Inflammation As a Mechanism and Therapeutic Target for In-stent Restenosis

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Restenosis following coronary stenting has long been attributed to neointimal proliferation, thrombosis, and negative remodeling. More recently, the important role of inflammation in vascular healing has also been increasingly well understood. From animal models and from clinical experience, we know that endothelial injury, platelet and leukocyte interactions, and subcellular chemoattractant and inflammatory mediators are pivotal in the development of the inflammatory response following stent implantation. By examining the specific mechanisms governing the inflammatory response to percutaneous coronary intervention, we may gain insight into potential therapeutic targets and strategies to prevent restenosis in clinical practice.

Introduction

Since the first angioplasty was performed nearly three decades ago, tremendous effort has been invested in understanding the biologic response to vascular injury [1]. As endovascular technique has evolved to embrace a nearly universal use of coronary stents, our paradigm for the understanding of the vascular biology has changed as well. We now appreciate that the original models that identified neointimal proliferation, thrombosis, and arterial constriction (or negative remodeling) as the key mediators of restenosis post-angioplasty omit the important role of inflammation in vascular wound healing [2,3]. With the chronic injury and foreign body response engendered by the endovascular implantation of a stainless-steel stent, inflammation plays an even more important role. By examining the mechanisms by which inflammation influences vascular wound-healing, we may improve our understanding of the pitfalls and successes of our current interventional techniques and develop insight into future strategies that could offer significant clinical advantages.

Atherogenesis

The greatest understanding of inflammation in vascular biology has been established through the study of atherogenesis [4,5]. Although the study of atherogenesis may serve as a scaffold upon which we may build our understanding of the mechanisms of restenosis, these two modes of vascular injury and wound-healing are clearly distinct. In our discussions of the respective pathophysiology, one major point of distinction is that atheroma develops in the context of dysfunctional endothelial cells, whereas restenosis most often occurs following endothelial denudation at the time of balloon-induced or stent-induced coronary injury. As such, the repertoire of cellular and subcellular components in these processes may diverge. Nevertheless, by applying the mechanistic insights derived from one model to the other, we may develop a more complete understanding of the vascular response to injury.

The first phases of atherogenesis are characterized by endothelial dysfunction, which is the result of chronic inflammatory stimuli. Many of the traditional cardiac risk factors promote metabolic, environmental, or physical stress, which disrupts the integrity of the natural barrier-like properties of the endothelium [4]. As a result, circulating inflammatory cells, which typically function in the capacity of immunologic defense, are recruited to the endothelial surface by way of a complex cascade of signaling events [6]. Initial adhesion and rolling of leukocytes on the endoluminal surface is mediated by the production of glycoproteins, such as P-selectin and vascular cellular adhesion molecule-1 (VCAM-1), which are expressed by dysfunctional endothelial cells [7,8]. Leukocyte activation, firm adhesion, and transplatelet and transendothelial migration are governed by interactions with chemoattractant factors such as monocyte chemoattractant protein-1 (MCP-1). As a result, leukocytes accumulate in the arterial wall. The production of oxidized low-density lipoprotein, angiotensin II, interleukin-1 (IL-1), and tumor necrosis factor-α (TNF-α) induce the production of VCAM-1 and MCP-1 by endothelial cells, which leads to further leukocyte recruitment, compounding the degree of inflammation and vascular injury [9].

Following the initial inflammatory burst, the developing atheromatous lesion typically enters a long period of

apparent clinical inactivity. During this time, the lesion grows and develops due to the apoptosis of recruited macrophages and T cells, creating the "necrotic core" [10]. Smooth muscle cell recruitment and proliferation leads to the development of a fibrous cap, which overlies the lesion and protects the contents from the potentially thrombotic circulating milieu.

