Atherosclerosis

3

Michael Hristov and Christian Weber

Atherosclerosis as an enduring inflammatory disease of the arterial wall is characterised by an imbalanced lipid metabolism and a maladaptive immune response resulting in sub-endothelial lipoprotein retention and endothelial activation with migration of immune and smooth muscle cells to the inflamed intima. This leads to formation of atheromas with chronic vascular stenosis and tissue ischemia which is often complicated by acute atherothrombotic events such as myocardial infarction or stroke. Even today, atherosclerosis and its consequences remain the leading causes of mortality and morbidity in the industrialised nations.

3.1 Traditional Risk Factors and Pathogenesis of Plaque Formation

Atherosclerotic lesions develop preferentially in vascular segments with slowed or disturbed blood flow, e.g. arterial bifurcations and branching sites. This arterial environment in combination with traditional risk factors such as hypertension, smoking and diabetes or other pathogens (immune complexes, toxins, viruses, etc.) may favour non-denuding endothelial dysfunction or even denuding injury with exposure of matrix proteoglycans and platelet activation. Accordingly, this so-called "response-to-injury" hypothesis has provided a fundamental framework in the pathogenesis of atherosclerosis to address numerous questions about cellular interactions and the nature of inflammatory mediators they produce [1].

M. Hristov • C. Weber (✉)
Department of Vascular Medicine, Institute for Cardiovascular Prevention,
Ludwig-Maximilians-University (LMU), Pettenkoferstr. 9, Munich 80336, Germany
e-mail: michael.hristov@med.uni-muenchen.de; christian.weber@med.uni-muenchen.de

A. Berbari, G. Mancia (eds.), *Arterial Disorders:*
Definition, Clinical Manifestations, Mechanisms and Therapeutic Approaches,
DOI 10.1007/978-3-319-14556-3_3

Early atherosclerotic lesions (known as *fatty streaks*) do not affect the vascular lumen and consist predominantly of focal lipid deposition, T lymphocytes and monocytes that transform to macrophages and lipid-loaded foam cells [1–4]. Indeed, almost each leukocyte sub-population ranging from lymphocytes, monocytes and neutrophils to dendritic or mast cells can be found at varying numbers inside the lesion during different stages [4]. Epidemiological data have further acknowledged leukocytosis as independent risk factor and predictor of cardiovascular events [5].

The continuous cell infiltration, proliferation and necrosis with accumulation of cell debris and cholesterol crystals result in lesion growth, and the *fatty streaks* transform gradually into stenotic plaques. These fibrous plaques contain an acellular necrotic core that is covered by a fibrous cap of dense connective tissue consisting of collagen, intimal smooth muscle cells (SMCs) and macrophages [1–3]. The "shoulder" regions of the plaques are often infiltrated by T lymphocytes and mast cells, which release proteolytic enzymes and proinflammatory mediators that may facilitate plaque destabilisation [3, 6]. Moreover, because of increasing plaque volume, the overlaying endothelial monolayer may be stretched quite thin and becomes more permeable or can even rupture.

Advanced stable fibroatheromas are covered by a dense, tension-resistant fibrous cap and contain few inflammatory cells [6, 7]. These plaques typically associate with chronic tissue ischemia that can be partially compensated by the growth of collateral vessels. In contrast, vulnerable plaques have a thin fibrous cap with more inflammatory cells than SMCs next to relative large and "soft" necrotic core. These plaques are more prone to erosion or rupture with subsequent occlusive atherothrombotic complications.

Dyslipidaemia with elevated circulating low-density lipoprotein (LDL) levels is another cardinal risk factor for the development and progression of atherosclerotic plaques. Due to increased endothelial permeability at predilection sites, LDL is easily embedded in the sub-endothelial space [2]. A variety of biochemical modifications occur there and enhance its pro-atherogenic action [2, 8]. Most important is the oxidation of LDL by reactive oxygen species or enzymes such as myeloperoxidase or lipoxygenases released from surrounding inflammatory cells [3]. Products of oxidised LDL (oxLDL) are chemotactic for monocytes but cytotoxic to endothelial cells. OxLDL is taken up at enhanced rates by macrophages, thus contributing to foam cell formation. Moreover, oxLDL can inhibit macrophage egress from the lesion but induce expression of adhesion molecules or deposition of chemokines on activated endothelial cells which further accelerates the recruitment of leukocytes. This accelerated leukocyte recruitment in conjunction with insufficient egress drives a continuous, oxLDL-mediated cell overload and lesion growth.

