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REVIEW ARTICLE

The role of inflammation in vascular injury and repair

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Summary. Inflammation plays a critical role in the vascular response to injury. In particular, mechanical injury using techniques such as balloon angioplasty and stenting results in complex inflammatory reactions which influence proliferation of vessel wall constituents such as endothelial cells, smooth muscle cells, and extracellular matrix proteins. Inflammatory cells are recruited to the injured vessel wall initially as a reparative mechanism; however, these same imflammatory processes are also pivotal in the development of restenotic lesions. Leukocytes serve as the primary inflammatory cells but we now know that platelets produce a number of important inflammatory mediators. This review describes the mechanisms that regulate endothelial cell migration, smooth muscle cell activation, and extracellular matrix protein production, all of which are key components in the inflammatory response to vascular injury.

Keywords: atherosclerosis, balloon angioplasty, inflammation, restenosis.

Atherosclerosis is responsible for approximately 50% of all deaths in the developed world. The concept that atherosclerosis develops in response to vascular injury and involves inflammation and vessel remodeling is now well accepted [1]. Spontaneous atherosclerosis results from vascular injury induced by multiple insults including hypercholesterolemia, diabetes, smoking, and hypertension. Effective treatment strategies for stenotic atherosclerotic lesions include percutaneous interventions such as balloon angioplasty and stenting; however, these procedures are associated with a significant recurrence rate. Mechanical injury has been shown to provoke a distinct pathobiological response that is significantly different from spontaneous atherosclerosis [2]. The processes invoked after mechanical vascular injury lead to intimal hyperplasia, which involves platelet deposition, proliferation and migration of smooth muscle cells (SMC), and synthesis and deposition of extracellular matrix. Mechanical injury may also result in vascular remodeling with vessel constriction and a reduced vascular lumen as a result of scarring of the outer layer of the vessel. These responses are collectively referred to as restenosis. There are many factors involved in the vascular response to injury; however, inflammatory mediators appear to play a key role in the initiation and progression of both spontaneous atherosclerosis and vascular injury secondary to mechanical manipulation. These processes involve a complex network of interactions that begin as a beneficial reparative process but may ultimately result in detrimental vascular changes. Inflammatory mediators include cell adhesion molecules, cytokines, chemokines and growth factors that direct the recruitment of inflammatory cells including monocytes/macrophages, neutrophils, and T-lymphocytes. We now know that platelets are not only involved in the thrombotic component of vascular repair but are also important in the elaboration of inflammatory mediators. These mediators have multiple and diverse effects on the constituents of the vessel wall, including endothelial cells (EC), SMC, and extracellular matrix (ECM) proteins. This review will focus on inflammatory processes that are involved in the development of restenotic lesions after mechanical injury such as balloon angioplasty or stent placement.

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Background

A normal muscular artery consists of three distinct layers (Fig. 1). The intima is a thin layer that lines the lumen of the vessel and is composed of an endothelial monolayer and underlying extracellular connective tissue. In a normal artery the endothelium creates a non-thrombogenic surface that functions as a selectively permeable barrier, which controls transport of solutes into the arterial wall. The media consists primarily of SMCs and is separated from the intima by the internal elastic lamina. In a normal adult artery the SMCs replicate at a very slow rate and function principally to establish and maintain vascular tone. Non-activated SMC in the media express a set of proteins and adhesion molecules that are characteristic of the contractile phenotype [3]. The outermost layer, know as the adventitia, contains fibroblasts, collagen bundles, proteoglycans, and the vasa-vasorum, all of which are separated from the media by the external elastic lamina.

Various animal models of mechanical injury have been described and have contributed to our understanding of the role of inflammation in the development of intimal hyperplasia

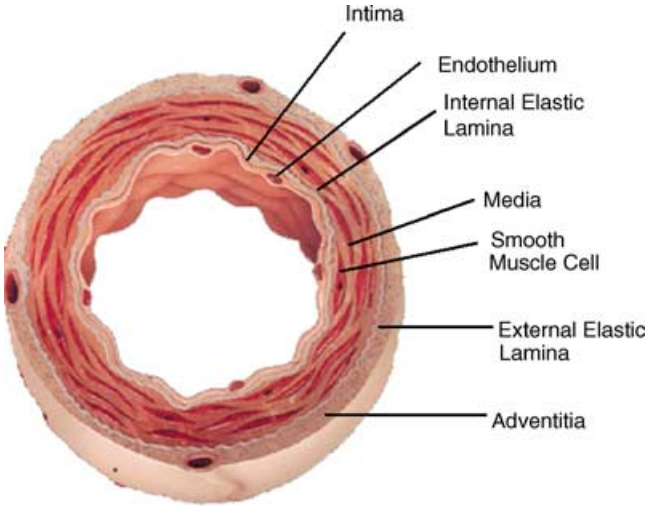

Fig. 1. Anatomy of the vessel wall.