Over time, however, lesion progression leads to the development of more pronounced inflammation and clinical nstability. Production of macrophage colony-stimulating factor, IL-1, and cytokines, as well as T-cell release of interferon-α and macrophage production of matrix metalloproteinases, may lead to the degradation of the fibrous cap by slowing the production of new collagen within the tissue layer and accelerating breakdown of the existing collagen [11,12]. In addition, some elements of the inflammatory process themselves may enhance local thrombogenicity (*eg*, the CD40 ligand may stimulate macrophage production of tissue factor, which itself catalyzes factor VIIa and leads to thrombin production and activation with consequent thrombosis) [13,14].

Percutaneous Coronary Intervention and Inflammation

Although early models of restenosis post-angioplasty established the importance of neointimal proliferation, thrombosis, and negative remodeling, only recently has the importance of inflammation been recognized. Following coronary intervention, there is disruption and denudation of the endothelial surface, which initiates a cascade of events, as originally proposed by Libby *et al*. [15] in 1992, that promote leukocyte recruitment, adhesion, and participation in the vascular healing response to injury (Fig. 1). Endothelial denudation promotes platelet adhesion to the injured surface of the arterial wall, and subsequently fibrin is deposited. The interaction between platelets and leukocytes is mediated by way of the platelet expression of P-selectin; leukocytes attach loosely and roll along the carpet of platelets lining the denuded abluminal surface. Leukocytes then adhere more firmly and migrate across the platelets by way of a Mac-1–mediated sequence of events, promoting progressive inflammation of the arterial wall [16].

The interactions between platelets and leukocytes trigger a cascade of events that promote inflammation: neutrophil activation is stimulated; expression of cellular adhesion molecules is upregulated; chemical signals are released that increase integrin activation; and chemokine synthesis is increased. From a more mechanistic standpoint, the early stages of inflammation are characterized by the production of intracellular adhesion molecule-1 (ICAM-1), VCAM-1, and TNF-α; in later stages, class II major histocompatibility complex (MHC) elevation may indicate a more chronic, immunologically mediated response to vascular injury [15]. In this regard, the interaction of platelets and leukocytes may be viewed as a sentinel event in the inflammatory response to percutaneous endovascular intervention. Therapies targeted to disrupt this interaction show promise in the prevention of restenosis and are discussed later.

Evidence from Animal Models Linking Inflammation with Restenosis

Following vascular injury, there is a cascade of events that promote inflammation and subsequent restenosis. The study in animal models of this sequence of events—and of therapeutic efforts to disrupt this sequence—has yielded significant insight into the pathophysiology of restenosis and into potential strategies for treatment. Although no single animal model completely replicates human physiology, each model may provide relative or analogous mechanistic insights that may shape our technique in clinical practice.

As described previously, the β2-integrin Mac-1 plays a critical role in the adhesion of leukocytes to platelets following endothelial denudation, as seen following percutaneous coronary intervention (PCI). This interaction is known to play a key role in the subsequent cascade of events that lead to restenosis, and thus may serve as a potential target for therapeutic intervention. In a murine model, mechanical dilation and endothelial denudation of the carotid artery lead to leukocyte adhesion and accumulation in the arterial wall, with consequent intimal proliferation and experimental restenosis. In the absence of Mac-1, however, as examined in a Mac-1 knockout mouse model, the same arterial injury results in significantly less leukocyte recruitment and neointimal thickening [17]. In a rabbit model, antibody blockade of Mac-1 function significantly reduced intimal thickening after stent implantation in the iliac artery. Collectively, this experimental evidence establishes the importance of leukocyte recruitment by way of a Mac-1-mediated interaction with platelets in the inflammatory cascade leading to restenosis [18]. Moreover, these experiments suggest a role for blockade of Mac-1 function in the prevention of restenosis.