To complement the above traditional risk factors, a search for genetic variants linked with coronary artery disease (CAD) has flourished. Several genome-wide association studies (GWASs) have created an enormous wealth of data on susceptibility loci. The characterisation of these CAD-associated loci (9p21.3, 10q11.21, etc.) should provide new insight in the molecular mechanisms driving atherosclerosis and its outcomes and should open new fields for cardiovascular research [9].

3.2 Inflammatory Mediators in Atherogenic Cell Recruitment

Atherogenic recruitment of leukocytes involves rolling, firm adhesion, migration and trans-endothelial diapedesis [10]. This sequential cascade is controlled by chemokines, which are chemotactic cytokines divided into four groups according to their conserved cysteines and corresponding G protein-coupled receptors: C, CC, CXC and CX_3C [11, 12]. Considering the diversity of various leukocytes recruited during the atherogenic process, the abundance in the chemokine system has been perceived to confer robustness and specificity [13]. At a site of inflammation, particular leukocyte subsets may be recruited by a signature combination of chemokines engaging in heterophilic interactions, facilitated by a local repertoire of proteoglycans with differential binding affinities for these chemokines [4, 12, 13]. Soluble chemokines mediate direct leukocyte recruitment while surface-immobilised chemokines on activated endothelial cells trigger leukocyte arrest and activation of integrins. Numerous studies using antagonists or gene-deficient mice for important chemokines and/or their respective receptors have generated valuable insights into receptor-ligand axes with specific and combined contributions to atherogenesis [12]. Some of these crucial axes are CCR2-CCL2 (also known as monocyte chemotactic protein-1) and CCR5-CCL5 (also known as RANTES). Other chemokines and receptors appear rather involved in cell homeostasis and associate with reduced atheroprogression and plaque stabilisation, e.g. CXCL12 (also known as stromal cell-derived factor-1) and CXCR4/CXCR7, CX_3CL1 (also known as fractalkine) and CX_3CR1 or the dendritic cell chemokine CCL17 [12–14]. Finally, the combined action of chemokines such as the CCL5-CXCL4 (also known as platelet factor-4) heteromer can result in synergistic effects during atherogenic leukocyte recruitment [15].

Advanced human atherosclerotic lesions show an up-regulation of macrophage migration inhibitory factor (MIF) in macrophages and endothelial cells, which express MIF also in response to oxLDL [16]. Multiple mouse studies have confirmed the atheroprogressive function of MIF, which is probably linked to its versatile proinflammatory properties at crucial checkpoints in atherogenesis [3]. Macrophages can secret further proinflammatory mediators with implication in atherogenesis such as interleukin-1-beta (IL-1β) and tumour necrosis factor-alpha (TNF-α) [2, 6]. They are another robust source of platelet-derived growth factor (PDGF), which is one of the more important candidates coordinating the proliferative response of intimal SMCs [17].

Type I interferons (IFNs) have been implicated in atherogenesis as other important myeloid effector cytokines. Hence, treatment of atherosclerosis-prone mice with IFN-β increases lesion size, macrophage content and CCL5 plasma levels, whereas myeloid deficiency of the receptor for type I IFNs (IFNAR1) was associated with stable plaques [18]. Signalling through IFN-β-IFNAR1 interaction triggers CCL5 production by macrophages, thus increasing the CCR5-mediated monocyte recruitment [18]. This explains a correlation between up-regulated type I IFN signalling and CCL5 expression in advanced human lesions [3, 18].

Co-stimulatory and co-inhibitory signalling molecules fine-tune immune reactions by regulating inflammatory phenotypes and responses of T lymphocytes and antigen-presenting cells. For the prominent co-stimulatory pair CD40-CD40L, both receptor and ligand are expressed by lesional cell types and highly up-regulated in advanced human plaques [3, 19]. Mouse studies have revealed a plaque-destabilising function for CD40-CD40L signalling, attributable to the induction of multiple inflammatory factors [19]. Notably, repeated injection of CD40L-deficient platelets specifically implicated platelet CD40L in atherogenesis by mediating proinflammatory leukocyte-platelet interactions and regulatory T-cell homeostasis without compromising systemic immune responses or inducing haemorrhage [20].

3.3 The Role of Scavenger and Toll-Like Receptors (TLRs)

Although the major uptake of modified LDL in macrophages is mediated by scavenger receptor A (SR-A) and CD36, the genetic deletion of these receptors has yielded varying effects on experimental atherosclerosis [3, 21]. Combined deficiency of SR-A and CD36 in *Apoe−/−* mice has implicated both receptors in atheroprogression by inducing proinflammatory gene expression and macrophage apoptosis rather than foam cell formation [22]. TLR4 and TLR6 cooperate with CD36 in this inflammatory response to atherogenic lipids: oxLDL sequestered by CD36 induces intracellular CD36-TLR4-TLR6 heteromerisation, thus activating the transcription factor NF-κB and the secretion of chemokines [23]. Accordingly, a sustained oxidative burst and apoptosis elicited by oxLDL with continuous stress of the endoplasmic reticulum through combined signalling of CD36 with TLR2-TLR6 may similarly occur in lesional macrophages with intracellular accumulation of free cholesterol [24, 25].

