Modulation of the Inflammatory Response in Cardiovascular Disease

D. Neil Granger, Thorsten Vowinkel, Thomas Petnehazy

Abstract—There is a growing body of evidence that inflammation might play an important role in the initiation and progression of cardiovascular diseases (CVDs). The designation of CVD as a chronic inflammatory process is further supported by evidence that the risk factors for CVD cause endothelial cells throughout the vascular tree to assume an inflammatory phenotype. These activated endothelial cells characteristically exhibit oxidative stress and increased adhesiveness for circulating leukocytes. Although initial efforts to define the mechanisms underlying the inflammatory phenotype in diseased endothelial cells have focused on the linkage between oxidative stress and adhesion molecule activation/expression, recent work has implicated a variety of additional factors that can modulate the magnitude and/or nature of the inflammatory responses in CVD. Platelets, angiotensin II, and the CD40/CD40 ligand signaling system are gaining recognition as contributors to the pathogenesis of CVD. These factors appear to converge with known pathways that link oxidative stress with adhesion molecule expression and help to explain the apparent integration of coagulation with inflammation in CVD. These factors also hold the promise of offering multiple sites for therapeutic intervention in CVD. (Hypertension. 2004;43:924-931.)

Key Words: oxidative stress ■ integrins ■ angiotensin II ■ adhesion molecules

Inflammation is gaining widespread attention for its role in the initiation and progression of cardiovascular disease (CVD). Epidemiological studies have revealed strong associations between biochemical markers of systemic inflammation and both the presence of and future risk for symptomatic CVD. Animal experimentation as well as clinical studies has provided convincing evidence that the known risk factors for CVD (hypertension, diabetes, hypercholesterolemia, and smoking) can elicit both an inflammatory and a prothrombogenic phenotype in the vascular system. The phenotypic changes are more pronounced in endothelial cells and can include oxidative stress, increased expression of endothelial cell adhesion molecules (CAMs), activation of cell signaling pathways (eg, the CD40/CD40 ligand [CD40L] dyad), and the consequent adhesion and activation of leukocytes and platelets. In the microcirculation, the inflammatory manifestations of CVD are more readily visible in postcapillary venules. There is growing evidence, however, that the endothelium-dependent arteriolar dysfunction often associated with CVD is also linked to the systemic inflammatory response.1–4

Because the expression of adhesion glycoproteins by activated endothelial cells is a rate-determining step in the recruitment of inflammatory cells, much attention has been devoted to the role of endothelial CAMs in CVD. This attention has produced considerable evidence for the: (1) altered endothelial CAM expression in animal models of CVD;5; (2) use of circulating levels of soluble endothelial CAMs (eg, soluble intercellular adhesion molecule-1 [sICAM-1]) as a marker for the severity of inflammation in clinical CVD6; (3) endothelial CAM expression as a critical determinant of the end-organ damage and vascular dysfunction associated with experimental CVD;7,8 and (4) linkage between endothelial CAM expression and several other factors (eg, oxidative stress) that have also been implicated in the development of CVD.7,8 Oxidative stress, platelets, the CD40/CD40L signaling system, and angiotensin II (Ang II) are some of the factors that influence endothelial CAM expression, and there is evidence for their involvement in the initiation and/or perpetuation of inflammation in CVD.8,9

Adhesion Molecules

Leukocytes are recruited to sites of inflammation by a highly coordinated and well-regulated process that involves the expression and/or activation of adhesion molecules on endothelial cells and circulating inflammatory cells.2,4 These CAMs ensure an orderly sequence of cell-cell interactions that initially involve slowing the leukocyte with a weak adhesive interaction that is manifested as rolling. As the adhesive forces between leukocytes and endothelial cells strengthen, the leukocytes become firmly attached and remain stationary on the vessel wall (adherence), where the process

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From the Department of Molecular and Cellular Physiology (D.N.G.), Louisiana State University Health Sciences Center, Shreveport; the Department of General Surgery (T.V.), University of Münster, Germany; and the University Clinic for Pediatric Surgery (T.P.), University of Graz, Austria.
Correspondence to D. Neil Granger, PhD, Department of Molecular and Cellular Physiology, LSU Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71130-3932. E-mail d-granger@lsuhsc.edu
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of transendothelial migration can occur. Each stage of leukocyte recruitment is mediated by different families of adhesion molecules that are expressed on leukocytes or endothelial cells (Table 1).