In the inflammatory cascade following PCI, the leukocyte glycoprotein ligand P-selectin also plays an important role in leukocyte adhesion and restenosis. In pre-injured pig coronary arteries, bolus administration of an antibody directed against P-selectin reduces experimental restenosis after stenting by 35% when compared with control animals. In addition, platelet and neutrophil adhesion were also dramatically reduced following administration of the antibody to P-selectin. In this preclinical model, evidence is presented that identifies the role of P-selectin in adhesion of leukocytes following vascular injury and implicates this step as vital to the inflammatory cascade that results in restenosis. Blocking P-selectin activity may hold promise for clinical reduction of restenosis following PCI [19].

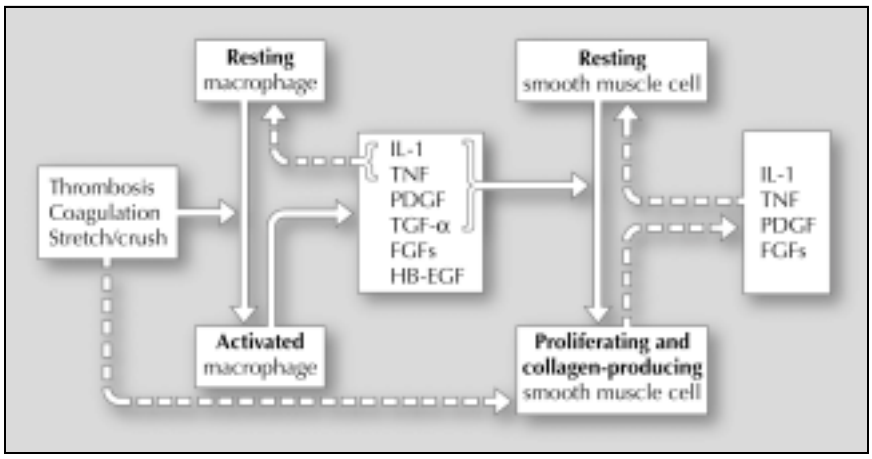

Figure 1. Diagram of the inflammatory cascade following vascular injury, which leads to the development of restenosis. (FGF—fibroblast growth factor; HB-EGF—heparin-binding epidermal growth factor; IL-1—interleukin-1; PDGF—platelet-derived growth factor; TGF-α—transforming growth factor-α; TNF—tumor necrosis factor.) (*Adapted from* Libby *et al*. [15].)

In animal models characterized by systemic inflammatory stress—exposure to atherogenic diet, induction of diabetes mellitus, and simulation of arterial wall shear stress—an overexpression of cellular adhesion molecules may be detected systemically [16]. The increased presence of adhesion molecules supports the notion that the recruitment of inflammatory cells is important in wound-healing. After balloon injury in the rabbit iliac artery, ICAM-1, VCAM-1, and class II MHC are elevated [20]. In the same model, after iliac stenting, inflammatory cells are seen at the site of endothelial injury immediately after intervention. The concentration of monocytes in the media after stenting is proportional to the subsequent neointimal thickening, corroborating the theory that the monocyte/macrophage lineage may play a causal role in restenosis. In addition, in vitro assays demonstrate that co-culture of neutrophils with smooth muscle cells—or merely the exposure to neutrophil medium—may increase smooth muscle cell proliferation, a major component of neointimal thickening in vivo [16,21,22].

Using anti-inflammatory medications to block the recruitment of monocytes early after balloon injury reduces neointimal thickening. As with any anti-restenotic therapy, though, the timing of administration must be coordinated to assure that the therapeutic agent is present and active at the appropriate site at the same time as its intended target. For example, following balloon injury of the rabbit iliac artery, brief (*ie*, hours) administration of heparin is sufficient to inhibit neointimal proliferation. In the same model, however, when a stent is implanted, brief administration of heparin does not inhibit neointimal proliferation; however, sustained (*ie*, days) administration of heparin does prevent proliferation. From this experience, we appreciate that the inflammatory and proliferative healing response to transient endovascular injury (*ie*, balloon angioplasty) may be combated with transient administration of therapy; however, the chronic endovascular injury engendered by stent implantation requires sustained therapy in order to inhibit the corresponding sustained inflammatory and proliferative wound-healing response [23,24].

