1. Introduction

The term 'atherosclerosis' is generally employed for a disease process characterized by the formation of atheromas (fibrofatty intimal plaques) resulting in progressive hardening of the arterial wall, loss of vascular elasticity, and luminal compromise. Different segments of the vascular tree vary in their susceptibility to develop atherosclerotic lesions: atheromas have been described characteristically in middle- and large-sized muscular and elastic arteries. Aorta and iliac, coronary and cerebral arteries are considered the prime targets, and so peripheral artery disease, myocardial infarcts, and cerebral infarcts are the most prominent clinical manifestations of the disease. There does not exist one definite 'microcirculatory manifestation' of atherosclerosis. Rather, there is an array of different involvements of the microcirculation in distinct processes with relevance to atherosclerosis (Fig. 1). For instance, the microcirculation shows functional alterations that are not necessarily associated with morphological changes of the microcirculatory network per se. This microcirculatory disturbance has been termed 'downstream dysregulation'. Besides diabetes and hypertension (resulting in various microvascular changes including hyaline arteriolosclerosis; please see respective articles by Hutchins et al. and Tooke et al., in this issue), the microcirculation may be affected by local or systemic pathophysiological challenges such as ischemia/reperfusion injury or the immunologic responses during acute and/or chronic allograft rejection of transplanted organs: transplant-associated accelerated allograft atherosclerosis. One section of this article will cover the microcirculation as it becomes relevant in vaso vasorum of complicated atherosclerotic lesions of larger vessels. Finally, this review will address the microcirculation as a research tool to study cell–cell interaction and other pathophysiologically relevant features of atherogenesis.

Arteriosclerosis is considered to be a disease of multifactorial etiology and incompletely understood pathophysiology. Several concepts have been put forward, trying to combine as many aspects as possible into unifying hypotheses. Based on the early proposals made by Virchow in 1847 [1], Ross and Glomset [2] formulated the 'response to injury' hypothesis of atherogenesis, according to which the sequelae of events originate from an injury to endothelial cells (e.g., mechanical, chemical, toxic, viral), leading to denudation or endothelial cell dysfunction. Without going into the details of this hypothesis—which is summarized in a recent update by Ross [3]—the general message of relevance for the understanding of the microcirculatory changes associated with atherosclerosis is the concept of a balance between damaging mechanisms affecting the vessel wall, on the one hand, and the protective defense mechanisms intrinsic to the vessel wall and/or the components of the blood, on the other hand. This concept covers both functional 'regulatory' mechanisms (such as the vasodilator/vasoconstrictor responses) and also morphological aspects (such as oxidative attacks on membrane lipids, leading to impaired endothelial cell homeostasis, breakdown of endothelial integrity, leakage of fluid and macromolecules, deposition of fat and extracellular matrix, etc.).
2. Downstream dysregulation

Clinical tests and imaging techniques have demonstrated that the microcirculation is functionally affected in patients with atherosclerosis of larger arteries. For instance, laser Doppler fluxmetry on toe pulp and leg skin has revealed marked retardation and complete loss of vasodilator response during postischemic reactive hyperemia in patients with lower limb atherosclerosis, contributing to intermittent claudication or critical ischemia [4]. Similar observations have been made in hypertriglyceridemic patients, in which postischemic reactive hyperemia is significantly impaired [5]. Of particular interest is the fact that these changes are entirely reversible by dietary or pharmacological correction of the hypertriglyceridemic state [5]. Others have performed experiments with various endothelium-dependent and endothelium-independent vasodilators and have suggested that endothelium-dependent vascular relaxation is abnormal in the coronary microcirculation of atherosclerotic animals [6,7]. These findings have been extended to the coronary microcirculation of patients, suggesting that the functional consequences of atherosclerosis extend into the microcirculation, well beyond the overt morphological lesions seen in the larger coronary vessels. In atherosclerotic Cynomolgus monkeys, Chilian et al. have demonstrated a significantly augmented vasoconstrictor response to serotonin, not only in atherosclerotic affected coronary arteries, but also in morphologically unaffected downstream microvessels [8]. This finding is of particular importance since the myocardial perfusion is regulated predominantly by resistance arterioles with a diameter of less than 200 μm. Several explanations have been put forward to interpret these observations: inhibition of the formation of vasodilatory prostacyclin from endothelial cells in atherosclerotic plaques [9] or inhibition of endothelium-derived vasodilation [6,7]. This latter concept was further supported by experiments in which the abnormal physiological and pharmacological response in arterioles of atherosclerotic pigs could be completely restored by the administration of L-arginine, the precursor of nitric oxide (= endothelial-derived relaxing factor) [10]. These findings have recently been extended to studies in patients with early atherosclerosis [11]. Using single photon emission computed tomographic imaging studies on patients without hemodynamically significant epicardial coronary artery lesions (not associated with angina pectoris), these authors could demonstrate that exercise-induced myocardial ischemia was associated with impaired endothelium-dependent vasodilation of the coronary microcirculation [11]. In agreement with the previously mentioned improvement of the postischemic hyperemic response by lipid-lowering agents [5], the reduced vasodilator response in the myocardial microcirculation of hypercholesterolemic patients could be restored by lipid-lowering therapy [12]. These and earlier studies [13,14] have attributed the impaired vasodilator response of microvessels in atherosclerotic subjects to the prevailing high levels of circulating lipid and cholesterol. Direct effects of hypercholesterolemia on the acetylcholine receptor or its pertussis-sensitive signal transduction pathway have been proposed to explain the loss of endothelial function in hypercholesterolemic patients [15]. Later, Kugiyama et al. showed that oxidatively modified low-density lipoprotein (LDL), but not native LDL, inhibits endothelium-dependent arterial...
relaxation [16]. In agreement with the presumption that oxidative inactivation of nitric oxide may contribute to the microcirculatory dysfunction in atherosclerosis [12]. Xiu et al. have shown that the antioxidant, butylated hydroxytoluene, effectively reverses the microcirculatory dysfunction induced by hypercholesterolemia. In particular, the antioxidant treatment restored the microvessel diameter of third-order arterioles without affecting blood lipids per se [17].

