7. Inflammation and atherosclerosis

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Abstract. Three decades ago the prevailing viewpoint envisaged atherosclerosis as a bland proliferative process. According to that concept, endothelial denuding injury led to platelet aggregation and release of platelet-derived growth factor which would trigger the proliferation of smooth muscle cells in the arterial intima, and form the nidus of the atherosclerotic plaque. The advent of the cell biological era supplanted the simplistic concept of the atheroma as a passive deposition of lipid debris on the artery wall. Atherosclerosis is associated to vascular impairment, endothelial dysfunction, prothrombotic tendency, and low-grade chronic inflammation. Indeed, today, atherosclerosis is considered an autoimmune disease that contributes to several vascular complications because inflammatory cells and immune mediators occur prominently in atherosclerotic lesions. Multiple independent pathways of evidence now pinpoint inflammation as a key regulatory process that links multiple risk factors for atherosclerosis and its complications with altered arterial biology. Inflammation drives the formation, progression, and rupture of atherosclerotic plaques. T cells contribute to inflammatory processes that promote thrombosis by stimulating production of collagen-degrading proteinases and the potent procoagulant tissue factor. Increased synthesis and release of different cytokines have been reported as associated with future atherothrombotic events Modulators

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of inflammation evoke production of acute phase reactants in the liver, implicated in thrombogenesis and clot stability. Inflammation is thus moving from a theoretical concept to a tool that provides practical clinical utility in risk assessment and targeting of therapy. This revolution the pathophysiology of atherosclerosis has provided clinical insight that may aid patient management.

I. Introduction

Atherosclerosis remains the most common cause of vascular complications, including stroke, acute myocardial infarction, and peripheral artery disease. Over the last years, the understanding of the importance of inflammation during all stages of atherosclerosis has greatly increased. Today, atherosclerosis is not merely a cholesterol storage disease that obstructs arteries [1], and our therapeutic goals now reach beyond addressing flow-limiting stenoses by invasive revascularization procedures. Much of the plaque’s peril lies in its thrombogenic potential, not just the degree of stenosis it causes. Inflammatory processes govern much of the biology of plaques and their clinical destiny [2]. Under normal conditions, the endothelial cells (ECs) maintain the liquid state of the blood and resist adhesion and aggregation of platelets and leukocytes and promote fibrinolysis. When activated by stimuli such as hypertension, smoking, an unhealthy diet, obesity, insulin resistance or inflammation, the ECs become dysfunctional and express adhesion molecules that recruit leukocytes. Blood monocytes, the most numerous of the inflammatory cells in the plaques, adhere to the surface of the dysfunctional ECs by binding to leukocyte adhesion molecules not expressed by normal ECs, but induced by mediators such as cytokines, angiotensin II, or oxidized lipoproteins. Once the monocytes adhere to the activated ECs, proinflammatory chemokines provide a chemotactic stimulus that induces them to enter the intima where they monocytes mature into macrophages expressing scavenger receptors that allow them to engulf modified lipoprotein particles. The cytoplasm becomes full of lipid giving the macrophages the appearance of foam cells inside atherosclerotic lesions. The macrophages proliferate within the intima, sustaining and amplifying the inflammatory process by releasing several growth factors and cytokines, including enzymes that can destroy the arterial extracellular matrix, such as matrix metalloproteinases (MMPs) and the procoagulant tissue factor (TF) [2]. The cytokine monocyte chemoattractant protein-1 (MCP-1) interacts with the monocyte chemokine receptor CCR2, recruiting them to the arterial endothelium and facilitating their entry between ECs by diapedesis [3]. One key mediator of monocyte maturation into macrophages within the intima, the macrophage colony-stimulating factor, increases in atherosclerotic lesions and induces scavenger receptor expression [4].
On the other hand, atherosclerosis is also an autoimmune disease in which autoantigens and autoantibodies affect the vasculature remodelling process. Processing and presentation of autoantigens and subsequent autoantibody production occur in lymphoid organs as well as in non-lymphoid tissues such as arteries. Many cells participate in human atherosclerotic lesions, including lymphocytes, macrophages, dendritic cells (DCs), mast cells, vascular smooth muscle cells (VSMCs), and ECs. Many of these cells are recruited from the lymphoid organs or the circulation and participate in antigen or autoantigen presentation and T cell activation.

II. The role of innate immunity in atherosclerosis

The monocyte/macrophage, the most prominent cellular component of the innate immune response, plays an early crucial role in atherogenesis. In human artery biopsy specimens and experimental models of atherosclerosis it has been shown that monocyte recruitment is an early event in atherogenesis. This recruitment involves attachment to activated ECs by leukocyte adhesion molecules. Several protein mediators known as chemokines are able to direct cell migration of monocytes into the intima. Maturation of monocytes into macrophages, their multiplication, and production of many mediators ensues [5].

Several new findings regarding monocyte recruitment to atherosclerosis have come to light. First, kinetics of monocyte recruitment in mouse atherosclerotic lesions suggests that monocyte entry occurs not just during the initial stages of lesion formation, but continues even in established lesions [6], a fact with promising implications for targeting monocyte recruitment for atherosclerosis treatment. Another recent recognition revolves around monocyte heterogeneity in atherosclerosis [7]. A body of evidence suggests a disease-relevant dimorphism of monocytes. Hyperlipidemia elicits an enrichment of a pro-inflammatory subset of monocytes in the mouse. These pro-inflammatory monocytes may correspond to a human monocyte subset marked by the presence of P-selectin glycoprotein ligand [8] that home to atherosclerotic lesions, where they propagate the innate immune response by expressing high levels of pro-inflammatory cytokines and other macrophage mediators including MMPs.

Recent evidence has also highlighted the atherogeneic potential of mast cells. Identified as a minority leukocyte population in the arterial adventitia and atherosclerotic intima, mast cells have several functions implicated in atherogenesis [9]. For example, they release vasoactive molecules (histamine and leukotrienes), as well as serine proteinases and heparin (a co-factor in growth factor action and angiogenesis). Recent studies have provided evidence for mast cell participation in mouse atherogenesis [10]. Since pharmacologic
agents can modulate mast cell functions in humans they may have also some therapeutic implications.

Many links exist between lipoproteins and innate immunity. Modified lipoproteins interact with scavenger receptors and may thus send proinflammatory signals. Oxidized phospholipids derived from modified lipoproteins may also drive inflammation. A lipoprotein-associated phospholipase currently targeted in clinical trials may generate pro-inflammatory derivatives of oxidatively modified lipoproteins [11]. Some data show that apolipoprotein CIII, a constituent of certain triglyceride-rich lipoproteins associated with poor clinical outcomes, incite inflammation by binding to TLR2 [12].

Another area in relation to innate immunity in atherosclerosis regards the links between thrombosis and inflammation. Previously considered independent pathways in host defense, current evidence supports considerable link [13]. For example, prostaglandins control inflammation as well as thrombosis. The major mediator of blood coagulation, thrombin, elicits the expression of pro-inflammatory cytokines from vascular ECs and VSMCs. Activated platelets secrete preformed pro-inflammatory cytokines and exteriorize and shed a multipotent pro-inflammatory stimulus, the CD40-ligand (CD154). Platelets also release a pro-inflammatory mediator known as myeloid-related protein 8/14 [14], a heterodimeric molecule that serves as a biomarker for atherothrombotic events in both healthy populations and in survivors of acute coronary syndromes [15]. Myeloid-related protein 8/14 can bind TLR-4 activating innate immunity through this pattern recognition receptor and is also able to promote EC apoptosis, a process implicated in plaque thrombosis [16]. All these observations suggest an intimate link between these two convergent pathways in atherosclerosis.