The selectins, which mediate leukocyte rolling, are expressed by leukocytes (L-selectin, platelets (P-selectin), and endothelial cells (E- and P-selectin)). L-selectin is constitutively expressed on leukocytes, yet it is shed after leukocyte activation. P-selectin is normally stored in granules within endothelial cells (Weibel-Palade bodies) and platelets (α-granules), where it can be rapidly mobilized to the surface of activated cells. E-selectin is not normally expressed on endothelial cells; however, cytokines, reactive oxygen species (ROS), and bacterial endotoxin can elicit E-selectin expression through transcription-dependent mechanisms.

β2-Integrins are present on different leukocyte populations, where they exhibit unique binding specificity and signaling properties that depend on the combination of α- and β-subunits. The β2-integrins share a common β2-chain (CD18) that is linked to 1 of 4 α-chains designated CD11a, CD11b, CD11c, and CD11d. In the nonactivated leukocyte, CD11a/CD18 (lymphocyte function–associated antigen-1 [LFA-1]) is in a nonadhesive state. Receptor-mediated leukocyte activation leads to conformational changes in LFA-1 structure; increased adhesiveness of the glycoprotein; and the subsequent adhesion, activation, and transendothelial migration of leukocytes. Although CD11b/CD18 (Mac-1) is constitutively expressed on circulating leukocytes, it is also stored in secretory granules from which the glycoprotein can be mobilized when leukocytes are activated. The leukocyte adhesion mediated by both Mac-1 and LFA-1 most likely involves an interaction with ICAM-1 or ICAM-2 expressed on endothelial cells.

Five members of the immunoglobulin supergene family have been implicated as mediators of leukocyte–endothelial cell adhesion: ICAM-1, ICAM-2, vascular cell adhesion molecule-1 (VCAM-1), platelet and endothelial cell adhesion molecule-1 (PECAM-1), and mucosal addressin CAM (MadCAM-1). ICAM-1 is constitutively expressed on endothelial cells in most regional vascular beds, and its expression can be significantly increased on endothelial cell activation with cytokines or bacterial endotoxins. ICAM-2 is expressed on resting endothelial cells, and its expression is not influenced by endothelial cell activation.

VCAM-1, which mediates the adhesion of lymphocytes and monocytes, exhibits a lower level of constitutive expression on endothelial cells than does ICAM-1. However, its cell surface expression can increase dramatically by way of transcription-dependent (nuclear factor [NF]-κB) mechanisms. PECAM-1 is constitutively expressed on endothelial cells, leukocytes, and platelets, where it can mediate heterotypic and homotypic cell interactions. This adhesion molecule, which does not respond to cytokine challenge, plays an important role in regulating the transendothelial migration of leukocytes. MadCAM-1 is mainly expressed on high endothelial venules, where it mediates the trafficking of lymphocytes in lymphoid tissues.
endothelial cells and platelets, thereby limiting its utility as a marker of endothelial cell activation.

There is a large body of evidence that implicates inflammation and adhesion molecules in the pathogenesis of CVD, including atherosclerosis, stroke, and myocardial infarction. Hypertension, diabetes, hypercholesterolemia, hyperhomocysteinemia, and smoking are all associated with increased CAM expression on endothelial cells and/or increased levels of circulating soluble CAMs. More direct evidence for the involvement of adhesion molecules has been generated from animal models of CVD. Monoclonal antibodies that block the function of specific CAMs and mutant mice that are genetically deficient in 1 or more CAMs have been used to demonstrate a critical role for inflammation and adhesion molecules in animal models of myocardial infarction and stroke. The comparable protective actions of P-selectin and ICAM-1 (or CD11/CD18) directed interventions in many of these models suggest that interference with either the rolling or firm adherence of leukocytes effectively blunts leukocyte accumulation and the resulting tissue necrosis. Similarly dramatic results with antiadhesion strategies have been reported in animal models of atherosclerosis. For example, the complete absence of P-selectin, E-selectin, or ICAM-1 or the partial absence of VCAM-1 in mice that are genetically predisposed to atherosclerosis results in a significant delay in the development of atherosclerotic lesions.