(Fig. 2). Although the pathobiology of lesions formed in these models differs somewhat from lesions found in human atherosclerotic arteries after mechanical injury, these models have provided useful information regarding the composition of restenotic lesions. These lesions consist primarily of ECM proteins, SMCs, and varying numbers of inflammatory cells.

Endothelial injury and activation

Normal physiological roles of the endothelium that are key in the vascular response to injury include regulation of leukocyte adhesion, platelet activation and adhesion, and hemostasis/thrombosis. To maintain these functions the endothelium expresses and responds to multiple biologically active substances including cytokines, chemokines and cell adhesion molecules. Mechanical injury of a vessel results in endothelial damage with compromise of its normal physiological role and the propagation of a number of inflammatory pathways which lead to leukocyte adhesion, platelet activation, and SMC proliferation, migration and ECM deposition, all of which will be further discussed.

Leukocyte adhesion

Animal vascular injury models utilizing various forms of mechanical intervention including wire withdrawal [4], air desiccation [5], balloon denudation [6], and intravascular stent deployment [7,8] have been used to incite endothelial damage. Vascular responses to these forms of mechanical injury may vary based on factors such as animal species and diet, but collectively these models have contributed significantly to our understanding of the pathobiological processes leading to intimal proliferation and subsequent restenosis.

Mechanical injury damages or denudes the intact endothelial monolayer and results in upregulation of cell adhesion molecules (CAM), particularly on the regenerating endothelium. There are three major classes of leukocyte cell adhesion molecules: selectins, integrins, and the immunoglobulin superfamily of cell adhesion molecules. The selectins include P-selectin, E-selectin, and L-selectin. P-selectin is stored in the α granules of platelets and the Weibel–Palade bodies of endothelial cells and can be rapidly mobilized to the cell surface upon stimulation [9]. P-selectin glycoprotein-1 (PSGL-1) is the primary ligand for P-selectin and is expressed on most leukocytes; however, it serves as a functional P-selectin ligand predominantly on neutrophils and monocytes where it undergoes post-translational modification including fucosylation, sialylation, and tyrosine sulfation [10]. P-selectin has been shown to be intensely expressed on both endothelial cells and platelets as early as 24 h after balloon denudation injury of rat carotid arteries [11]. E-selectin is not constitutively expressed on endothelial cells but is transcribed and mobilized to the cell surface in response to such stimuli as tumor necrosis factor (TNF)-α, interleukin (IL)1-β, bacterial toxins, and oxidants. E-selectin can also bind PSGL-1, but is thought to have other unknown ligands. E-selectin is also upregulated on endothelial cells after mechanical injury [12]. L-selectin is constitutively expressed on myeloid cells and a large subset of lymphocytes [13]. L-selectin ligands include glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1), mucosal addressin cell adhesion molecule (MAdCAM-1), CD34, and PSGL-1. The L-selectin/PSGL-1 interaction is thought to be important in the process of 'secondary tethering' which involves neutrophils and lymphocytes rolling on leukocytes already attached to the vessel wall [14,15] .

The immunoglobulin superfamily of cell adhesion molecules includes intracellular adhesion molecule-1 (ICAM-1), ICAM-2, and vascular cell adhesion molecule-1 (VCAM-1), which are relevant to vascular injury. ICAM-1 is basally expressed on many cell types including endothelial cells and smooth muscle cells; however, VCAM-1 is not constitutively expressed [16–18]. In an apolipoprotein-E-deficient mouse model of vascular injury both ICAM-1 and VCAM-1 have been shown to be

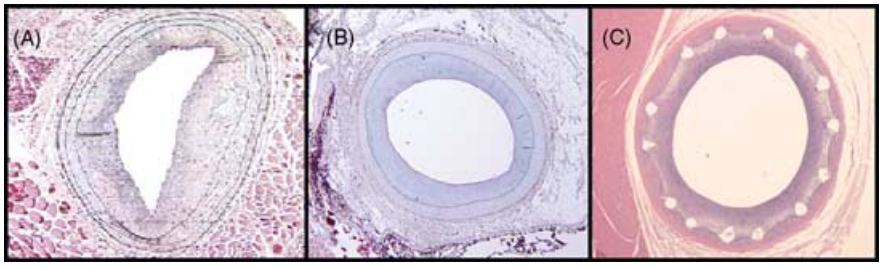

Fig. 2. Intimal hyperplasia in mouse carotid wire injury (A), rabbit femoral air desiccation injury (B), and pig coronary stent injury (C).