III. The role of acquired immunity in atherosclerosis

The adaptive immune response has arisen more recently in evolution. This arm of host defenses, in contrast to the innate immune response, requires “education” of the immune system. The clinical experience illustrates the lag time in developing an adaptive immune response. For example, an antibody response or a cell immune response sometimes requires even months following vaccination with an antigen. Contrasting with the innate immune response, the adaptive arm displays high specificity. Instead of recognizing just hundreds of molecular patterns, the repertoire of antibodies and T-cell receptors can recognize many millions of specific structures.

Interacting with DCs, a special subset of mononuclear phagocytes specialized in antigen presentation, T cells encounter antigens and mount a
cellular immune response. The DCs populate atherosclerotic plaques and regional draining lymph nodes where they can present antigens to T cells with co-stimulatory molecules that incite this key afferent limb of adaptive immunity. Putative antigens that stimulate T cells in the context of atherosclerosis include certain heat shock proteins (HSP), components of plasma lipoproteins, and potentially microbial structures as well. The clone of T cells that recognizes antigen in this context will proliferate to amplify the immune response. Upon renewed exposure to the specific antigen, these T cells produce cytokines, trigger inflammation, and some T cells have mechanisms specialized for killing cells. This amplification accounts for the delay in the typical adaptive immune response that is slower and much more structurally specific than the “fast and blunt” innate immune response described above.

Various functionally distinct classes of T cells exist. Helper T cells spearhead antigen recognition, and fall into two major functional subtypes known as Th1 and Th2. Humans may have less accentuated polarization of Th1 vs. Th2 cells than inbred mice. In human atherosclerotic lesions, Th1 cells expressing the cytokines interferon-γ (IFN-γ) and interleukin 2 (IL-2) prevail over IL-4, IL-5- and IL-10-producing Th2 cells. T cells produce high amounts of IFN-γ but low amounts of IL-4 [17], a cytokine that may promote humoral immunity. Th1 responses generally amplify pro-inflammatory pathways by secretion of cytokines such as IFN-γ. The Th1 response appears to aggravate atherosclerosis. A more recently recognized T cell subset, Th17 cells, may also exert particularly pro-inflammatory actions. Whereas the role of Th2 in atherosclerosis is controversial some, but not all, evidence suggests that Th2-slanted responses may drive aneurysm formation [18].

Another T cell subtype, known as regulatory T cells (Treg), appears to play an intriguing modulatory role in atherosclerosis. Treg can dampen inflammatory responses. Genetic manipulations that interfere with Treg functions mediated by transforming growth factor β (TGF-β) augment atherogenesis in mice, yield lesions with signs of heightened inflammation, and even trigger thrombosis [19]. Thus, Treg cells and Th2 vs. Th1 and Th17 cells can counterbalance the pro-atherogenic effects of Th1 cells indicating the yin/yang complexity of cellular immunity.

The types of T cells just described express the surface marker CD4 and recognize antigen presented by DCs and macrophages. One third of all T cells in human lesions are of a different type that carries the CD8 marker and recognizes antigens bound to HLA molecules on many different cell types, typically viral antigens on infected cells. When activated, CD8+ T cells kill neighbor cells via cell/cell contact. Several mediators produced in lesions can recruit CD8+ T cells capable of killing smooth muscle cells and...
macrophages, processes linked to lesion growth and complication [20]. All these T cells share the capacity to recognize protein antigens bound to HLA molecules on cell surfaces. The NKT cells, in contrast, reacts towards lipid antigens presented by CD1 molecules on antigen-presenting cells. Once activated, the NKT cells produce proinflammatory cytokines that promotes atherosclerosis [21].

III.a. Another inflammatory cells present in atherosclerotic lesions

The most studied inflammatory cells in atherosclerotic lesions include T cells, B cells, DCs and macrophages. Besides T cells, all other inflammatory cells can present antigens (antigen presenting cells) to assist T-cell activation, essential for the early progression of atherosclerosis. In atherosclerotic lesions, T cells include mainly CD4+ and CD8+ cells, although CD4+ cells dominate in number. It has been suggested that T cell activation occurs initially in lymphoid organs such as spleen and lymph nodes and then migrates to the aorta for secondary activation by antigen presenting cells that present the same antigens. In turn, activated T cells can stimulate other inflammatory cells such as macrophages to secrete inflammatory cytokines, proteases and tissue factors [22]. B cells, which are much fewer than T cells, appear in the fatty streak or the more external layer of the aortic wall [22]. Both T and B cells are critical in atherogenesis since reduction of T and B cells lead to decreased plaque development [22]. With CD4+ T-cell transfer from immunocompetent to immunodeficient apolipoprotein E knockout Apoe-/- mice, disease increases dramatically, whereas atherosclerosis-prone but immunodeficient animals show reduced development of early lesions with the fatty streaks [23]. By contrast, low-density lipoprotein (LDL) receptor-deficient LDLr-/- mice lacking B cells had 30-40% increase in atherosclerosis, resulting in decreased serum anti-oxidated-LDL (ox-LDL) autoantibodies, a fact that suggests that the B cells that make autoantibodies are anti-atherogenic. This explains why mice that go through splenectomy have enhanced atherosclerosis, a phenomenon that splenic B-cell reconstitution can reverse [24].

DCs occur in the sub-endothelial space with other immunocompetent cells. There, they capture autoantigens then migrate to lymphoid stations, where they present autoantigens to T cells, provoking their responses [25]. Thus, T cells can respond to specific autoantigens while DCs and macrophages can present such autoantigens, giving rise to clonal expansion of their specific T cells and autoantibodies. Compared with macrophages or lymphocytes, mast cells constitute minor inflammatory cells in human atherosclerotic lesions but they are not insignificant. Mast cell inactivation reduces atherosclerotic lesion development in Apoe-/-mice [26]. Using mast cell-deficient KitW-sh/W-sh
mice, these minor inflammatory cells are indeed critical to diet-induced atherosclerosis in LDLr/- mice. KitW-sh/W-sh mice in the background of LDLr/- are protected from atherogenesis. Using mast cell reconstitution techniques, mast cells release pro-inflammatory cytokines IL-6 and IFN-γ to stimulate vascular cell release of matrix-degrading MMPs [10]. Therefore, different inflammatory cells serve different roles in atherogenesis.