3. Transplantation-associated accelerated allograft atherosclerosis

Accelerated allograft atherosclerosis (AAA) was first described in an orthotopic cardiac transplant model in dogs and later in humans [18]. Accelerated allograft atherosclerosis affects the major epicardial vessels along their entire length, and—unlike 'classical' atherosclerosis—includes small epicardial branches and even the intramyocardial microvessels (less than 200 µm in diameter). The fact that the microcirculation is not spared as in 'classical' atherogenesis precludes coronary artery bypass grafting or other interventional procedures. Because small intramyocardial branches of the coronary tree are affected, they are often occluded before the luminal occlusion of major epicardial vessels, and this results in multiple stellate infarcts. Sudden death, therefore, is not uncommon in cardiac transplant recipients. While AAA has been detected angiographically in over 90% of cardiac allograft recipients at 5 years, it may cause death as early as a few months after transplantation. In fact, AAA is the number one cause of death in cardiac allograft recipients after the first few months post-transplantation. AAA is not confined to cardiac transplantation. In liver transplantation, up to 50% of graft failures are ascribed to AAA with features of arteriolar and bile duct loss [19], and also in renal transplantation, AAA accounts for the majority of late graft losses [20]. An important feature of AAA is the selective involvement of the engrafted organ's arteries, but sparing the host's own vessels. An immune-mediated mechanism has been proposed to account for this peculiar localization of AAA. Evidence suggesting that AAA is immune-mediated has been derived from the presence of T-cell infiltrates in the affected vessels [21], and from the apparent association of AAA with recurrent rejection episodes. It has been proposed that the development of AAA may represent the response of the cells within the donor arteries to stimuli released by a chronic, localized form of delayed hypersensitivity to alloantigens on graft-derived vessel wall cells [22]. However, while improvement in the immunosuppressive treatment regime of transplant patients has significantly improved 1-year graft survival, it has utterly failed to substantially reduce the incidence and severity of AAA and the loss of allografts after the first year of transplantation [23]. Indeed, an increase in incidence and severity of AAA has been described since the introduction of cyclosporine [20]. This suggests that factors other than immune-mediated events contribute substantially to the development of AAA. Cytomegalovirus infection has been proposed [24], as well as the damage inflicted upon the allograft by ischemia and reperfusion during transplantation [25–27]. Leukotrienes, platelet-activating factor, oxygen radicals, and numerous cytokines are generated in organ allografts in response to cold storage and reperfusion by the recipient's blood. The tissue damage induced by reperfusion injury is characterized by accumulation and emigration of circulating leukocytes [28,29]. While in reperfusion injury and acute rejection, infiltrating leukocytes—either directly or through the release of enzymes, inflammatory mediators, and oxygen free radicals—contribute to the breakdown of endothelial integrity and microvascular perfusion, the release of leukocyte-derived mediators, growth factors, cytokines, and oxygen free radicals may contribute to peroxidative damage to endothelial cells and the proliferative response of various cell types within the vessel wall. The contribution of reperfusion injury to AAA has been dramatically illustrated in animal experiments [30,31], as well as in several clinical trials. In these studies, the severity of allograft injury in the immediate post-transplant period was significantly linked to the incidence of AAA and allograft loss in heart [32] and kidney [27] transplant recipients. Of particular interest in this respect is a clinical trial in kidney transplant recipients, in which long-term allograft survival was significantly improved in patients who had received only a single injection of superoxide dismutase immediately before reperfusion of the organ graft during surgery [26]. While the pathologic mechanisms underlying this correlation of ischemia/reperfusion injury and AAA are still incompletely understood, the implications for the clinical management of transplant patients are impressive and offer hope for a significant improvement in long-term outcome after organ transplantation.