Despite the immense success seen with antiadhesion strategies in animal models of CVD, the efficacy of antiadhesion therapy has not been as encouraging in the clinical setting. The limited numbers of phase II and III clinical trials based on antiadhesion therapy for CVD have not shown significant protection against either myocardial infarction or stroke. Nonetheless, comparable reagents have shown efficacy in clinical trials for chronic inflammatory diseases, such as psoriasis, rheumatoid arthritis, and inflammatory bowel disease, suggesting that either leukocyte–endothelial cell adhesion is not a critical component of human CVD or that better drugs or improved drug delivery strategies are needed to realize the potential of antiadhesion therapy in CVD. Opportunities to address the latter possibility are likely to result from ongoing industry efforts to develop improved humanized antibodies and small-molecule CAM inhibitors.

**Platelets**

An important function of the vasculature is to prevent the adhesion of platelets and subsequent formation of microthrombi, which can lead to impaired tissue perfusion. Endothelial cells help create this antithrombogenic surface. Accordingly, endothelial denudation or injury will lead to immediate platelet adhesion and aggregation at the site of injury. The binding of platelets to exposed regions of the subendothelial matrix is mediated by specific adhesion glycoproteins expressed on platelets that bind to ligands embedded in the matrix, such as von Willebrand factor, collagen, and fibronectin. There is evidence that platelets can also bind to the surface of activated endothelial cells and to leukocytes that are already adherent to the vessel wall. These adhesive interactions between platelets and endothelial cells can be mediated by a variety of glycoproteins that are expressed on activated platelets, including P-selectin, PECAM-1, von Willebrand factor, and β3-integrins (glycoprotein IIb/IIIa).

There is growing recognition that platelets not only are involved in hemostasis and thrombosis but also can modulate acute and chronic inflammatory responses. The binding of activated platelets to endothelial cells and/or leukocytes can influence the intensity of an inflammatory response through the release of different bioactive compounds. Platelets release factors that might either inhibit (soluble P-selectin, nitric oxide [NO]) or activate (oxygen radicals, leukotrienes, thromboxane A2) neutrophils. Activated platelets can release and/or activate a variety of inflammatory molecules (Table 2) that can elicit endothelial activation. Platelets can also enhance the recruitment of leukocytes into inflamed tissue by serving as a P-selectin–rich platform on the endothelium for leukocyte adhesion and by reducing shear rates.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Membrane phospholipids and products</th>
<th>Inflammatory Function</th>
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<tbody>
<tr>
<td>PAF</td>
<td>Increases IL-1β production, triggers and amplifies inflammatory and thrombotic cascades</td>
<td></td>
</tr>
<tr>
<td>Thromboxane A2</td>
<td>Reduces shear, enhances recruitment of leukocytes</td>
<td></td>
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<tr>
<td>Chemokines</td>
<td>Chemotaxis of monocytes, lymphocytes, and eosinophils</td>
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<td>RANTES</td>
<td>Chemotaxis of monocytes and lymphocytes</td>
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<tr>
<td>PF4</td>
<td>Facilitates macrophage differentiation, leukocyte chemotaxis?</td>
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<tr>
<td>β-TG</td>
<td>Leukocyte chemotaxis?</td>
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<tr>
<td>IL-8</td>
<td>Chemotaxis of neutrophils</td>
<td></td>
</tr>
<tr>
<td>ENA-78</td>
<td>Chemotaxis of neutrophils</td>
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<tr>
<td>MIP-1α</td>
<td>Chemotaxis of monocytes and lymphocytes</td>
<td></td>
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<tr>
<td>MCP-1</td>
<td>Chemotaxis of monocytes and lymphocytes, monocyte differentiation</td>
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<tr>
<th>Adhesion molecules</th>
<th>Membrane phospholipids and products</th>
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<tr>
<td>P-selectin</td>
<td>Platelet-endothelial and platelet-leukocyte interactions, RANTES deposition</td>
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<tr>
<td>GPIb/IIIa</td>
<td>Platelet aggregation, platelet-endothelial and platelet-leukocyte interactions (via fibrinogen)</td>
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<td>Transmembrane proteins</td>
<td>CD40L</td>
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<td></td>
<td>Upregulates endothelial CAM expression and proinflammatory chemokines</td>
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<td>AT1 receptor</td>
<td>Activation leads to increased P-selectin expression on platelets</td>
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</tbody>
</table>