**III.b. Monocyte heterogeneity**

Like ECs and VSMCs, monocytes/macrophages exhibit heterogeneity, falling into 2 distinct subsets [7]. Mice with normal blood cholesterol have approximately equal numbers of circulating monocytes bearing low or high levels of the marker Ly-6C. When fed a high-fat Western diet, apolipoprotein-E deficient (apoE/-) mice have a striking increase in monocytes with high levels of this marker (Ly-6Chi), but no change in the numbers of monocytes with low levels of Ly-6C. Ly-6Chi monocytes adhere preferentially to activated ECs, accumulate in atherosclerotic plaques, and rapidly become lesional macrophages [7]. In comparison with Ly-6Clo cells, Ly-6Chi monocytes have heightened proinflammatory properties, producing higher levels of proinflammatory cytokines, myeloperoxidase, and some proteinases. Ly-6Chi monocytes produce higher levels of than Ly-6Clo monocytes, which contributes to their homing to and rolling on the arterial ECs prior to penetrating into the intima [8]. Although human monocytes do not express Ly-6C, high expression of PSGL-1 may identify a proinflammatory population of monocytes. These studies support a particular role for proinflammatory monocytes in the inflammatory process that takes place in atherosclerotic plaques. In addition to macrophages, atheromata contain a smaller population of T lymphocytes. Although numerically a minority of the leukocytes in plaque, these master cells of the adaptive immune response appear to exert decisive regulatory roles by instructing the more abundant monocytic effectors of the innate immune response. Thus, the relationship of the T cells to the mononuclear phagocytes may be like the conductor of an orchestra or the general of an army [22]. The T cells in the plaque also display heterogeneity of functions. Some subsets appear to be proinflammatory (eg, Th1 cells), while others tend to mute inflammation (eg, Treg and Th2 cells). The putative antigens that activate the plaque T cells, and the regulation of the balance between the T cell subsets, remain unknown [22]. B lymphocytes, the key cells in the humoral arm of the adaptive immune response, seem to have a net inhibitory effect on atherogenesis, a property being explored in the development of vaccines to mitigate atherosclerosis [27].
III.c. Humoral immunity and atherosclerosis

B lymphocytes secrete antibodies that like T cells can perhaps recognize billions of diverse structures. Convergent lines of experimental evidence suggest that humoral immunity can attenuate rather than promote atherogenesis. For example, splenectomy, ablating an important B cell compartment, aggravates atherosclerosis [24]. Hypercholesterolemic mice develop a strong humoral response directed against epitopes characteristic of ox-LDL [28]. Immunization of rabbits or mice with oxi-LDL attenuates atherosclerosis. Interestingly, the antibodies elicited in mice in response to ox-LDL also recognize a pneumococcal antigen [29]. This finding underscores the view that host defenses against infectious agents can overlap with inflammatory pathways involved in atherogenesis. The observation that humoral immunity against ox-LDL might protect against atherosclerosis has inspired therapeutic explorations of vaccination against ox-LDL to mitigate this disease.

III.d. Autoantigens and atherosclerosis

Autoantigens refer to those generated by structure modification on specific moieties of endogenous molecules. Although these antigens occur in human atherosclerotic lesions, they are not aorta-specific. In atherosclerotic lesions, ox-LDL, HSPs and β2-glycoprotein I are the most common autoantigens. In early atherosclerotic lesions, LDLs are trapped in the sub-endothelial space and subsequently oxidized [30]. Two aldehydes with strong immunogenic properties develop: malondialdehyde and 4-hydroxynonenal aldehyde with anionic valence. This oxidation process associates with major structural modifications of LDL, including fragments of apo B-100 and generation of various aldehyde and phospholipid adducts to apo B-derived peptides. DCs and macrophages uptake ox-LDL and they use major histocompatibility complex (MHC) class II molecules to present ox-LDL for recognition by specific CD4+ T cells; then, they lead to ox-LDL-specific T-cell expansion [31]. Indeed, about 10% of lesional T cells are ox-LDL-specific [31] which also appear in circulation. Transfer of ox-LDL-reactive T cells to T-cell-deficient Apoe-/-scid/scid mice occurs more efficiently at lesion acceleration than transfer of T cells without antigen specificity [32]. Besides stimulating T cells via antigen presenting cells, ox-LDL can promote inflammation by attracting monocytes and more T cells to the lesions. Ox-LDL can also be cytotoxic to the ECs (causing a prothrombotic endothelial surface dysfunction) and stimulate the release of various soluble inflammatory and adhesion molecules. Macrophage scavenger receptors remove ox-LDL, leading to ox-LDL
macrophage intracellular accumulation and foam cell formation. Clinical studies showed the presence of IgM against malondialdehyde-modified apo B-100 peptide and association with plasma ox-LDL levels in humans [33].

Autoantigen HSP shows high-sequence homology from bacteria to humans. For example, mycobacterial and chlamydial HSP65 show considerable mimicry with human HSP60 [34]. Most known risk factors or stressors evoke HSP expression on ECs, VSMCs and macrophages to maintain cell viability. In turn, HSP induces production of inflammatory cytokines and proteases from macrophages and mediates monocyte adhesion to ECs. In subjects with established atherothrombotic disease, antibodies against HSP60/65 increase and predict further development of the disease [35]. These antibodies specifically react with cells in the plaques and mediate lysis of stressed ECs and macrophages. Chlamydial HSP65 isolated from human serum are cytotoxic to heat-stressed human ECs, and antibodies to microbial HSP65 recognize specific epitopes on human HSP60 present in the arterial wall of healthy young people [34]. Intralesional cells have significantly increased T cell reactions against human HSP60 compared with peripheral T cells, and T cells isolated from human atherosclerotic lesions showed significant reaction against human HSP60 as well as chlamydial and mycobacterial HSP [36]. On challenge with human HSP60, plaque T cells express Th1 functions, including cytotoxicity and monocyte TF production [36].

High levels of autoantigen β2-glycoprotein-I alongside CD4+ and CD8+ T cells appear in atherosclerotic lesions. β2-glycoprotein-I binds strongly to negatively charged molecules such as phospholipids and to activated platelets and apoptotic cell membranes, which mediate clearance of apoptotic cells from the circulation. In atherosclerotic lesions, β2-glycoprotein-I co-localizes with ox-LDL and often forms covalent complexes that occur in autoimmune diseases. In patients with systemic lupus erythematosus (SLE) or antiphospholipid syndrome (APS), serum complexes and anti-β2-glycoprotein-I-ox-LDL complex autoantibodies are elevated [37]. Similarly, such complexes and antibodies present in the bloodstream of patients with vascular complications, such as acute myocardial infarction and unstable angina, strongly associate with arterial thrombosis. These complexes inhibit ox-LDL uptake by macrophages through scavenger receptors, but are more rapidly internalized by macrophages as IgG immune complexes in the presence of anti-β2-glycoprotein-I autoantibodies. These macrophages will then present β2-glycoprotein-I antigenic peptides (on MHC class II) to specific T cells. Therefore, circulating IgG immune complexes containing ox-LDL and β2-glycoprotein-I can be pro-atherogenic. Indeed, adoptive transfer of β2-glycoprotein-I-reactive lymphocytes enhances early atherosclerosis in Ldlr -/- mice [38].
III.e. Autoantibodies and atherosclerosis