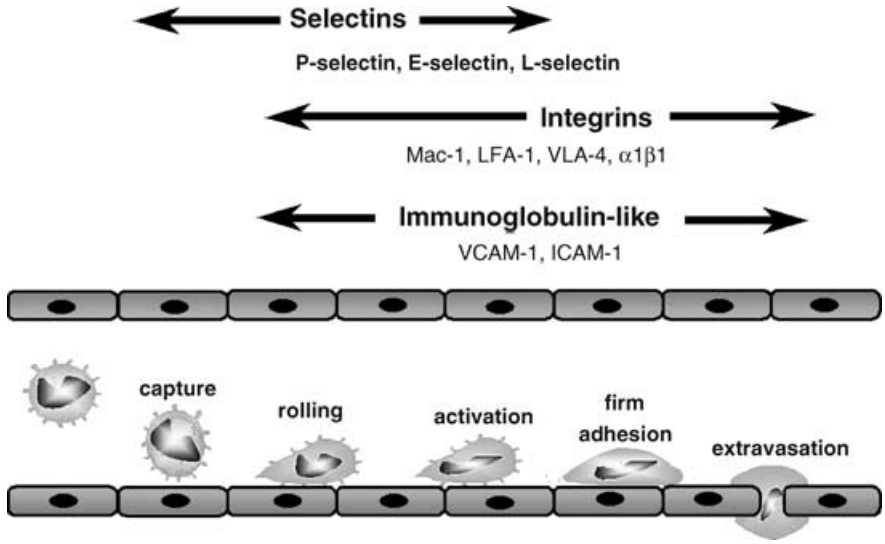

Fig. 3. Selectins mediate the initial capture and tethering of leukocytes to the endothelium. As the leukocytes roll along the activated endothelium various chemokines and cytokines are released from both cell types. Firm adhesion and leukocyte extravasation are mediated by members of the integrin and immunoglobulin families of cell adhesion molecules. Adapted from [133] with permission.

upregulated after wire withdrawal injury. Furthermore, the sites of ICAM-1 and VCAM-1 upregulation were associated with increased macrophage infiltration [19]. Similar increases in ICAM-1 and VCAM-1 expression were also noted in a rabbit balloon injury model [20]. These cell surface proteins are primarily regulated through transcriptional activation by nuclear factor (NF)-κB and/or AP-1 [21]. (NF)-κB and AP-1 are both transcription factors which are activated by proinflammatory cytokines [21,22]. (NF)-κB is also activated by balloon injury, shear stress, and oxidized LDL [23]. (NF)-κB is a central mediator regulating many other inflammatory pathways in response to vascular injury. In addition to the upregulation of cell adhesion molecules, activation of (NF)-κB promotes cytokine and nitric oxide (NO) production, stimulates smooth muscle cell (SMC) migration and proliferation, and modulates cell cycle pathways [23].

The integrin family of CAMs are heterodimeric proteins that are composed of α and β subunits. There are currently 15 α and eight β subunits known. VLA-4 ($\alpha_4\beta_1$), is the major β_1-integrin on leukocytes and is expressed on eosinophils, monocytes, lymphocytes, natural killer cells, and transmigrated but not blood neutrophils [24]. VLA-4 serves as the ligand for VCAM-1 on endothelial cells [25]. Monoclonal antibody blockade of VLA-4 attenuates leukocyte recruitment and neointimal formation after carotid air desiccation injury in the ApoE-deficient mouse [26]. LFA-1 (CD11a/CD18) and Mac1 (CD11b/CD18) are β_2-integrins that are expressed on leukocytes and bind ICAM-1 [27]. Mac1 is also capable of binding other vascular wall components including extracellular matrix proteins such as fibronectin, laminin, collagen and vitronectin, as well as coagulation proteins such as fibrinogen, factor X, and denatured proteins [28,29]. Both Mac1 and LFA-1 expression has been shown to be upregulated on leukocytes after balloon angioplasty [30] and play an integral role in firm adhesion and transmigration of the leukocyte through the activated endothelium. $\alpha_v\beta_3$ is another important integrin expressed on endothelial cells, SMC, platelets and leukocytes [31]. In multiple animal models, expression of $\alpha_v\beta_3$ is upregulated on EC and SMC after vascular injury [31]. Blockade of $\alpha_v\beta_3$ results in reduction of macrophage infiltration and decreased neointimal formation in several balloon angioplasty models of vascular injury [20,32,33], but the mechanisms are unknown.