As suggested by the different responses to balloon angioplasty and stenting in the rabbit iliac artery, the cellular physiology of healing after these two types of vascular injury are quite different. Following balloon injury alone, macrophage infiltration of the media is not dramatically increased. Following stenting, however, there is a substantial increase in macrophage presence in the media. In a primate model of vascular injury, the levels of MCP-1 (a monocyte chemokine) and IL-8 (a neutrophil chemokine) were examined. After angioplasty, both MCP-1 and IL-8 were overexpressed for a matter of hours after the intervention. Following stenting, however, IL-8 and (to a lesser extent) MCP-1 were sustained for up to 14 days after the intervention. Of interest, blockade of monocyte recruitment in this model—achieved through antibody-mediated blocking of the CCR2 receptor and, therefore MCP-1 production—did not reduce neointimal thickening after angioplasty alone, but did reduce neointimal thickening after stenting [25••]. This finding suggests that monocyte/macrophage lineage cells may play an important role after stenting, but not after balloon angioplasty alone. Inhibition of CD18 (a neutrophil chemokine) did reduce neointimal thickening after angioplasty alone, however, suggesting that neutrophil lineage cells may play an important role in the inflammatory and proliferative response following balloon injury.

Clinical Evidence Linking Inflammation with Restenosis

In many instances, the physiologic mechanisms elucidated in animal models have helped us to identify equivalent processes in humans, where inflammation has been found to play an important role in restenosis. Several clinical trials have shown that restenosis rates are higher in patients who have elevated systemic markers of inflammation. High levels of CRP predict greater rates of restenosis [26,27]. Following PCI, systemic elevation of ICAM, VCAM, L-selectin, and P-selectin predicts a greater incidence of restenosis [28–31]. High levels of IL-1 prior to percutaneous transluminal coronary angiography (PTCA) predict late lumen loss [32]. Elevation of

MCP-1 levels following PTCA is proportionally related to the rate of restenosis [33]. These findings support the hypothesis that inflammatory mechanisms contribute to restenosis in clinical practice.

In an angioplasty study where coronary blood was collected just proximal to and just distal to the site of intervention, L-selectin and CD11b (a Mac-1 subunit) levels were higher downstream from the lesion than they were upstream from the lesion [34]. This finding suggests that these two leukocyte adhesion factors may be produced at the site of injury and that they likely precede the recruitment of leukocytes post-angioplasty. In another study, the amount of CD11b was measured on circulating neutrophils and monocytes; higher levels of CD11b were proportional to more frequent adverse events following angioplasty, suggesting the potential impact of inflammation on clinical outcomes [16,35]. In a clinical study of over 1200 patients, individuals with a polymorphism of the gene coding for CD18 (a subunit of Mac-1) were found to have lower rates of restenosis following coronary stenting than those without the genetic polymorphism. The findings of this large clinical trial suggests that, as demonstrated in animal models, Mac-1 plays a critical role in the development of restenosis after coronary stenting, and that inflammation is a major determinant of both experimental and clinical restenosis [36•].

In a study by Moreno *et al.* [37], where coronary tissue was extracted at the time of PCI using directional coronary atherectomy, analysis of the tissue revealed abundant macrophages at the site of the lesion. Moreover, measuring the concentration of macrophages present in the tissue revealed that greater macrophage presence at the time of intervention was proportional to a higher subsequent risk of restenosis [37].

Clinical evidence suggests that the actual technique of stent deployment and expansion may contribute to the degree of inflammation and, perhaps, propensity to restenosis after intervention. Farb *et al.* [38] examined pathology specimens, including 55 stents implanted in 35 human coronary arteries. They found that increasing depth of stent-strut penetration, with increasing medial injury and increasing penetration of the lipid core with stent struts, resulted in a greater degree of inflammation. In addition, the neointimal area increased with a greater ratio of stent area to reference lumen area (*ie*, stent oversizing, with excessive post-dilatation, led to increased in-stent restenosis in the specimens examined). This study provides a link between the degree of mechanical injury at the time of stenting and subsequent inflammation and restenosis [38].