4. Vaso vasorum

In normal arteries, vaso vasorum are present as an adventitial network which extends into the outer media of only the very largest vessels such as the aorta and the pulmonary arteries [33]. With the development of atherosclerosis, however, adventitial vessels proliferate and penetrate the media to form a microvascular plexus in the diseased intima [34,35]. A correlation has been established between the extent of the vascular network of vaso vasorum and the degree of intimal thickening. Vaso vasorum
originate from the adventitia, commonly from sites proximal to the atherosclerotic lesion, and in some instances from intramural branches or even from the lumen of the atherosclerotically affected vessel. Although some investigators have reported that oxygen consumption of atherosclerotic vessels exceeds that of their normal counterparts [36], it is unknown whether the oxygen demand could account for this marked increase in vaso vasorum. If vaso vasorum are required to sustain the viability of the plaque and the underlying media, the frequent occurrence of necrosis in advanced lesions may represent temporary ischemia, resulting from the inability of the plaque vaso vasorum to meet the metabolic requirements of the enlarging lesion. Current evidence suggests that isolated plaque fragments possess angiogenic activity per se. Alpem-Elran et al. demonstrated that fragments of human plaque tissue induced angiogenesis when implanted into rabbit corneas [37]. Boiled plaque was not angiogenic. Later studies have identified elevated levels of growth factors in atherosclerotic plaques, such as fibroblast growth factor [38], transforming growth factor beta [39], and tumor necrosis factor [40]. In particular, fibroblast growth factor was found concentrated around vaso vasorum, suggesting a potential role in angiogenesis [38]. Besides the strong positive correlation between intimal microvessel density and intimal thickening, immunohistochemical studies have documented that these microvessels leak plasma albumin into the artery wall, exudate fibrinogen, and are thus thought to contribute significantly to the development of advanced atherosclerotic plaques [41].

5. The microcirculation as a research tool for the study of cell-cell interactions and other pathophysiologically relevant features of atherogenesis: oxLDL-induced microcirculatory dysfunction

The ‘response to injury’ hypothesis of atherogenesis recognizes leukocyte adhesion and emigration as an important, early step in the formation of foam cells, fatty streak lesions, and atherosclerotic plaques [3]. Searching for leukocyte attractant and adhesion-promoting factors, Alderson et al. [42] and others [43] demonstrated that exposure of cultured endothelial cells to human LDL or to very-low-density lipoprotein (VLDL) from cholesterol-fed animals enhanced their adhesivity for monocytes. Froste-gard et al. later found that the adhesion-promoting effect of LDL on endothelial cells was markedly increased by prior in vitro oxidation of the LDL particles [44]. This finding is consistent with earlier observations that oxidation infers chemotactic activity to otherwise inactive lipids [45] and that oxidized LDL exerts direct chemotactic effects on monocytes [46]. Berliner and co-workers reported that minimally modified LDL—a form of oxLDL more likely to be found in human plasma or atherosclerotic lesions—induces mRNA for a murine equivalent to human monocyte chemoattractant protein-1 (MCP-1) [47] and significantly increases monocyte adhesion to cultured endothelial cells [48].

In order to provide in vivo evidence for the adhesion-promoting effect of oxLDL, we have exposed hamster to native and oxidized LDL and documented the effect on
leukocyte/endothelial cell interaction by intravital microscopy [49]. In these studies, we could demonstrate that intravenous injection of oxLDL, but not native LDL, elicited the rolling (not shown) and subsequent adhesion of circulating leukocytes to the microvascular endothelium, both of venules and of arterioles (20–60 μm in diameter: Figs. 2 and 3). Electron micrographs and differential leukocyte counts revealed that the adhesion-promoting effect of oxLDL actually affects all leukocyte subpopulations with no specific preference for monocytes [50]. Similar in vivo effects of oxLDL and minimally modified LDL have later been documented in a rat mesenteric model for intravital microscopy [51] (Fig. 2). These authors could also demonstrate that leukocyte adhesion in response to oxidized LDL was associated with increased permeability of fluorescently stained macromolecules across the endothelium, suggesting an impairment of the endothelial barrier function at these sites [51]. Parallel to the induction of leukocyte adhesion, we also observed the formation of leukocyte/platelet aggregates tumbling through the microvessels and engaging in firm adhesion to the endothelial lining [52] (Fig. 2). Finally, we used scanning electron microscopy to document that the adhesion-promoting effect of oxLDL was not confined to the microcirculation, but was also seen on the aortic endothelium, in particular in the vicinity of interepithelial junctions [53] (Fig. 2).

