of the atherosclerotic plaque acting in an autocrine/paracrine manner. IGFs stimulate VSMC proliferation, migration, and extracellular matrix synthesis. In macrophages, IGFs promote excess LDL cholesterol uptake, release of proinflammatory cytokines, and chemotaxis. This inflammatory environment digests the fibrous cap that overlies the lipid-rich core, thus leaving the plaque prone to rupture. IGFs also stimulate endothelial cell migration and organization, forming vascular conduits that may become newly formed capillary networks under additional angiogenic stimuli. SMC indicates smooth muscle cells; ACE-I, angiotensin-converting enzyme inhibitors; HMGCoA reductase-I, 3-hydroxy-3-methyl-glutaryl-coenzyme-A reductase inhibitors; and TNF-α, tumor necrosis factor-α.
Inflammatory angiogenesis occurs in atherogenesis and involves both endothelial cells and macrophages. Animal studies of inflammation-linked angiogenesis produced after microembolization of a coronary artery showed alterations in gene expression of IGF-I and the binding proteins 3, 5, and 6 in macrophages.65

Restenosis
Coronary restenosis remains a major clinical problem after all percutaneous revascularization procedures. Morphology in the pig stent model66 and after human coronary stenting demonstrates early thrombus formation and inflammation followed by neointimal growth.67 Smooth muscle cell accumulation is key to neointimal formation after angioplasty. Several studies indicate that IGF-I is involved in local cellular events leading to restenosis after coronary angioplasty.3,68 IGF-I immunoreactivity is present in medial VSMCs and in the neointima after arterial injury in the rat.68 IGF-I paracrine effects in vascular repair are also evident by aortic IGF-I gene induction associated with receptor downregulation after balloon injury.69 RT-PCR analysis revealed increased mRNA levels of IGF-I and IGFBP-1 to -5 in human restenotic specimens.1999. IGF-I production and action in the media is stretched and damaged by balloon inflation, it is.

IGFs also serve beneficial effects at the vessel wall after injury. A number of studies show that VSMC elastogenesis is far higher than in normal coronary VSMCs.28 However, restenotic tissue obtained several months after the intervention showed no IGF-I mRNA expression.3 The predominant IGF-I production and action in arterial media suggests that IGF-I has a growth-promoting effect on VSMCs after balloon injury. In fact, transgenic mice with IGF-I overexpression show vascular smooth muscle hyperplasia.70 Furthermore, mice with paracrine overproduction of IGFBP-4 developed smooth muscle hypoplasia,71 supporting the concept that IGFBP-4 inhibits IGF-I action. IGFBP-4 proteolysis by highly specific IGFBP-4 proteases remains an important issue, as tissue bioactive IGF-I rises after vascular injury. We recently found that VSMC injury increases the expression and proteolytic activity of PAPP-A (IGFBP-4 protease), which in turn releases free IGF-I and appears to be intimately involved in the development of neointimal hyperplasia (A. Bayes-Genis et al, unpublished data, 1999).

Thus, it remains to be demonstrated that somatostatin analogues are an effective anti-restenosis strategy.

In conclusion, this review of experimental and clinical studies supports the importance of the IGF axis in atherosclerosis and restenosis. The IGF axis is complex. Many possible interactions of its components account for functional diversity, the extent of which is only now beginning to be understood. Alterations in the balance of the components of the IGF axis in the vessel wall influence the cell growth, survival, migration, and extracellular matrix synthesis that modulate atherosclerotic plaque progression and neointimal formation of restenosis. A better understanding of IGF axis dynamics could identify new targets to limit or prevent these vascular pathologies.

References
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