PAF indicates platelet-activating factor; IL, interleukin; RANTES, regulated upon activation normal T cell expressed and secreted; PF, platelet factor, β-TG, β−thromboglobulin; ENA, epithelial neutrophil-activating protein; MIP, macrophage inflammatory protein; and MCP, monocyte chemotactant protein. Other abbreviations are as defined in text.
in venules through the release of potent vasoconstrictors (e.g., thromboxane A). Thrombin is another link between coagulation and inflammation. When thrombin binds to its receptor, protease-activated receptor-1, on endothelial cells, it induces the expression of P-selectin, E-selectin, VCAM-1, and ICAM-1 through activation of NF-κB.24

There are several lines of evidence that support an intimate connection between the hemostatic and inflammatory systems in CVD. Hypercholesterolemia appears to exert a profound influence on platelet reactivity to aggregating stimuli. Platelet activation in human subjects with elevated serum cholesterol levels is evident from the increased expression of P-selectin on platelets25 and the corresponding increase in plasma levels of soluble P-selectin.26 Mice maintained on a cholesterol-enriched diet exhibit P-selectin–dependent rolling and adhesion of platelets in postcapillary venules.27 Platelet accumulation in atherosclerotic lesions is also well documented.28–30 Recent studies in hypercholesterolemic apolipoprotein E–/– mice indicate that platelets adhere to the carotid artery endothelium long before the development of atherosclerotic lesions and that prolonged blockade of platelet adhesion reduces leukocyte recruitment into the arterial wall and attenuates atherosclerotic lesion formation.29,30

Although the link between hemostasis and inflammation is less clear in hypertension, this disease is associated with altered platelet function. Platelets from hypertensive subjects have an increased tendency to aggregate,31 an increased expression of P-selectin,32,33 and increased intraplatelet calcium (a measure of cell activation).34 Antiplatelet agents have also been shown to confer a degree of benefit to “high-risk” hypertensive patients.35

**Oxidative Stress**

Oxidative stress is a characteristic feature of the inflammatory response. There are several enzymes that can generate ROS and result in oxidative stress, including the mitochondrial oxidases, xanthine oxidase, NAD(P)H oxidase, nitric oxide synthase (NOS), cytochrome P450, lipoxigenase, and cyclooxygenase.7,36 The dominant ROS-producing enzyme in leukocytes is NAD(P)H oxidase, whereas endothelial cells have the capacity to generate ROS from several of the aforementioned enzymes. In normal tissues, ROS are detoxified by antioxidant enzymes like superoxide dismutase (SOD) and catalase and by the free radical scavengers normally present in extracellular fluid (e.g., bilirubin, uric acid). The consumption of superoxide by basally generated NO appears to be of equal or greater quantitative importance (relative to SOD) in preventing oxidative stress.36

During inflammation, ROS can promote the adhesion of blood cells to vascular endothelium by (1) eliciting the production of inflammatory mediators, (2) activating nuclear transcription factors that bind to genes encoding endothelial CAMs or cytokines, or (3) mobilizing preformed adhesion molecules to the endothelial cell surface.7,36 Hydrogen peroxide can generate platelet-activating factor and leukotriene B4, both of which will upregulate/activate β2-integrins on leukocytes. The ROS-sensitive nuclear transcription factors NF-κB and activation protein-1 (AP-1) are present in the promoter regions of the genes for endothelial CAMs, such as E-selectin, ICAM-1, and VCAM-1. Oxidative stress has been linked to the activation of both NF-κB and AP-1. ROS can also mobilize preformed pools of adhesion molecules in leukocytes (CD11b/CD18) and endothelial cells (P-selectin), which might explain the rapid recruitment of rolling and adherent leukocytes in inflamed tissue.