These CAMs all have distinct roles in inflammatory cell recruitment to the damaged vessel wall. Leukocyte recruitment is a coordinated process involving rolling, adhesion, and transmigration of these cells (Fig. 3). The selectins are responsible for the initial leukocyte/endothelial interactions often termed 'tethering.' These weak interactions can result in leukocyte rolling along the endothelium of the vessel. Studies utilizing animal models have confirmed the role of endothelial selectins in leukocyte rolling [34,35] and the ultimate development of restenotic lesions after balloon angioplasty or stenting [11,36–38]. As the leukocytes 'roll' along the endothelium, chemokines and other chemoattractants, as well as adhesion molecule engagement, activate these cells. Leukocyte integrins redistribute on the cell surface and acquire an activated conformational change facilitating adherence to the upregulated immunoglobulin family of CAMs, thus promoting firm adhesion. Once adhered to the endothelium, the leukocytes begin the process of transmigration into the subendothelial space via endothelial cell–cell junctions or directly through endothelial cells [39]. The importance of these integrin-mediated events in leukocyte trafficking is illustrated by the reduction in neointimal hyperplasia in Mac1-deficient mice and in rabbits treated with a monoclonal antibody to Mac1 after balloon injury [5,40].

Both circulating and tissue inflammatory cells are involved in the vessel response to injury. Historically, monocytes/macrophages have been described as the primary white blood cells involved in the vascular response to injury and the development of neointimal hyperplasia; however, recent studies suggest that lymphocytes, particularly T-lymphocytes [41–43], and neutrophils [44] are also important in the response to injury. However, there are inconsistencies in the data regarding the role of lymphocytes. Several studies have provided evidence that lymphocytes upregulate the proinflammatory cytokines such as interferon (IFN)-γ, ultimately resulting in intimal hyperplasia

[43]. Other reports support the notion that lymphocytes provide a protective role after vascular injury by inhibiting smooth muscle proliferation via unclear mechanisms [44]. The predominant inflammatory mediator may be dependent on the mechanism and type of injury. Until recently, neutrophils had not been considered important mediators of neointimal growth in response to vascular injury. Studies have now shown that early after balloon injury, neutrophils appear to be the main cellular infiltrate; in contrast, following stent-induced injury, monocytes play a more prominent role [44].

Platelet activation and thrombus formation

Vascular injury resulting from mechanical instrumentation results in disruption or denudation of the intact endothelial monolayer and subsequent exposure of circulating blood cells to the subendothelial matrix. The exposed subendothelial matrix contains numerous platelet activating factors including thrombin, ADP, thromboxane, platelet activating factor (PAF), epinephrine, serotonin, and collagen. Activated platelets in turn express many proinflammatory molecules such as cell adhesion molecules, cytokines, chemokines, and other growth factors and membrane-bound proteins which influence leukocyte recruitment, smooth muscle activation, and arterial remodeling after vascular injury (Table 1).

Platelets are thought to be recruited to the injured vessel wall via platelet glycoprotein (GP) Ib interaction with von Willebrand factor in the subendothelial matrix. Recently, however, GP VI has been found to play a critical role in platelet–collagen interaction and recruitment to the injured vessel wall. Monoclonal antibody blockade of GP VI resulted in an 89% reduction in platelet tethering to the exposed subendothelium in a mouse carotid injury model [45]. Furthermore, ligation of GP VI can shift $\alpha_{IIb}\beta_3$ and $\alpha_2\beta_1$ integrins from a low to a high-affinity state, resulting in stable arrest of platelets on the injured vessel wall.

As previously discussed, leukocyte chemotaxis is a key component of the vessel response to injury. Platelet deposition on the subendothelial matrix after endothelial denudation has been shown to play an important role in leukocyte chemotaxis and transmigration into the intima of the injured vessel. Mac1 has recently been shown to mediate platelet–leukocyte interactions via a novel counterreceptor known as junctional adhesion molecule 3 (JAM-3) [46]. Platelet–leukocyte interactions are important in the development of both spontaneous atherosclerosis [47] and neointimal hyperplasia after mechanical injury [5] as these interactions are thought to result in direct transmigration of leukocytes across the platelet monolayer into the developing neointima.