Immunization of atherosclerosis-prone animals with ox-LDL, regardless of adjuvant selection, demonstrated the importance of autoantibodies in atherosclerosis animal models [39]. The most extensively characterized anti-ox-LDL autoantibody is an IgM called EO6, which reacts against an oxidized phospholipid in modified LDL that has been identified as 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphorylcholine. An ox-LDL monoclonal antibody recognizes the phosphorylcholine epitope in oxidized phospholipids and those on apoptotic cell surfaces from B-cell hybridoma from Apoe-/- mice. This autoantibody is indistinguishable from the natural antibody T15, which is secreted by a specialized subclass of B lymphocytes termed B-1 cells in the peritoneal cavity and spleen. Functionally, these autoantibodies block the selective binding to ox-LDL and apoptotic cells by scavenger receptors in a dose-dependent manner and thereby prevent macrophage lipid accumulation. In addition to IgM, IgG also inhibits ox-LDL uptake by macrophages. In malondialdehyde-LDL-immunized Apoe-/- mice, the IgG titres against malondialdehyde-LDL correlate inversely with plaque size and serum cholesterol levels [40]. These antibodies neutralize the many pro-atherogenic effects of ox-LDL and minimally oxidized LDL and inhibit apoptotic blebs to induce monocyte adhesion by ECs. In humans, antibodies present in the plasma target a series of malondialdehyde-modified peptide sequences in apo B-100. Clinical studies indicated that IgM against malondialdehyde-LDL and ox-LDL associated inversely with carotid or femoral intima-medial thickness (IMT), and IgG against ox-LDL also correlated inversely with carotid IMT. In a nested case-control study, these IgM predicted the risk of developing acute myocardial infarction and causing cardiac death.

By contrast, anti-bacterial HSP65 antibody levels correlate positively with the presence of sonographically visible atherosclerotic lesions in the carotid arteries and established coronary atherosclerosis, and associate with elevated levels of coronary calcification independent of established coronary artery disease risk factors. A subsequent follow-up study confirmed that such correlation held true in a 5-year period of observations, especially in those with progressive carotid atherosclerosis [35]. Human serum HSP65 antibodies react with recombinant form of human HSP60 and those present in ECs, macrophages and VSMCs in atherosclerotic lesions. Purified anti-HSP65 antibodies are indeed cytotoxic to ECs. Similarly, antibodies targeting human HSP60 also associate with severity of coronary artery disease. Increased levels of soluble HSP60 and antibodies to HSP60 predict increased risk of coronary artery disease. A large population-based study showed the presence of serum soluble HSP60 in patients with carotid atherosclerosis, a correlation that held independent of age, sex and other risk factors [41].
Bacterial infection-associated atherosclerosis has been linked to HSP60/65. Anti-HSP60 antibody correlates strongly with human IgA to *Chlamydia pneumoniae* and with IgG to *Helicobacter pylori*. Infection with *C. pneumoniae* showed pro-atherogenic effect. Research suggests that bacterial HSP60 product exerts a direct pro-atherogenic effect by stimulating tumor necrosis factor-α (TNFα) and MMP production [42] and IgA anti-HSP60 and anti-*C. pneumoniae* increases the risk of coronary events 7-fold [43]. Serum IgG antibody against particular sequences of the *H. pylori*-derived HSP60 appeared predominantly in patients with atherothrombotic disease. *H. pylori* infection induces atherosclerosis in mice by causing autoimmunity to endogenous HSP60 due to molecular mimicry and enhancing HSP-specific Th1 immune responses. Antibiotics or anti-HSP60 antibodies reduce atherosclerosis in these mice [44]. Similar to antibodies to HSP60/65, those against autoantigens β2-glycoprotein-I or cardiolipin also positively correlate with atherothrombotic events. Case-control studies demonstrated the association of anti-cardiolipin antibodies with stroke and acute myocardial infarction [45] and IgG/IgM/IgA anticardiolipin antibodies and IgA for anti-β2-glycoprotein-I associate with increased risk of ischaemic stroke, arterial thrombosis, atherosclerotic immune process, acute myocardial infarction and peripheral vascular diseases. Although the exact mechanisms remain unknown, anti-β2-glycoprotein-I was thought to interact with β2-glycoprotein-I on the EC membrane and induce pro-inflammatory and procoagulant endothelial phenotype that is pro-atherogenic [46].

**IV. Atherosclerosis, inflammation, and thrombosis**

Fracture of the plaque’s fibrous cap and subsequent thrombosis causes most cases of fatal acute myocardial infarction events [47]. Inflammation regulates the fragility of the fibrous cap, as well as the thrombogenic potential of the plaque [2]. In addition to macrophages, T cells play an important role in the inflammatory process leading to thrombosis. T cells enter the intima by binding to vascular cells adhesion molecule-1 (VCAM-1) and in response to the IFN-γ-inducible chemokine ligands (CXCLs), IFN-γ-inducible protein-10 (IP-10), monokine induced by IFN-γ (MIG), and IFN-γ-inducible T-cell α-chemoattractant (I-TAC) [48]. These chemokines bind to the chemokine receptor CXCR3, expressed on T cells in the plaque.

When activated in the intima, the T cells produce pro-inflammatory cytokines, including the CD40 ligand, CD154. Ligation of CD40 by CD154 induces production of MMPs and the potent procoagulant TF [49]. TF initiates the coagulation cascade, enhancing the thrombogenicity of the plaque’s lipid core. Inflammation also influences the metabolism of collagen, the key
extracellular matrix molecule that confers strength and stability on the fibrous cap. IFN-γ produced by T cells in the plaque inhibits production of collagen by VSMCs [50]. T lymphocytes also promote degradation of collagen indirectly by local production of cytokines, including CD40L, that boost the elaboration of MMPs by neighboring macrophages [49]. Thus, inflammation contributes to all phases of atherosclerosis, from its initiation to its ultimate complication of thrombosis.

**IV.a. Role of obesity in atherogenesis, thrombosis and inflammation**

Some atherogenic conditions such as central obesity are associated with alterations of vascular function (including EC dysfunction), pro-thrombotic tendency, low-grade chronic inflammation, and oxidative stress: these defects, frequently associated as a cluster, are the main pathogenetic link between obesity and the increased risk of atherothrombotic events [51]. Some molecules directly synthesized by adipocytes and called “adipokines” may control energy balance and appetite, and influence insulin sensitivity via endocrine mechanisms. Also, they are able to modulate adipocyte size/number and angiogenesis via paracrine mechanisms, thus exerting a major role in the regulation of fat mass [52]. Furthermore, they can also exert a role in the control of blood pressure, lipoprotein metabolism, coagulation, immunity and inflammation [52]. Other peptides belonging to the cytokine group, produced and released by the stromal vascular components of adipose tissue (i.e., lymphocytes, fibroblasts, macrophages, ECs, and preadipocytes) [53] are mainly involved in local and systemic inflammation [54]. The increase in abdominal adipose tissue mass dysregulates both, adipokine and adipocytokine secretion patterns [54]. With the exception of the insulin sensitizing peptide adiponectin, adipokine production and secretion are increased in central obesity [55]: this fact plays a pivotal role both in the pathogenesis of vascular damage through adverse effects on hemostatic balance and vascular function [52], and in the amplification of inflammatory processes in vascular and nonvascular tissues [52]. Also, cytokine release is enhanced: this phenomenon is attributable to increased prevalence of hypertrophied adipocytes with altered adipokine synthesis and secretion, local hypoxia, as well as activation of resident inflammatory cells and macrophages [55]. In particular, adipose tissue from individuals with central obesity synthesizes and releases increased amount of proinflammatory chemokines and cytokines, such as MCP-1, macrophage migration inhibitory factor, TNF-α, and interleukins, including IL-1β and IL-6 [55]; procoagulant and proinflammatory mediators such as TF and plasminogen activator inhibitor-1; vasoactive substances such as angiotensinogen and endothelin-1 (ET-1) [56]; molecules involved in the
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pathogenesis of insulin resistance, such as TNF-α and resistin [57]. Central obesity is characterized by an enhanced atherothrombotic risk, and it is known that dysfunctions of platelets and VSMCs are deeply involved in atherothrombosis [58].