Therapeutic Targets and Future Directions

As reviewed in detail previously in this article, the role of inflammation in the development of restenosis after vascular injury encompasses a complex cascade of events. With numerous cellular and subcellular mediators, there are many opportunities for therapeutic intervention using anti-inflammatory treatments. For years, however, all attempts to translate strategies that had been successful in animal models into clinical practice had met with frustration. In some cases, the failure was due to the use of the wrong drug, because some targets identified in animal models do not play as critical a role in human physiology. In other cases, treatments failed because the dosing or the pharmacokinetic and pharmacodynamic determinants did not permit appropriate drug delivery to the intended site of action at the time when the therapeutic target is present and active. Also, other therapies did not succeed in humans because the preclinical success was achieved following balloon angioplasty alone, and in humans the overwhelming majority of PCI procedures involve implantation of a stent.

Recent studies, however, have identified a number of anti-inflammatory agents with therapeutic potential in the prevention of restenosis. Many drugs in our current antithrombotic armamentarium also possess anti-inflammatory characteristics. Aspirin, currently used for its antiplatelet effects and for prevention of thrombosis post-stenting, has well-known anti-inflamatory properties. Heparin possesses antiproliferative and anti-inflammatory attributes [21,39,40]. In addition to targeting platelets, the glycoprotein IIb/IIIa blockers such as abciximab may also interact with other integrin molecules such as Mac-1, which are important in the association of the inflammatory response and restenosis [41–43]. Some studies have demonstrated potential benefit with probucol and tranilast, although these therapies have not been embraced in wide-scale practice [44,45]. In patients with systemic evidence for inflammation, as measured by an elevated C-reactive protein (CRP) at baseline, the administration of oral steroid anti-inflammatory therapy post-stenting dramatically reduces the rate of restenosis, as reported by Versaci *et al.* [27].

One of the most important recent developments in coronary intervention is the widespread use of drug-eluting stents. The stent platforms currently approved by the US Food and Drug Administration use sirolimus or paclitaxel, both of which have protean effects on the blood vessel wall. Sirolimus was originally used as an immunosuppressive agent. Its profound impact on restenosis prevention is felt to reflect both its properties as an antiproliferative as well as an anti-inflammatory agent. In porcine coronary arteries, sirolimus has been found to reduce the arterial wall expression of MCP-1 and IL-6 [46]. Paclitaxel, a microtubule-stabilizing agent, interferes with the cytoskeletal interactions with integrin cellular adhesion molecules. In addition, paclitaxel may have direct impact on impairment of leukocyte function and consequent inflammation [47,48].

Case reports have shown a rare but profound paradoxic proinflammatory response from drug-eluting stents, however. In a case reported by Virmani *et al.* [49], a 58-year-old patient developed stent thrombosis 18 months

after PCI using two overlapping sirolimus-eluting stents. The event was fatal, and at autopsy the stented segment showed evidence of vessel enlargement with severe localized hypersensitivity comprised primarily of eosinophils and T cells [49]. Such cases of hypersensitivity are felt to represent a patient-specific reaction to the drug or the polymer in the stent platform, and underscore the important role of inflammation in vascular wound-healing and the attendant risks of restenosis or thrombosis.

Conclusions

Inflammation is now recognized as playing an important role in the vascular response to injury, particularly following PCI. Although our current treatment paradigm emphasizes "spot treatment" of obstructive lesions with coronary stents, perhaps the future may hold a strategy combining mechanical technologies with systemic anti-inflammatory therapy. With continued study of such combined therapy, both in clinical practice and in preclinical models, we may gain insight into the role of inflammation on restenosis and expose potential targets for therapeutic intervention.