Endothelial damage compromises the delicate balance between antithrombotic and prothrombotic factors and results in thrombus formation. Proinflammatory cytokines such as TNF, IL-1 and IL-6 stimulate one of the most potent prothrombotic agents, tissue factor (TF). In atherosclerotic vessels, TF is primarily expressed by lipid-laden macrophages; however, after arterial intervention such as balloon angioplasty TF is also upregulated on endothelial cells and SMC [48]. TF stimulates the extrinsic coagulation cascade resulting in thrombin production and ultimately formation of a fibrin-rich thrombus.

Thrombin, a serine protease, binds its receptor PAR-1 (protease-activated receptor-1) on endothelial cells and platelets and promotes leukocyte transmigration by upregulating endothelial P-selectin, E-selectin, VCAM-1 and ICAM-1 expression through activation of NF-κB [49]. Thrombin also stimulates local secretion of chemoattractants such as MCP-1 and numerous growth factors from the endothelium [49]. Finally, thrombin mediates the release of PAF, a potent proinflammatory agent and vasodilator, from endothelial cells. PAF, in a paracrine fashion, increases endothelial permeability and monocyte chemotaxis [49].

Platelet P-selectin plays an important role in platelet–leukocyte interactions after vascular injury as monocytes, neutrophils, and T-lymphocytes all express the P-selectin ligand, PSGL-1 [10,50]. Apolipoprotein-E-deficient mice transplanted with bone marrow from mice deficient in both ApoE-E and P-selectin developed significantly smaller lesions after wire denudation injury, illustrating the importance of platelet P-selectin in the development of neointimal hyperplasia [38]. In addition,

Table 1 Platelet-associated inflammatory mediators

	Family	Systematic name	Function
P-selectin	Selectin family of cell adhesion molecules	CD62-P	Platelet–leukocyte, platelet–endothelial interactions, mediates deposition of RANTES, facilitates constrictive remodeling
PF4	C-X-C chemokine	CXCL4	Leukocyte chemotaxis?
βTG	C-X-C chemokine	Precursor to CXCL7	Leukocyte chemotaxis?
IL-8	C-X-C chemokine	CXCL8	Leukocyte chemotaxis and arrest
MIP-1α	C-C chemokine	CCL3	Leukocyte chemotaxis, monocyte differentiation?
RANTES	C-C chemokine	CCL5	Leukocyte chemotaxis, arrest of rolling monocytes
MCP-1	C-C chemokine	CCL2	Leukocyte chemotaxis, migration, monocyte differentiation
IL-1	Cytokine		Stimulates chemokines? endothelial CAM expression
PDGF	Growth factor		Chemotaxis, SMC mitogen, vasoconstriction
CD40L	Transmembrane protein, TNF subfamily	CD154	?Endothelial CAM expression, re-endothelialization
GPIIbIIIa	β_3-integrin	CD41/CD61	Platelet aggregation, platelet–neutrophil interaction via fibrinogen

PF4, Platelet factor 4; βTG, β-thromboglobulin; IL-8, interleukin-8; MIP-1α, macrophage inflammatory protein-1α; RANTES, Regulated Upon Activation, Normal T-cell Expressed and presumably Secreted; MCP-1, monocyte chemoattractant protein-1; IL-1, interleukin-1; PDGF, platelet-derived growth factor.

P-selectin or PSGL-1 blockade using a monoclonal antibody strategy results in a significant reduction in neointimal growth [51–53]. Other animal studies using P-selectin gene deletion have also shown a clear role of P-selectin expressed on endothelial cells and platelets in the development of neointimal hyperplasia after vascular injury [35,36,38].

Leukocyte adhesion and rolling are also facilitated by various chemokines produced by activated platelets. Chemokines are thought to provide the signals that convert the low-affinity, selectin-mediated interaction into the higher affinity, integrin-mediated interaction that leads to extravasation of leukocytes [54]. Platelet factor 4(PF4) and β-thromboglobulin (βTG), RANTES (regulated upon activation, normal T-cell-expressed and presumably secreted), MIP1-α (macrophage inflammatory protein) and platelet-derived growth factor (PDGF) are all α-granule constituents that are chemotactic for leukocytes and/or SMC [55]. PF4 and βTG are both members of the C-X-C family of chemokines, while RANTES and MIP1-α are members of the C-C chemokine family. Monocyte chemoattractant protein-1 (MCP-1), another member of the C-C chemokine family, and RANTES are both upregulated in animal models of vascular injury and appear important mediators of macrophage infiltration and subsequent neointimal formation [56,57]. Deposition of RANTES at the site of vascular injury has been shown to be dependent on P-selectin expression and specific RANTES blockade results in decreased macrophage infiltration and neointimal formation after arterial injury [57]. Platelets can also mediate inflammatory reactions through the production of cytokines and chemokines such as IL-1 and IL-8 [58,59].