3.4 Activated Platelets

Chronic inflammation associates with elevated platelet counts and platelet activation. Similar to leukocytosis, the thrombocytosis has been considered as risk factor for atherosclerosis. Platelets are activated by shear flow, oxLDL or hyperglycaemia. Upon activation they release membrane microparticles next to a broad arsenal of soluble components with relevance not only to coagulation and wound healing but also to immune response [26, 27]. The intravenous injection of activated platelets leads to a rapid progression of atherosclerotic lesions in mouse models that can be explained by a transfer of inflammatory chemokines such as CXCL4 and CCL5 [28]. As mentioned above, both chemokines may form a heteromer with synergistic effect on monocyte recruitment [15]. Disrupting this interaction by a designed synthetic peptide in vivo resulted in decreased atherosclerotic lesion formation, and the genetic deficiency of CCL5 and CXCL4 has been found to reduce plaque formation

in mice [15]. Furthermore, the P-selectin-mediated adhesion of platelets to monocytes under coronary flow conditions results in circulating complexes with increased adhesion to endothelial cells and immigration into the arterial wall [29]. These monocyte-platelet aggregates are increased in patients with CAD end especially after myocardial infarction [30].

3.5 Concluding Remarks and Perspectives for Prevention and Therapy

The currently available non-invasive therapy against atherosclerosis and especially CAD is usually restricted to controlling body weight, blood pressure, glycaemia, LDL cholesterol and triglyceride levels as well as platelet aggregation to prevent advanced thrombotic complications. Patients but also healthy individuals are further encouraged to follow general lifestyle recommendations including physical activity, smoking cessation and balanced diet. So far, the positive dietary effects of cold-water oily fish, red wine and olive oil consumption on CAD have been well documented [31, 32].

However, these established strategies do not fully address the inflammatory and immune mechanisms driving atheroprogression. Thus, emerging and future concepts in the treatment of atherosclerosis are urgently required. In this regard, several lessons from experimental animal models could help to develop novel, more powerful therapeutics such as inhibitors of chemokine heteromerisation, receptor antagonists, artificial lipoprotein complexes or even vaccination [13, 33–35]. Such a pioneering drug development requires stringent evaluation of effectiveness which has to rely on surrogate markers. Although a variety of cellular, soluble, imaging or functional biomarkers are available, their predictability remains partially limited. Hence, new biomarkers should be developed to complement existing ones and multiple biomarker panels should be combined in an integrated approach with higher predictive potential. Associated GWASs and transcriptional, proteomic or metabolic profiling may facilitate this search.

Moreover, there is an urgent need for improved imaging techniques, as current modalities (intravascular ultrasound, carotid intima-media thickness, quantitative coronary angiography and computed tomography) are often invasive and provide only quantitative but incomplete morphological data of atherosclerotic lesions. As cellular and molecular composition better reflects plaque pathology compared to size, highly advanced imaging techniques, such as high-resolution magnetic resonance and positron-emission tomography, will be required. The development of new molecular imaging with specific biological functions could add another dimension to plaque imaging, including monitoring the activation of endothelial cells and macrophages. If successful, this will be of tremendous value for drug development and testing efficacy, allowing detailed mechanistic insights into therapeutic effects at early stages, as well as imaging-based clinical outcome studies.