The importance of ROS in the initiation of leukocyte– and platelet–endothelial cell adhesion has been demonstrated in studies of reagents that either scavenge (e.g., SOD or catalase) or inhibit (e.g., allopurinol) the production of ROS.37,38 Similarly, mice that are genetically deficient in critical protein subunits (e.g., p47phox) of NAD(P)H oxidase or transgenic mice that overexpress SOD exhibit attenuated leukocyte adhesion responses in models of oxidative stress.39 Studies demonstrating that treatment of otherwise normal animals with inhibitors of NO40 and endothelial NOS–knockout mice41 exhibit increased leukocyte adhesion support the view that a critical determinant of whether the vasculature assumes a proinflammatory or an anti-inflammatory (and therefore, a prothrombogenic or an antithrombogenic) phenotype is the balance between ROS and NO. Although NO and superoxide per se are often ascribed anti-inflammatory and proinflammatory (and antithrombogenic and prothrombogenic) roles, respectively, the products of their chemical interaction (RNOS) can yield either phenotype, depending on whether there is net oxidation or nitrosation of specific molecular targets that regulate the inflammatory response (Figure 1).3,36

There is evidence implicating oxidative stress in the pathogenesis of stroke,42 myocardial infarction,43 myocardial stunning,44 atherosclerosis,4,45 and congestive heart failure.46 Similarly, there is evidence that implicates ROS in the deleterious effects of hypercholesterolemia,3,7 hypertension,47 diabetes mellitus,48 cigarette smoke,49 and hyperhomocysteinemia.50 How much of the protective effect afforded by antioxidant therapy can be attributed to an attenuated inflammatory response remains unclear for many models of experimental CVD. However, large-scale clinical trials of antioxidant vitamin supplementation have failed to confirm the benefits predicted from animal studies.51

**Renin-Angiotensin System**

Whereas Ang II has long been appreciated for its role in the regulation of blood pressure and salt balance, there is evidence that implicates the renin-angiotensin system (RAS) in the inflammation associated with CVD. A key response of endothelial cells and inflammatory cells to Ang II is increased production of ROS and the consequent imbalance between ROS and NO47,52 This pro-oxidative effect of Ang II results from its engagement to the high-affinity angiotensin II type 1 (AT1) receptor, which leads to the activation of NAD(P)H.53 The proinflammatory AT1 receptor is found on endothelial cells and circulating blood cells, including neutrophils, monocytes, T lymphocytes, and platelets. AT1 receptor antagonists block the oxidative stress induced in these cells by Ang II.54,55

The redox-sensitive nuclear transcription factor NF-κB appears to be an important link between AT1 receptor activation, oxidative stress, and the inflammatory phenotype that is assumed by Ang II–responsive cells.56 Activation of
NF-κB by the AT1 receptor induces redox-sensitive genes for certain endothelial CAMs (eg, VCAM-1), cytokines (eg, tumor necrosis factor [TNF]-α), and chemokines (monocyte chemoattractant protein-1).57 Ang II can also engage the AT1 receptors on leukocytes to promote β2-integrin upregulation.47 Superfusion of the rat mesentery with subvasoconstrictor doses of Ang II induces an AT1 receptor–dependent rolling, firm adhesion and emigration of leukocytes, and an enhanced production of ROS in postcapillary venules.58 These effects of Ang II appear to result from an ROS-dependent mobilization of preformed P-selectin to the endothelial cell surface.58