More recently, activated platelets have also been found to express CD40 ligand (CD40L, also known as CD154, gp39, TRAP), an integral membrane protein and member of the TNF gene superfamily which binds CD40 expressed on a number of cells including endothelial cells [60]. CD40L upregulates endothelial CAM expression as well as a number of proinflammatory chemokines including IL-8, MCP-1, and RANTES [60,61]. CD40L is rapidly cleaved from the platelet membrane to form soluble CD40L (sCD40L). Whether sCD40L maintains the proinflammatory properties of the membrane-bound CD40 is unclear, but levels of sCD40 have been found to be elevated in patients with acute coronary syndromes and after percutaneous coronary interventions [62]. Recently sCD40L has also been shown to inhibit endothelial cell migration by increasing production of endothelial reactive oxygen species, thus decreasing re-endothelialization and allowing for further SMC proliferation with resultant intimal hyperplasia after vascular injury [63].

Vascular smooth muscle cell response

In response to vascular injury, SMCs exhibit many changes that ultimately result in the pivotal processes of proliferation, migration and apoptosis. The SMC is thought to undergo a phenotypic change from a contractile SMC exhibiting SMC-specific contractile and cytoskeletal proteins including SMC α-actin [3], vinculin, and desmin [64–69] to an activated secretory state. This activated state is characterized by changes in affinity of SMC integrins for their ligands [70], expression of adhesion molecules such as ICAM-1 and VCAM-1 [71,72], production of numerous cytokines and growth factors including IL-1, MCP-1, bFGF, transforming growth factor (TGF)-α, TNF-α, VEGF [73,74], programmed cell death [75–77], and conversion to a dedifferentiated state [78]. This phenotypic switch results in the synthesis of more cytokines and growth factors that act as paracrine mediators to activate other SMCs, facilitate leukocyte chemotaxis and infiltration into the vessel wall [79–82], upregulate adhesion molecule expression on endothelial cells, and stimulate production of ECM components such as collagen, elastin, and proteoglycans [83–86]. After vascular injury, SMCs exhibit upregulated expression of VCAM-1 and MCP-1 via a NF-κB-regulated mechanism [71]. Activated SMCs are characterized by changes in morphology, with decreased cytoskeletal myofilaments, reduced levels of contractile proteins such as α-actin, and increased synthetic organelles such as Golgi apparatus and rough endoplasmic reticulum [65–68]. Activated SMCs also proliferate and are thought to migrate to the intima, where the inflammatory milieu promotes the maintenance of the activated state with continued production of cytokines [87,88], growth factors and ECM, ultimately contributing to the developing neointima. The size of the neointima is determined in large part by the SMC and ECM content [89]. Inhibition of the SMC ability to synthesize ECM proteins such as elastin has been shown to decrease neointimal size [90]. There is also evidence that these migrating neointimal SMCs behave differently from resident medial SMCs. They may be more sensitive to noxious stimuli, and more likely to undergo apoptosis [91]. SMC activation and phenotypic switch is not a terminal, irreversible change, as SMCs in the neointima have been shown to increase their α-actin content in chronic experiments [92,93], implying that their phenotype may switch back to the contractile phenotype.

Proliferation

After vascular injury, SMCs proliferate under the influence of various mediators including oxidative stress [94,95], signaling from damaged endothelium [96], growth factors released from leukocytes and platelets including bFGF and PDGF, interactions with infiltrating leukocytes [97], interactions between the endothelial cells and the SMCs [96], and interactions of SMCs with the ECM [98,99]. Two proinflammatory cytokines, TNF-α and IL-1β, are upregulated after balloon injury and have been demonstrated to induce SMC proliferation [100]. These cytokines are thought to act by upregulation of PDGF from endothelial cells. However, other mechanisms are likely to be involved [100]. The cellular mechanisms that regulate SMC proliferation are not fully understood but involve complex regulation of entry into the cell cycle at multiple levels [101–104]. The cell cycle is a set of tightly regulated steps that control DNA synthesis and mitosis. The resting SMC is maintained in a non-proliferative gap phase (G_0); however, after injury the SMC enters the G_1 phase where the necessary elements are assembled that then allow entry into the synthetic phase (S phase) of DNA