IV.b. Role of platelets in inflammation

Platelets are anuclear cell fragments released from megakaryocytes, hematopoietic cells that differentiate and undergo endomitosis [59]. Despite the lack of nucleus, they are able to carry de novo synthesis of different mediators involved in the regulation of inflammatory and coagulant pathways including IL-1β, plasminogen activator inhibitor-1, and TF [60]. Platelets play a pivotal role in the response to vascular injury through adhesion to exposed subendothelial layer triggered by different collagen types and adhesive proteins such as von Willebrand factor, fibronectin, laminin, fibulin and thrombospondin [60]. Activation of platelets by components of the subendothelial matrix is linked to exposure of membrane glycoprotein receptors including glycoprotein Ib/V/IX (GPIb/V/IX) complex which interacts with von Willebrand factor, integrin αIIbβ3 (GPIIb/IIIa) able to bind Arg-Gly-Asp domain of von Willebrand factor and fibrinogen, and GPVI which ensures a stable anchorage with subendothelial matrix by direct interaction with collagen [61]. Platelet activation and formation of aggregates are triggered also by thrombin, endogenous mediators released from storage granules and erythrocytes such as ADP, and de novo synthesis of platelet activating factor and thromboxane A₂ (TxA₂) [61]. Activated platelets also release inflammatory mediators from granules, including platelet-derived growth factor (PDGF) and platelet factor 4 (PF-4) [62].

Beyond acute activation as a consequence of vascular injury, circulating platelets are actively involved in all phases of the atherogenic process, from atherosclerotic plaque formation to plaque inflammation and rupture [63]. In these conditions, platelet reactivity is increased by reactive oxygen species (ROS) produced as a consequence of oxidative stress, by reduction of endothelial antithrombotic properties and by the increased availability of proinflammatory mediators, such as cytokines and chemokines [64]. Actually, platelets release several mediators linking thrombosis and vascular inflammation such as the RANTES (regulated on activation, normal T cell expressed and secreted) chemokine, PDGF, PF-4, TGF-β, CD40 ligand (CD40L, CD154), P-selectin, and TxA₂ [62]. RANTES recruits monocytes and T cells; in conjunction with P-selectin this chemokine can be immobilized on inflamed ECs, thus inducing monocyte arrest and migration [65]. PDGF, the major growth factor contained in platelets [62], stimulates both migration and
proliferation of VSMCs by cooperation with serotonin, and TGF-β [62], and is chemotactic for monocytes [63]; its effects on VSMCs are critical for the development of atherosclerotic process [63]. PF-4 is a member of the C-X-C chemokine subfamily that exerts chemotactic effects on monocytes, promotes monocyte-to-foam cell differentiation [62], enhances the binding of ox-LDL to vascular wall, and inhibits LDL degradation through LDL receptors [66].

CD40L is a trimeric protein structurally related to TNF-α superfamily stored in α-granules of resting platelets [67]. After platelet activation, it is rapidly exposed on cell surface and cleaved to release the soluble fragment (sCD40L) able to increase the stability of new platelet aggregates, and to activate vascular inflammatory mechanisms by inducing production of ROS, enhancing expression of adhesion molecules like VCAM-1, Intercellular Adhesion Molecule-1 (ICAM-1), and E-selectin in ECs and VSMCs, and increasing secretion of cytokines, chemokines, MMPs, and procoagulant factors [68]. Several cell types including ECs, VSMCs, monocytes, neutrophils, B cells and fibroblasts, bind CD40L through the specific receptor CD40 [68]. This receptor has been recently identified also in adipocytes where it plays a relevant role in the crosstalk with resident lymphocytes [69]; furthermore, circulating sCD40L levels have been found at abnormally high levels in patients with obesity, type 2 diabetes mellitus and atherothrombotic diseases [70]. P-selectin is stored in platelet α-granules [62] and, after activation, rapidly translocated upon cell surface becoming accessible to circulation; this phenomenon strengthens initial rolling contact between platelets and vessel wall and promotes RANTES deposition on ECs, thus increasing monocyte recruitment [65]. Platelet stimulation by agonists or exposure to high shear stress leads to formation and release of platelet-derived membrane-coated vesicles termed platelet microparticles (PMPs) [71], which influence the activities of other cell types both regionally and systemically [71]. PMPs are lipid-protein complexes with a diameter <1 μm, composed by vesicular fragments of the plasma membrane and α-granules [72]: their protein content plays a relevant role in both hemostasis and inflammation, by facilitating coagulation, promoting platelet and leukocyte adhesion to the subendothelial matrix, supporting angiogenesis and stimulating VSMCs [71]. These effects may contribute to the chronic inflammatory state which characterizes atherosclerosis [71]; in particular, a portion of platelet-derived IL-1β associated with PMPs stimulates the production of chemoattractant molecules and cytokines and the expression of specific adhesion molecules in ECs, thus enhancing their interaction with circulating leukocytes and, therefore, their ability to trigger inflammatory responses [73]. Also, RANTES is delivered to sites of EC injury via PMPs to promote monocyte recruitment [74].
IV.c. Platelet abnormalities in atherosclerotic conditions

Platelet hyperactivity is deeply involved in the increased atherothrombotic risk of patients with central obesity and type 2 diabetes mellitus [75]. A variety of defects of platelet function mainly related to increased adhesiveness and activability in vivo and reduced sensitivity to physiological antagonists has been identified [75]. Mean volume of circulating platelets, a parameter directly related to in vivo platelet activation relevant to predict myocardial infarction occurrence and mortality and re-stenosis following coronary angioplasty [76], is increased in several atherothrombotic risk factors [76]; furthermore, a positive correlation between body mass index (BMI) and mean platelet volume has been found in obese individuals [76], whereas weight loss may lead to a decrease in platelet size and reactivity [77]. Increasing evidences indicate that the size of circulating platelets deeply influences their hemostatic potential being the response of larger platelets to aggregating stimuli more rapid and the amount of released mediators increased. Platelet volume as well as other platelet parameters is mainly determined during megakaryocyte fragmentation in bone marrow [78]. Even though the mechanisms influencing megakaryocytopoiesis are not completely understood, an involvement of inflammatory cytokines (including interleukins -1, -3, -6, -8, -11, and -18), and of nitric oxide (NO) has been shown in some studies [79]; this phenomenon allowed to hypothesize that the increased production and release of proinflammatory cytokines, as well as EC dysfunction, might influence megakaryocytopoiesis and circulating platelet volume [80].