References

1. Ross R (1999) Atherosclerosis-an inflammatory disease. N Engl J Med 340:115–126
2. Hansson GK (2005) Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 352:1685–1695
3. Weber C, Noels H (2011) Atherosclerosis: current pathogenesis and therapeutic options. Nat Med 17:1410–1422
4. Weber C, Zernecke A, Libby P (2008) The multifaceted contributions of leukocyte subsets to atherosclerosis: lessons from mouse models. Nat Rev Immunol 8:802–815
5. Madjid M, Awan I, Willerson JT et al (2004) Leukocyte count and coronary heart disease: implications for risk assessment. J Am Coll Cardiol 44:1945–1956
6. Hansson GK, Libby P (2006) The immune response in atherosclerosis: a double-edged sword. Nat Rev Immunol 6:508–519
7. Libby P, Aikawa M (2002) Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. Nat Med 8:1257–1262
8. Witztum JL, Berliner JA (1998) Oxidized phospholipids and isoprostanes in atherosclerosis. Curr Opin Lipidol 9:441–448
9. Schunkert H, König IR, Kathiresan S et al (2011) Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet 43:333–338
10. Weber C, Fraemohs L, Dejana E (2007) The role of junctional adhesion molecules in vascular inflammation. Nat Rev Immunol 7:467–477
11. Luster AD (1998) Chemokines–chemotactic cytokines that mediate inflammation. N Engl J Med 338:436–445
12. Zernecke A, Weber C (2014) Chemokines in atherosclerosis: proceedings resumed. Arterioscler Thromb Vasc Biol 34:742–750
13. Koenen RR, Weber C (2010) Therapeutic targeting of chemokine interactions in atherosclerosis. Nat Rev Drug Discov 9:141–153
14. Weber C, Meiler S, Döring Y et al (2011) CCL17-expressing dendritic cells drive atherosclerosis by restraining regulatory T cell homeostasis in mice. J Clin Invest 121:2898–2910
15. Koenen RR, von Hundelshausen P, Nesmelova IV et al (2009) Disrupting functional interactions between platelet chemokines inhibits atherosclerosis in hyperlipidemic mice. Nat Med 15:97–103
16. Bernhagen J, Krohn R, Lue H et al (2007) MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. Nat Med 13:587–596
17. Doran AC, Meller N, McNamara CA (2008) Role of smooth muscle cells in the initiation and early progression of atherosclerosis. Arterioscler Thromb Vasc Biol 28:812–819
18. Goossens P, Gijbels MJ, Zernecke A et al (2010) Myeloid type I interferon signaling promotes atherosclerosis by stimulating macrophage recruitment to lesions. Cell Metab 12:142–153
19. Lievens D, Eijgelaar WJ, Biessen EA et al (2009) The multi-functionality of CD40L and its receptor CD40 in atherosclerosis. Thromb Haemost 102:206–214
20. Lievens D, Zernecke A, Seijkens T et al (2010) Platelet CD40L mediates thrombotic and inflammatory processes in atherosclerosis. Blood 116:4317–4327
21. Greaves DR, Gordon S (2009) The macrophage scavenger receptor at 30 years of age: current knowledge and future challenges. J Lipid Res 50:S282–S286
22. Manning-Tobin JJ, Moore KJ, Seimon TA et al (2009) Loss of SR-A and CD36 activity reduces atherosclerotic lesion complexity without abrogating foam cell formation in hyperlipidemic mice. Arterioscler Thromb Vasc Biol 29:19–26
23. Stewart CR, Stuart LM, Wilkinson K et al (2010) CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. Nat Immunol 11:155–161
24. Seimon TA, Nadolski MJ, Liao X et al (2010) Atherogenic lipids and lipoproteins trigger CD36-TLR2-dependent apoptosis in macrophages undergoing endoplasmic reticulum stress. Cell Metab 12:467–482

25. Tabas I (2010) Macrophage death and defective inflammation resolution in atherosclerosis. Nat Rev Immunol 10:36–46
26. Weber C (2005) Platelets and chemokines in atherosclerosis: partners in crime. Circ Res 96:612–616
27. von Hundelshausen P, Weber C (2007) Platelets as immune cells: bridging inflammation and cardiovascular disease. Circ Res 100:27–40
28. Huo Y, Schober A, Forlow SB et al (2003) Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. Nat Med 9:61–67
29. Freedman JE, Loscalzo J (2002) Platelet-monocyte aggregates: bridging thrombosis and inflammation. Circulation 105:2130–2132
30. Tapp LD, Shantsila E, Wrigley BJ et al (2012) The CD14++CD16+ monocyte subset and monocyte-platelet interactions in patients with ST-elevation myocardial infarction. J Thromb Haemost 10:1231–1241
31. Saravanan P, Davidson NC, Schmidt EB et al (2010) Cardiovascular effects of marine omega-3 fatty acids. Lancet 376:540–550
32. Hernáez Á, Fernández-Castillejo S, Farràs M et al (2014) Olive oil polyphenols enhance high-density lipoprotein function in humans: a randomized controlled trial. Arterioscler Thromb Vasc Biol 34:2115–2119
33. Zhao Q (2010) Dual targeting of CCR2 and CCR5: therapeutic potential for immunologic and cardiovascular diseases. J Leukoc Biol 88:41–55
34. Nissen SE, Tsunoda T, Tuzcu EM et al (2003) Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. JAMA 290:2292–2300
35. Nilsson J, Hansson GK, Shah PK (2005) Immunomodulation of atherosclerosis: implications for vaccine development. Arterioscler Thromb Vasc Biol 25:18–28