replication. SMCs subsequently enter a second gap phase (G_2) during which proteins that are used in mitosis are synthesized. Cell cycle regulators at various points along this cascade ensure an orderly progression. There are numerous molecular regulators of the cell cycle including the cyclin class of proteins (cyclins A–H), the cyclin-dependent kinases (CDKs), and the cyclin-dependent kinase inhibitors (CDKIs) such as p21, p27 and p57. The cyclins and CDKs are positive regulators of the cell cycle, whereas the CDKIs are important negative regulators [105]. Recent successful therapeutic strategies to prevent restenosis after coronary stenting using rapamcyin and paclitaxel are directed at cell cycle regulation [105,106]. Growth factors also play a key role in regulation of SMC proliferation. For example, in animal studies antibodies that block bFGF or PDGF have been shown to reduce SMC proliferation after balloon injury [78,107,108].

Smooth muscle cell migration

SMC adhesion receptors such as integrins, syndecans, and cadherins function to anchor the SMC cytoskeleton to the ECM, thereby allowing functional contraction of the vessel wall [70]. After vascular injury, dedifferentiated SMCs are motile and express an altered set of adhesion receptors. The relationship with the ECM that binds the SMC cytoskeleton to the extracellular vessel scaffolding is altered, and is thought to allow SMC migration to the intima. The SMC integrins undergo conformational changes that alter their affinity for their receptors. In uninjured arteries, β_1-integrins are found in an active state and play a role in maintaining the contractile phenotype [109,110]. After injury, activated β_1-integrin expression decreases, allowing increased proliferative and migratory activity of the SMC [111,112]. In addition, blocking the β_3-integrin with abciximab, a monoclonal antibody to β_3, in the rat carotid balloon injury model, decreased matrix metalloproteinase (MMP) production, SMC migration, and neointimal size [113]. Although not constitutively expressed, ICAM-1 and VCAM-1 have been detected in SMCs after injury [72,114]. VCAM-1 is coexpressed with MCP-1 [114] and induces macrophage infiltration into the vessel wall. ICAM-1 expression affects not only adhesion of monocytes, but also expression of tissue factor and procoagulant activity of monocytes [115]. VCAM-1 and ICAM-1 expression leads to augmented adhesion of α_4-integrin-positive lymphocytes and β_2-integrin-expressing monocytes [116,117]. As discussed above, these adhesion molecules are thought to play an important role in leukocyte trafficking in the vessel wall.

Apoptosis

Apoptosis, or programmed cell death, is an integral part of the cellular response to vascular injury. Endothelial cells, leukocytes, and SMC may undergo apoptosis but the net effects are still controversial. Immediately after balloon injury, extensive SMC death occurs, and the remaining SMCs then proliferate and migrate [95]. Medial SMC apoptosis occurred as early as 30 min after balloon injury in both rat carotid and rabbit iliac arteries [118]. In a rat carotid balloon injury model as many as 14% of resident SMCs underwent apoptosis 24 h after balloon injury [77]. The remaining SMCs respond to modulatory cytokines such as TNF-α and IL-1β, as previously mentioned, from the damaged endothelium, extracellular matrix, and paracrine signals from neighboring SMCs to proliferate and migrate to the neointima [77]. Whether the overall effect of SMC apoptosis is beneficial or detrimental is not clear. After injury, apoptosis may be beneficial in that it counteracts SMC proliferation that, if left unchecked, could lead to an exaggerated neointima formation and significant luminal narrowing. However, in the later stages of the vascular response, remnants of apoptotic SMCs may cause oxidative damage to surrounding cells and result in the influx of tissue macrophages in an attempt to clear the damaged SMCs and engulf the remaining cellular debris. This macrophage infiltration in turn leads to increased metalloproteinase activity and decreased collagen content, which may lead to plaque instability. The regulation of SMC apoptosis is not completely understood, although it appears to be a tightly regulated process [119]. There is evidence that macrophages may induce SMC apoptosis via direct interaction between CD95 (Fas) on SMCs and Fas-ligand (Fas-L) expressed on activated macrophages. Furthermore this Fas/Fas-L-mediated SMC apoptosis may be nitric oxide-dependent [120]. There is also evidence that SMC apoptosis may be mediated by inflammatory cytokines such as TNF-α [121], but a definitive role has not been shown.