The increased in vivo activation of circulating platelets is mirrored by the enhanced expression of activation-dependent adhesion molecules, and by increased plasma concentrations of sP-selectin; another index of in vivo platelet activation, that is, urinary excretion of 11-dehydro-TxB$_2$, the major enzymatic metabolite of TxA$_2$, is increased in women affected by visceral obesity, compared to non-obese women [81]. Relevant defects of platelet function in atherogeneic states are related also to a reduced sensitivity to mediators, such as insulin, prostacyclin (PGI$_2$), and NO, which in healthy subjects reduce platelet sensitivity to proaggregating stimuli [82]. Insulin, which physiologically reduces platelet responses to agonists both in vitro [83] and in vivo [84], mainly through a NO-dependent mechanism mediated by the increase of intraplatelet cyclic nucleotides 3’5’-cGMP and 3’5’-cAMP [83], exhibits a deeply impaired antiaggregating effect in insulin resistant states, such as central obesity, type 2 diabetes mellitus with obesity and hypertension [84]. Furthermore, platelets from obese subjects or obese type 2 diabetic patients show defective responses to the NO/cyclic nucleotide/protein kinase pathway including the ability of NO and NO donors to increase cGMP, the
ability of cGMP to reduce platelet calcium and consequently aggregation [85]; similarly, also the ability of PGI₂ to increase cAMP and of cAMP to reduce platelet function are impaired in these patients [85]. As previously mentioned, a relevant factor causing platelet dysfunction is the increased oxidative stress [64], which is present in atherogenic states, as a consequence of imbalance between ROS production and reduced levels of substances able to protect from the damage of free radicals and peroxides [86]. For example, several metabolic abnormalities of the patients with visceral obesity, that is, excess of circulating free fatty acids, of ox-LDL, and of proinflammatory adipokines and cytokines, contribute to ROS production [86]. Some adipokines and inflammatory cytokines such as TNF-α and leptin are involved in enhanced oxidative stress [86]; furthermore, the increase in fat mass, as well as the pathological secretion pattern of adipocytes decrease the availability of antiinflammatory proteins such as adiponectin and ghrelin, which exert also protective effects against oxidative stress [55]. High concentrations of ROS influence platelet function by different mechanisms, including decreased NO bioavailability, calcium mobilization abnormalities and over-expression of membrane glycoproteins [64].

Isoprostanes are a family of prostaglandin-like metabolites produced in vivo from esterified arachidonic acid of cell membrane phospholipids or lipoproteins through a ROS-dependent mechanism [87], completely independent of cyclooxygenase-1 (COX-1) activity. Once released, they circulate in plasma and are available for receptorial interaction with platelets and cells of the vascular wall; in particular, 8-iso-prostaglandin-F2α, which is an abundant isoprostane compound, formed in vivo in humans, induces vasoconstriction and amplifies the adhesive reactions and the aggregating responses of human platelets to agonists [88]. Several studies showed a stimulatory interaction of isoprostanes with TxA₂ receptors [89], which is prevented by TxA₂ receptor antagonism but not by COX-1 inhibition [90]. Recently, a further isoprostane binding site, responsible of cAMP reduction, has been recognized [89]. The increased levels of F₂-isoprostanes observed in several pathological entities can be involved both in the persistent platelet activation in vivo, and in the resistance to antiplatelet effects of aspirin [82].

IV.d. Influence of cytokines on platelets

As previously mentioned the altered pattern of adipocyte-derived hormones and cytokines is deeply involved in the chronic proinflammatory state characterizing patients with central obesity and partially accounts for their increased atherothrombotic risk [91]. Changes in adipocyte-derived factors enhance oxidative stress by activating oxidases, may interfere with NO
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availability and influence cell proliferation and apoptosis [92]. Several evidences indicated that adipokines and cytokines influence platelet production and responses and VSMC function.

IV.d.1. IL-6

A multifunctional proinflammatory cytokine produced by different cell types, which contributes to up to 35% of circulating IL-6 levels [93]. In healthy individuals IL-6 expression is due to a tight regulation dependent on a complex hormonal network related to glucocorticoid and catecholamine secretion [93]. Increased IL-6 expression and circulating levels are associated with a variety of metabolic and vascular diseases, such as central obesity, metabolic syndrome, type 2 diabetes mellitus, atherosclerosis, and coronary artery disease [93]. IL-6, together with other cytokines, is a key risk factor for atherothrombotic diseases due to its effect in plaque development and destabilization via release of other proinflammatory cytokines, oxidation of lipoproteins by phospholipases, stimulation of acute phase protein secretion, release of prothrombotic mediators, and activation of MMPs [94]. Moreover, the increased ROS formation by vascular enzyme systems under proinflammatory conditions may play a critical role in the cross talk between IL-6 and other vasoactive substances, such as angiotensin II and catecholamines.

As far as megakaryocytopoiesis is concerned, IL-6 acts synergistically with thrombopoietin, other interleukins, and growth factors in promoting the maturation of megakaryocyte precursors [95]; actually, in vivo administration of IL-6 to humans increases circulating platelet counts [95]. This effect may explain the association between the increased markers of chronic inflammation and the elevated platelet count in obese women [96]: this phenomenon has been considered prothrombotic, since higher platelet counts are associated with adverse clinical outcomes in patients with acute coronary events [97]. IL-6 is responsible of an acute prothrombotic state through mechanisms involving: (a) increased expression of TF, fibrinogen, factor VIII and von Willebrand factor; (b) activation of ECs; (c) reduced levels of hemostasis inhibitors, such as antithrombin and protein S [98]. Furthermore, IL-6 influences platelet function by enhancing thrombin-induced activation [98], and modulates platelet responses by increasing ROS production.

IV.d.2. Tumor Necrosis Factor-α (TNF-α)

A pleiotropic, proinflammatory cytokine, produced as 17 kDa protein and secreted as a 51 kDa trimer by adipose tissue, macrophages, natural killer cells,
T cells, ECs, and VSMCs [99]. It has been recognized in human atheroma [100]. Adipose tissue has been identified as one of the main sources of TNF-α since the majority of TNF-α produced in adipose tissue is derived from infiltrating macrophages and not from mature adipocytes [101]. Increased TNF-α circulating level are found in obese non-diabetic subjects and in type 2 diabetic patients [101]. As far as the TNF-α effect on vascular function are concerned, it has been observed that its acute administration exerts per se a vasodilatory effect, but impairs EC-dependent vasodilation in response to insulin and acetylcholine in healthy humans [102]; furthermore, it inhibits the vasodilating actions of insulin in vessels of rat skeletal muscle. The TNF-α interference with EC-mediated vasodilation is also due to shortening the half-life of NO synthase (eNOS) mRNA in ECs [103] and to the increase of ET-1 synthesis and spillower [103]. TNF-α induces inflammatory changes in vessel wall by activating the transcription factor Nuclear factor-κβ (NF-κβ), which increases the expression of ICAM-1 and VCAM-1, and the production of MCP-1 and monocyte- colony stimulating factor from ECs and VSMCs. Plasma TNF-α concentrations predict vascular damage, since they are associated with early atherosclerosis in middle-aged healthy men [104]; furthermore, elevations of TNF-α in the stable phase after acute myocardial infarction were associated with an increased risk of recurrent coronary events.