Acknowledgments

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References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Grunzig AR, Senning A, Siegenthaler WE: **Nonoperative dilatation of coronary-artery stenosis: percutaneous transluminal coronary angioplasty.** *N Engl J Med* 1979, **301:**61–68.
2. Mintz GS, Popma JJ, Pichard AD, *et al.*: **Arterial remodeling after coronary angioplasty: a serial intravascular ultrasound study.** *Circulation* 1996, **94:**35–43.
3. Post MJ, Borst C, Kuntz RE: **The relative importance of arterial remodeling compared with intimal hyperplasia in lumen renarrowing after balloon angioplasty. A study in the normal rabbit and the hypercholesterolemic Yucatan micropig.** *Circulation* 1994, **89:**2816–2821.
4. Ross R: **Atherosclerosis—an inflammatory disease.** *N Engl J Med* 1999, **340:**115–126.
5. Libby P, Ridker P, Maseri A: **Inflammation and atherosclerosis.** *Circulation* 2002, **105:**1135–1143.
6. Cybulsky MI, Gimbrone MA: **Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis.** *Science* 1991, **251:**788–791.
7. Dong ZM, Chapman SM, Brown AA, *et al.*: **The combined role of P- and E-selectins in atherosclerosis.** *J Clin Invest* 1998, **102:**145–152.
8. Cybulsky MI, Iiyama K, Li H, *et al.*: **A major role for VCAM-1, but not ICAM-1, in early atherosclerosis.** *J Clin Invest* 2001, **107:**1255–1262.
9. Libby P: **Current concepts of the pathogenesis of the acute coronary syndromes.** *Circulation* 2001, **104:**365–372.
10. Geng YJ, Libby P: **Evidence for apoptosis in advanced human atheroma. Colocalization with interleukin-1 beta-converting enzyme.** *Am J Pathol* 1995, **147:**251–266.
11. Amento E, Ehsani N, Palmer H, *et al.*: **Cytokines positively and negatively regulate intersitial collagen gene expression in human vascular smooth muscle cells.** *Arteriosclerosis* 1991, **11:**223.
12. Libby P, Simon DI: **Inflammation and thrombosis: the clot thickens.** *Circulation* 2001, **103:**1718–1720.
13. Mach F, Schonbeck U, Bonnefoy JY, *et al.*: **Activation of monocyte/macrophage functions related to acute atheroma complication by ligation of CD40: induction of collagenase, stromelysin, and tissue factor.** *Circulation* 1997, **96:**396–399.
14. Henn V, Slupsky JR, Grafe M, *et al.*: **CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells.** *Nature* 1998, **391:**591–594.
15. Libby P, Schwartz D, Brogi E, *et al.*: **A cascade model for restenosis. A special case of atherosclerosis progression.** *Circulation* 1992, **86:**III47–III52.
16. Welt FG, Rogers C: **Inflammation and restenosis in the stent era.** *Arterioscler Thromb Vasc Biol* 2002, **22:**1769–1776.
17. Simon DI, Chen Z, Seifert P, *et al.*: **Decreased neointimal formation in Mac-1(-/-) mice reveals a role for inflammation in vascular repair after angioplasty.** *J Clin Invest* 2000, **105:**293–300.