Losartan decreases P-selectin expression on platelets,59 suggesting that Ang II might also exert some of its proinflammatory actions in vivo by activating platelets. Platelets normally express AT1 receptors, and AT1 receptor antagonists have been shown to attenuate platelet adhesion60 and aggregation59 in vitro and to mediate an antithrombotic effect in vivo.59 This action might be linked to NO production, because losartan is known to enhance NO release from platelets.61

Although there is limited evidence for the involvement of inflammation-dependent processes in the protective effects of AT1 receptor antagonists in myocardial infarction62 and stroke,63 a more compelling case can be made for the involvement of Ang II and AT1 receptors in the inflammatory responses associated with hypercholesterolemia and atherosclerosis.64 Hypercholesterolemia is associated with an increased density of AT1 receptors on endothelial cells, circulating leukocytes, and platelets.45,54,55 This response is prevented by statin treatment through a mechanism that is independent of the drugs’ lipid-lowering effect.54,55 The possibility that Ang II and AT1 receptors are involved in eliciting the oxidative stress and leukocyte–endothelial cell adhesion of hypercholesterolemia is supported by studies of both large and microscopic blood vessels.52,65 Furthermore, surrogate markers for endothelial activation and inflammation (eg, sVCAM-1) indicate that RAS-directed drugs might exert some of their beneficial effects by controlling the inflammatory response that accompanies CVD.

**CD40/CD40L Interactions**

CD40/CD40L signaling might represent an important communication system that enables blood cells to amplify the endothelial cell responses to inflammation and contribute to the regulation of hemostasis.66 CD40, a member of the TNF receptor family of proteins, is constitutively expressed on platelets and endothelial cells. CD154 (or CD40L), the ligand for CD40, is found on cells of the immune system (eg, T lymphocytes) and on activated platelets.67 CD40L also exists in a soluble form (sCD40L), which is shed from lymphocytes and platelets within minutes to hours after cell activation.68 Engagement of CD40L with CD40 on endothelial cells results in phenotypic changes in the endothelial cell that are similar to those induced by TNF-α, ie, increased expression of E-selectin, ICAM-1, and VCAM-1; increased secretion of the chemokines interleukin-8, interleukin-6, and monocyte chemoattractant protein-1;66,68; and enhanced production of ROS.69

There are several lines of evidence that implicate CD40/CD40L signaling in the vascular pathology associated with hypercholesterolemia. Patients with moderate hypercholesterolemia exhibit significantly increased expression of CD40L and P-selectin on platelets and elevated CD40 expression on monocytes.25 Statin treatment significantly reduces CD40 and CD40L expression and lowers sCD40L in patients with...
familial hypercholesterolemia. Blockade of CD40L by neutralizing antibodies or genetic disruption of CD40L in mice appears to prevent the initiation and progression of atherosclerosis and induces a more stable plaque with fewer inflammatory cells, less lipids, and more smooth muscle cells and collagen. Elevated levels of soluble and membrane-bound forms of CD40L have been reported for patients with angina, after cardiopulmonary bypass surgery, and with congestive heart failure. Elevated blood levels of sCD40L even appear to be associated with the risk of future cardiovascular events in otherwise healthy women and predict patients with high-risk atherosclerotic lesions.

Conclusions

Preclinical and clinical research has provided considerable evidence for the involvement of inflammation in CVD. Parallel efforts to better understand the processes of hemostasis and coagulation have revealed an intimate link between platelet function and the inflammatory response. These revelations have fueled an intense interest in defining the nature and consequences of the interactions of blood cells with the vessel wall in CVD. Although the adhesion molecules that mediate these interactions are now well defined, the mechanisms that underlie the activation and/or increased expression of these adhesion molecules in CVD remain incompletely understood. Four distinct lines of investigation dealing with platelets, oxidative stress, Ang II receptor activation, and the CD40/CD40L signaling system are now merging (Figure 2) as a result of the effort to understand the events that initiate adhesion molecule involvement in CVD. These relative newcomers to the field of cardiovascular research have led to novel predictors for cardiovascular risk and hold the promise of offering multiple sites for therapeutic intervention in CVD, which should be considered in the design of future clinical trials.

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References


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