In vitro studies showed that TNF-α promotes platelet aggregation [105] and ROS production mainly through activation of the arachidonic acid pathway [106]. Other data indicate that TNF-α influences platelet function also by increasing the secretion of leptin, which acts as proaggregating hormone. Today, the results from in vivo studies are not conclusive and there are not evidences to identify TNF-α as prothrombotic or antithrombotic [107].

**IV.d.3. Leptin**

A 167-amino acid adipokine primarily synthesized and released by mature adipocytes, although expressed also in many other tissues including muscle, placenta and gastric epithelium. Its circulating levels are highly correlated with BMI [108]. Leptin receptors have been identified both in the hypothalamus and in extrahypothalamic tissues and its main role is to inform the brain regarding the amount of stored fat, thus primarily regulating food intake and energy expenditure [109]; however, in obese humans increased leptin levels are unable to induce weight loss: this phenomenon is attributed to a selective resistance to its metabolic actions. Leptin, which has a structural and functional relation to proinflammatory cytokines such as IL-6, also influences angiogenesis, inflammation, arterial blood pressure and secretion of other adipokines [110]. In animal models, chronic hyperleptinemia is involved in oxidative stress by
decreasing plasma levels of the antioxidant enzyme paraoxonase-1, an activity linked to circulating lipoproteins [111]. This leptin effect is followed by increased plasma and urinary concentration of isoprostanes reflecting an increased oxidative stress [111]. Evidences for an involvement of leptin in atherosclerosis have been recently provided by direct leptin administration in apolipoprotein deficient mice and by the finding that ob/ob mice which lacked functioning leptin gene are resistant to atherosclerosis despite the presence of obesity and diabetes [112]. Also in humans, the vascular actions of leptin are considered proatherogenic and the increase of its circulating levels due to adiposity has been involved in the pathogenesis of vascular damage [113]. At present, clinical investigations considered leptin as an independent risk factor for atherothrombotic diseases, evidencing that its plasma concentrations are independently associated with the IMT of the common carotid artery and with the degree of coronary artery calcification in patients with type 2 diabetes mellitus, after controlling for adiposity and CRP [114].

The pro-thrombotic actions of leptin in vivo are related to an influence on platelet function, and on coagulation/fibrinolysis balance, resulting in enhanced agonist-induced platelet aggregation and increased stability of arterial thrombi [115]. The long form of the leptin receptor (Ob-Rb) is present in human platelets and can be related to platelet activation by a specific pathway downstream of leptin-induced Janus kinase 2 (JAK2) activation including PI3-K and phospholipases Cγ2 and A2, which influence cAMP hydrolysis, GPIIb/IIIa expression, and thromboxane synthesis [115]. These findings suggest that circulating platelets as a major target of leptin action and a possible direct link between obesity and thrombotic complications [115]. Studies in vitro showed that leptin synergizes with sub-threshold concentrations of agonists (such as ADP and thrombin), to induce platelet aggregation [116], but is unable to directly aggregate platelets. The involvement of leptin in the increased platelet activation in human obesity is not universally accepted, since recent studies provided conflicting results about platelet responsiveness to leptin in overweight and obesity [117]. Normal weight subjects undergoing complete caloric deprivation have an increased sensitivity of hemostatic responses to leptin [118]: this phenomenon indicates that the sensitivity of leptin receptors on platelet membranes is influenced by body composition [118]. In light of this, the resistance to leptin in overweight or obese patients could represent a protective mechanism against the excess pro-thrombotic stimulation produced by obesity-related hyperleptinemia [119]. However, other mechanisms by which leptin may contribute to vascular damage, such as inflammation, oxidative stress, EC dysfunction, as well as increased sympathetic tone, are preserved in obese subjects and can contribute to pro-thrombotic action of leptin.
IV.d.4. Adiponectin

This is a 30-kDa protein collagen-like molecule that shares substantial homology with subunits of complement factor C1q [110]. It is expressed almost exclusively in mature adipocytes where is the most abundant adipokine synthesized and released locally and in the blood stream. It accounts for 0.01% of the total plasma proteins in form of trimer, hexamer and high molecular weight 12-to 18-mers [110]. As opposite to the other adipokines, circulating adiponectin is negatively related to the increase of fat mass likely owing to the abnormal hormonal milieu mainly caused by the inhibitory effects exerted by the increased local TNF-α levels, by the oxidative stress and by the proinflammatory state which prevail in central obesity [110]; its secretion is restored as a consequence of weight loss. Adiponectin exerts insulin-sensitizing effects by increasing glucose uptake, NO production, and free fatty acid oxidation [120] and shows an antiinflammatory activity mainly through a cAMP-mediated interference with NF-κB signaling. In vascular wall, the antiinflammatory properties of adiponectin, which account for its anti-atherogenic effects, reduce cell expression of adhesion molecules and scavenger receptors. This phenomenon is mediated by the inhibition of the effects of TNF-α and angiotensin II on both ECs (expression of adhesion molecules, protection, increase of permeability, production of ROS) and macrophages (decrease of cytokine production mediated by NF-κB signaling) [120]. The vasculo-protective effects of adiponectin have been recently confirmed in clinical studies, by showing that its decreased levels contribute to the metabolic and vascular abnormalities in obese subjects [120].

In animals, adiponectin plays a role as antithrombotic factor [121]. Actually, there is an accelerated thrombus formation after carotid arterial injury in adiponectin knockout mice in comparison to wild-type ones: the potential involvement of platelets in this effect is suggested by the presence of active adiponectin receptors AdipoR1 and AdipoR2 in wild-type mice and by the enhanced platelet response to ADP and collagen in adiponectin knockout animals [121]. The same receptors are present in isolated human platelets and in human megakaryocytic cell lines; in humans however, adiponectin does not influence platelet activation by ADP and collagen. It has been hypothesized that adiponectin exerts indirect anti-thrombotic effects by decreasing the circulating concentrations of TNF-α and IL-6 and by interfering with their pro-thrombotic activities, mainly dependent on increased oxidative stress and decreased NO bioavailability [75].

IV.d.5. Resistin

It is represented by a 12.5 kDa cysteine-rich protein of 108 amino acids [122]. It is produced by adipose tissue and may act both in paracrine and in
endocrine fashion [122]: however, only a low level of expression has been found in mature adipocytes in humans. Furthermore, resistin expression has been demonstrated in bone marrow, trophoblastic cells of placenta, pancreas, synovial tissue, and circulating blood cells [123]. Resistin is an important regulator of glucose homeostasis, adipogenesis, and, potentially, inflammation [123]; in particular, it can induce insulin resistance by regulating adipose tissue deposition through a negative feedback mechanism [123], and exerts proinflammatory effects through activation of NF-κβ. The interplay between resistin and vascular wall cells can potentially contribute to the development of atherosclerotic lesions; in particular, it favors angiogenesis by inducing EC growth activation and migration, mainly by increasing ET-1 release [124], and potentiating the effect of CD40L; furthermore, it is involved in lipid storage in macrophages [123]. Resistin induces proliferation of cultured human aortic VSMCs through both ERK 1/2 and Akt signaling pathways [125]. Furthermore, hypoxia increases resistin expression in cultured rat VSMCs [125].