18. Rogers C, Edelman ER, Simon DI: **A mAb to the beta2-leukocyte integrin Mac-1 (CD11b/CD18) reduces intimal thickening after angioplasty or stent implantation in rabbits.** *Proc Natl Acad Sci U S A* 1998, **95:**10134–10139.
19. Tanguay JF, Geoffroy P, Sirois MG, *et al.*: **Prevention of in-stent restenosis via reduction of thrombo-inflammatory reactions with recombinant P-selectin glycoprotein ligand-1.** *Thromb Haemost* 2004, **91:**1186–1193.
20. Tanaka H, Sukhova GK, Swanson SJ, *et al.*: **Sustained activation of vascular cells and leukocytes in the rabbit aorta after balloon injury.** *Circulation* 1993, **88:**1788–1803.
21. Rogers C, Welt FG, Karnovsky MJ, Edelman ER: **Monocyte recruitment and neointimal hyperplasia in rabbits. Coupled inhibitory effects of heparin.** *Arterioscler Thromb Vasc Biol* 1996, **16:**1312–1318.
22. Welt FG, Edelman ER, Simon DI, Rogers C: **Neutrophil, not macrophage, infiltration precedes neointimal thickening in balloon-injured arteries.** *Arterioscler Thromb Vasc Biol* 2000, **20:**2553–2558.
23. Rogers C, Edelman ER: **Controlled release of heparin reduces neointimal hyperplasia in stented rabbit arteries: ramifications for local therapy.** *J Interv Cardiol* 1992, **5:**195–202.
24. Edelman ER, Karnovsky MJ: **Contrasting effects of the intermittent and continuous administration of heparin in experimental restenosis.** *Circulation* 1994, **89:**770–776.
25. •• Horvath C, Welt FG, Nedelman M, *et al.*: **Targeting CCR2 or CD18 inhibits experimental in-stent restenosis in primates: inhibitory potential depends on type of injury and leukocyte targeted.** *Circ Res* 2002, **90:**488–494.
In a primate model, blockade of CCR2 (MCP-1) function inhibits leukocyte recruitment after stenting, with reduction of neointimal thickening. The same therapy does not affect experimental restenosis after angioplasty alone. The study indicates that the inflammatory response depends on the type of vascular injury, and that potential treatment modalities must be tailored to match the type of inflammatory response.
26. Gaspardone A, Crea F, Versaci F, *et al.*: **Predictive value of C-reactive protein after successful coronary-artery stenting in patients with stable angina.** *Am J Cardiol* 1998, **82:**515–518.
27. Versaci F, Gaspardone A, Tomai F, *et al.*: **Predictive value of C-reactive protein in patients with unstable angina pectoris undergoing coronary artery stent implantation.** *Am J Cardiol* 2000, **85:**92–95, A8.
28. Kamijikkoku S, Murohara T, Tayama S, *et al.*: **Acute myocardial infarction and increased soluble intercellular adhesion molecule-1: a marker of vascular inflammation and a risk of early restenosis?** *Am Heart J* 1998, **6:**231–236.