Extracellular matrix production and remodeling

The extracellular matrix serves as the scaffold of the vessel wall that provides an architectural framework onto which other vascular components (SMCs, endothelial cells, etc.) are organized. The ECM is predominantly produced by SMC and fibroblasts, and is composed of collagen, elastin, and proteoglycans. It makes up a majority of the neointimal volume of restenotic lesions [122]. ECM production is primarily regulated by TGF-α1 and PDGF [83–86]. TGF-α1 increases the synthesis of fibronectin, fibrillar collagens, elastin, thrombospondin, and proteoglycans [83–86], all of which are increased after vascular injury [122]. SMCs in the neointima produce more ECM than those in the media [123–125]. Macrophages that have traversed the vessel wall influence ECM composition and SMC migration via degradative proteases known as matrix metalloproteinases (MMPs) [126]. Activated macrophages also secrete proinflammatory cytokines such as IL-1 and TNF-α that regulate MMP gene expression in vascular cells [126]. Changes in the composition of ECM can alter SMC proliferation and migration, as well as influence the leukocytes that have migrated into the vessel wall [99,127]. MMPs are thought to be important for resorption of ECM to facilitate SMC migration from the media across the internal elastic lamina into the intima. Indirect evidence such as decreased SMC migration and subsequent neointimal proliferation after administration of a nonselective MMP inhibitor in a rat balloon injury model supports the role of

MMPs in the development of neointimal hyperplasia [126]. Recently, targeted disruption of the MMP-9 (gelatinase) gene in a mouse model of arterial injury resulted in decreased SMC migration and intimal hyperplasia [128]. MMP-9-deficient SMC also had decreased capacity to contract collagen [128], suggesting decreased constrictive remodeling effects. Tenascin-C is an extracellular matrix glycoprotein thought to be involved in cell release and migration. In a rat and porcine model of balloon arterial injury, tenascin-C expression is increased in the adventia early after injury, but expression is then noted in the neointima over the next 7–14 days, suggesting that this glycoprotein is important in SMC migration [129].

Vascular remodeling, particularly constrictive or negative remodeling, after mechanical injury has been linked to increased expression of inflammatory mediators. Monoclonal antibody blockade of P-selectin results in less adventitial inflammation and fibrosis with an overall decrease in vessel constriction after balloon injury in the rat carotid artery [11]. ECM glyproteins such as osteopontin and thrombospondin are now known to have mediating effects on SMCs. Thrombospondin is synthesized and secreted by activated platelets, macrophages, SMCs, and fibroblasts, and thrombospondin accumulation is seen in restenotic arteries [130]. In rat carotid arteries, antithrombospondin-1 blocking antibody suppressed neointima formation after balloon injury [131]. Osteopontin levels are also increased after arterial injury, and may mediate SMC migration [132].

Once thought to be a passive bystander, the ECM is now known to play an active and significant role in both the intimal hyperplasia and vascular remodeling that occur in response to injury. The ECM constituents are influenced by numerous interactions of inflammatory mediators, many of which have yet to be elucidated.

Conclusion

Over the past decade, percutaneous coronary and peripheral interventions have become a common therapy for stenotic atherosclerotic arteries. The immediate outcomes of these procedures are successful, but by virtue of their mechanisms of luminal enlargement they incite further vascular injury. The pathobiological response of these arteries with underlying atherosclerotic disease to mechanical injury is complex and may result in restenosis. It is now clear that inflammatory processes play a pivotal role in the vascular response to mechanical injury. As discussed, inflammatory mediators are involved in the key components of the development of restenotic lesions, including leukocyte and platelet adhesion to the damaged endothelium or subendothelium, migration and proliferation of SMCs, ECM synthesis, and constrictive vascular remodeling. Many of the inflammatory mediators, such as P-selectin, are now known to have multiple roles in the response to vascular injury. In fact, many have overlapping functions, which often make it difficult to delineate a specific mechanism for a particular inflammatory mediator. Importantly, we now also recognize that activated platelets are not only important for thrombus formation but also provide a host of proinflammatory mediators that influence adhesion molecule expression, cytokine and chemokine release from endothelial cells and leukocytes, and ECM synthesis and degradation. Numerous animal studies have demonstrated the role of inflammatory processes in the vascular response to mechanical injury. As these pathways are further defined, improved treatment strategies for atherosclerotic vascular disease will probably include therapies to modulate postprocedure inflammation and subsequent intimal proliferation.

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