IV.d.6. Endothelin

Increased circulating levels of ET-1 have been observed in patients affected by central obesity and metabolic syndrome. ET-1 elevation is proportional to hyperinsulinemia and weight loss by diet intervention reduces both serum insulin and ET-1 [126]. The increase of ET-1 accounts for a prevailing vasoconstrictive effect of insulin in insulin resistant states, in which the insulin-induced, PI3-K-mediated increase of NO is impaired [127]; furthermore, ET-1 contributes per se to vasoconstriction by influencing calcium fluxes, by activating the renin-angiotensin system, and by inducing VSMC hypertrophy [128]. Platelets are a potential target of circulating ET-1. However, as recently reviewed, ET-1 effects on platelets are still conflicting: some studies showed that in vitro exposure to ET-1 induces platelet activation or increases platelet responses to aggregating agonists [128], other studies, however, failed to detect any direct effect or even showed a decrease in platelet responses [129]. These conflicting results may be due to complex interactions between platelet ET(A) and ET(B) receptors. On the basis of the large number of reviewed studies, a relevant role can be recognized to impairment of platelet and VSMCs functions in the pro-thrombotic tendency, proinflammatory state and accelerated atherogenesis.

V. Autoimmune diseases and atherosclerosis

Atherosclerosis shares many similarities with other chronic autoimmune diseases such as SLE, rheumatoid arthritis (RA), APS, vasculitis and type I
diabetes mellitus. They all have evidence of activation of macrophages, lymphocytes and ECs; alteration in the Th1/Th2 ratio; and elevation of inflammatory cytokines. Vascular inflammation in autoimmune diseases may cause LDL oxidation and interaction of ox-LDL with various plasma proteins such as β2-glycoprotein-I. These events may favor autoantibody production and accelerate arterial thrombosis. Patients with SLE have 5 to 6 times more coronary events than the normal population. In young women, the risk can increase to 50-fold [130]. A large case-control study of non-hospitalized SLE patients with no signs of renal failure showed that the presence of plaques was much more common among SLE patients than among controls. A recent case-control study indicated that coronary artery calcification was also more frequent in patients with SLE than in controls [131]. Anti-ox-LDL antibody titres associate with disease activity in SLE. Increased atherosclerosis was also found in lupus mice. SLE-susceptible LDLr-/ mice develop atherosclerosis under moderate dyslipidemia and overt accumulation of atherogenic lipoproteins can enhance SLE disease [132].

RA also associates with accelerated vascular risk, resulting in early mortality and excess morbidity. RA patients can have up to double the risk of an atherothrombotic event irrespective of the traditional atherothrombotic risk factors. Many of the same cells that comprise the inflammatory infiltrates in an RA joint lining are likewise found in the atherosclerotic plaques. Both aberrant cellular and humoral immune responses are integral to the pathogenesis of the two conditions. The immune de-regulation that characterizes RA seems to have a key role in the accelerated form of atherosclerosis present in this autoimmune disease [133]. Examination of carotid IMT shows greater values in RA patients than in controls. A recent study also showed an association between ox-LDL autoantibodies and atherothrombotic disease in RA [134].

One in three APS patients develops arterial thrombosis (e.g., acute myocardial infarction, stroke and angina) during the disease evolution. β2-glycoprotein-I appears to constitute the major antigenic target for anti-phospholipid antibodies and plays a central role in the development of clinical complications of APS. Vasculitis is a chronic inflammatory disease of the large (aorta, coronary, pulmonary and cervical arteries), medium-sized (subclavian, mesenteric, iliac and temporal arteries) and small (skin, intestine, kidney, respiratory tract) vessels. Like atherosclerotic lesions, vasculitic lesions also contain T cells, monocytes, DCs and mast cells. These pro-inflammatory cells are critical to the pathogenesis. For examples, depletion of DCs abrogates large-vessel vasculitis whereas stimulation of these cells in normal temporal arteries induces vasculitis [135]. Similar to atherosclerotic patients, those with large-vessel vasculitis show high lesion Th1 cell contents while defect in Treg and their serum MMPs levels often increase, but anti-inflammatory IL-10
Inflammation and atherosclerosis decreased compared to control subjects [136]. Like other autoimmune diseases, vasculitis often associates with increased IgG autoantibodies against ox-LDL, HSP and β2-glycoprotein-I [137]. Besides those inflammatory signatures, vasculitic patients demonstrate EC injury, high carotid IMT, arterial stiffness or thoracic dilatation. Therefore, patients with vasculitis have high risk of developing atherosclerosis. Indeed, both populations share similar risk factors, such as diabetes, hypertension and high serum levels of C reactive protein (CRP) and IgG autoantibodies against ox-LDL, HSP and β2-glycoprotein-I.

VI. Atherosclerosis and inflammation biomarkers

Following on the ferment in the basic science laboratory regarding inflammation in atherosclerosis, we have now entered an era of clinical translation of inflammation biology to the clinic. The description of inflammatory pathways above identified several new potential therapeutic avenues. Many existing systemic anti-inflammatory strategies such as glucocorticoids, non-steroidal anti-inflammatory drugs, or anti-cytokine agents exert unwanted actions that render them less than ideal candidates for evaluation as long-term therapeutics for modulation of atherosclerosis. Of many promising more specific anti-inflammatory agents in development for atherosclerosis, none appear sufficiently validated for clinical use at present. In contrast, the use of inflammatory biomarkers to predict risk, to monitor treatments, and to guide therapy has shown substantial potential for clinical applicability.

The contemporary literature now contains numerous reports of the relationship between various biomarkers of inflammation and prospective cardiovascular risk, in apparently well individuals as well as in patients with coronary heart disease or heart failure. The clinical utility of a biomarker for risk prediction depends on practicability, ease, cost, and reproducibility of the measurement, and the ability to add to the predictability of existing biomarkers such as those incorporated in the Framingham algorithm. Many reviews have highlighted this fast moving field [138]. Among the many biomarkers of inflammation proposed for diagnostic use, myeloperoxidase, lipoprotein-associated phospholipase A2, pentraxin-3, cytokines such as IL-6, proteases such as MMP 9, and CRP measured by a highly sensitive assay have generated considerable attention. For a variety of reasons, CRP has emerged as a leading biomarker of inflammation for clinical application. In well individuals without acute infections or inflammatory diseases (e.g., RA), levels of high sensitivity CRP remain stable over long periods of time with a year-to-year and decade-to-decade variability comparable to that of cholesterol [139]. CRP has
considerable chemical stability, requires no special precautions for sampling, and has a relatively long half-life without the diurnal variation that plagues certain other biomarkers.

**VII. Conclusion**

Studies in mice and in humans show that inflammation drives all phases of atherosclerosis, including initiation, progression, and thrombotic complications of the lesion. Inflammation provides a common link between many risk factors for atherosclerosis and altered arterial biology. Emerging evidence from clinical trials supports the use of inflammatory status as a guide to therapy that can reduce cardiovascular events in apparently healthy people who would otherwise evade detection as standing to benefit from treatment. Indeed, the concept of inflammation in atherosclerosis has emerged from the realm of theory and laboratory investigation to assume a promising role as a useful tool in the clinic to aid the prevention and management of atherothrombotic disease.

**VIII. References**

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