29. Inoue T, Hoshi K, Yaguchi I, *et al.*: **Serum levels of circulating adhesion molecules after coronary angioplasty.** *Cardiology* 1999, **91:**236–242.
30. Ishiwata S, Tukada T, Nakanishi S, *et al.*: **Postangioplasty restenosis: platelet activation and the coagulation-fibrinolysis system as possible factors in the pathogenesis of restenosis.** *Am Heart J* 1997, **133:**387–392.
31. Tsakiris DA, Tschopl M, Jager K, *et al.*: **Circulating cell adhesion molecules and endothelial markers before and after transluminal angioplasty in peripheral arterial occlusive disease.** *Atherosclerosis* 1999, **142:**193–200.
32. Pietersma A, Koffard M, de Wit LE, *et al.*: **Late lumen loss after coronary angioplasty is associated with the activation status of circulating phagocytes before treatment.** *Circulation* 1995, **91:**1320–1325.
33. Cipollone F, Marini M, Fazia M, *et al.*: **Elevated circulating levels of monocyte chemoattractant protein-1 in patients with restenosis after coronary angioplasty.** *Arterioscler Thromb Vasc Biol* 2001, **21:**327–334.
34. Neumann FJ, Ott I, Gawaz M, *et al.*: **Neutrophil and platelet activation at balloon-injured coronary artery plaque in patients undergoing angioplasty.** *J Am Coll Cardiol* 1996, **27:**819–824.
35. Mickelson JK, Lakkis NM, Villarreal-Levy G, *et al.*: **Leukocyte activation with platelet adhesion after coronary angioplasty: a mechanism for recurrent disease?** *J Am Coll Cardiol* 1996, **28:**345–353.
36.• Koch W, Bottiger C, Mehilli J, *et al.*: **Association of a CD18 gene polymorphism with reduced risk of restenosis after coronary stenting.** *Am J Cardiol* 2001, **88:**1120–1124.
Clinical trial in which patients with polymorphism in gene for CD18 (Mac-1 subunit) were found to have lower rate of restenosis after coronary stenting. This study corroborates findings of animal research implicating Mac-1 and, therefore leukocyte recruitment in the development of restenosis.
37. Moreno PR, Bernardi VH, Lopez-Cuellar J, *et al.*: **Macrophage infiltration predicts restenosis after coronary intervention in patients with unstable angina.** *Circulation* 1996, **94:**3098–3102.
38. Farb A, Sangiorgi G, Carter AJ, *et al.*: **Pathology of acute and chronic coronary stenting in humans.** *Circulation* 1999, **99:**44–52.
39. Sy MS, Schneeberger E, McCluskey R, *et al.*: **Inhibition of delayed-type hypersensitivity by heparin depleted of anticoagulant activity.** *Cell Immunol* 1983, **82:**23–32.
40. Clowes AW, Clowes MM: **Inhibition of smooth muscle cell proliferation by heparin molecules.** *Transplant Proc* 1989, **21:**3700–3701.
41. Simon DI, Xu H, Ortlepp S, *et al.*: **7E3 monoclonal antibody directed against the platelet glycoprotein IIb/IIIa cross-reacts with the leukocyte integrin Mac-1 and blocks adhesion to fibrinogen and ICAM-1.** *Arterioscler Thromb Vasc Biol* 1997, **17:**528–535.
42. Plescia J, Conte MS, VanMeter G, *et al.*: **Molecular identification of the cross-reacting epitope on alphaM beta2 integrin I domain recognized by anti-alphaIIb beta3 monoclonal antibody 7E3 and its involvement in leukocyte adherence.** *J Biol Chem* 1998, **273:**20372–20377.
43. Altieri DC, Edgington TS: **A monoclonal antibody reacting with distinct adhesion molecules defines a transition in the functional state of the receptor CD11b/CD18 (Mac-1).** *J Immunol* 1988, **141:**2656–2660.
44. Cote G, Tardif JC, Lesperance J, *et al.*: **Effects of probucol on vascular remodeling after coronary angioplasty. Multivitamins and protocol study group.** *Circulation* 1999, **99:**30–35.
45. Holmes DR Jr, Savage MP, LaBlanche JM, *et al.*: **Results of Prevention of REStenosis with Tranilast and its Outcomes (PRESTO) trial.** *Circulation* 2000, **106:**1243–1250.
46. Suzuki T, Kopia G, Hayashi S, *et al.*: **Stent-based delivery of sirolimus reduces neointimal formation in a porcine coronary model.** *Circulation* 2001, **104:**1188–1193.
47. Drachman DE, Edelman ER, Seifert P, *et al.*: **Neointimal thickening after stent delivery of paclitaxel: change in composition and arrest of growth over six months.** *J Am Coll Cardiol* 2000, **36:**2325–2332.
48. Straubinger R: **Biopharmaceuticals of paclitaxel (Taxol): formulation, activity, and pharmacokinetics.** In *Taxol: Science and Applications.* Edited by Suffness M. Boca Raton, FL: CRC Press; 1995:237.
49. Virmani R, Guagliumi G, Farb A, *et al.*: **Localized hypersensitivity and late coronary thrombosis secondary to a sirolimus-eluting stent: should we be cautious?** *Circulation* 2004, **109:**701–705.