Searching for the mediator systems involved in oxLDL-induced leukocyte adhesion, experiments were performed in the same model to test whether inhibition of well-established mediators of leukocyte adhesion would interfere with leukocyte adhesion (summarized in Fig. 3). The involvement of leukotrienes was documented in experiments in which leukotriene biosynthesis was blocked pharmacologically (via inhibition of 5-lipoxygenase activity or translocation to its membrane-bound activator protein) or by dietary fish oil [50,54,55] (Fig. 3). The involvement of reactive oxygen species was evidenced in inhibition experiments using superoxide dismutase and vitamin C [53,56] (Fig. 3). The lipid-soluble antioxidants, vitamin E and probucol, failed to inhibit oxLDL-induced leukocyte adhesion, suggesting the involvement of water-soluble reactive oxygen species in oxLDL-induced leukocyte adhesion, rather than the sequela of lipid peroxidation [53] (Fig. 3). Blockade of the receptor for platelet-activating factor likewise significantly reduced leukocyte adhesion, and also the formation of platelet/leukocyte aggregates [57], suggesting the formation and involvement of platelet-activating factor, or of platelet-activating-factor-like lipids [58] in oxLDL-induced leukocyte adhesion. Neither the stimulation of leukocyte/endothelium interaction by oxLDL nor the attenuation of this phenomenon by the applied inhibitors could be ascribed to changes in

![Fig. 3. oxLDL-induced leukocyte adhesion to venular and arteriolar endothelium were assessed by intravital microscopy using the dorsal skinfold chamber preparation in hamsters [49]. The effect of oxLDL was compared to native LDL, the copper solution used for LDL oxidation, and normal saline (top row) [49,50]. These control experiments ruled out potential non-specific artifacts (i.e., species incompatibility of human LDL in the hamster, phototoxic effects of the fluorescent leukocyte marker). The second and third row show experiments performed to document the mediator role in oxLDL-induced leukocyte adhesion of leukotrienes and PAF/PAF-like lipids [50,54,55,57] and of water- but not lipid-soluble reactive oxygen species [53,56], respectively. The last row documents the involvement of adhesion receptors in oxLDL-induced leukocyte adhesion [52,59], and the inhibitory effect of pentoxifylline, which effectively downregulates the cell surface presentation of CD11b/CD18 [60]. The data in all panels are means ± s.d. of n = 7 animals per group, except for the anti-PECAM experiments, which were only repeated 3 times. A Wilcoxon rank sum test was applied to test for statistically significant differences between oxLDL and the various experimental conditions († P < 0.05,  †† P < 0.01 in venules, †'P < 0.05,  ††'P < 0.01 in arterioles, †'P < 0.05,  ††'P < 0.01 versus baseline leukocyte adhesion).
microhemodynamic parameters. Finally, we turned our attention to the adhesion receptors involved in the cell–cell interactions induced by oxLDL. As expected from findings in other pathophysiological conditions, blockade of P-selectin using a polyclonal antiseraum effectively prevented leukocyte adhesion and platelet aggregate formation in response to oxLDL [52] (Fig. 3). Since functionally blocking antibodies directed towards integrins were not available for use in hamsters, we used hairless mice to demonstrate that CD11b/CD18 adhesion receptors are likewise of crucial importance for oxLDL-induced leukocyte adhesion [59] (Fig. 3). In addition, the drug, pentoxifyllin, which effectively inhibits oxLDL-induced upregulation of CD11b/CD18 adhesion receptors on leukocytes [60], significantly inhibited oxLDL-induced leukocyte adhesion in hamsters, suggesting that these adhesion molecules are likewise involved in oxLDL-induced leukocyte adhesion in the hamster [60] (Fig. 3). CD11b/CD18 adhesion receptors are expressed solely on neutrophils and monocytes, but not on lymphocytes. The observation that anti-CD11b/CD18 antibodies inhibited the adhesion of all leukocyte subpopulations (Fig. 3) suggests a role for neutrophil/mastocyte adhesion (followed by activation, degranulation, release of mediators, etc.) in the recruitment of more leukocytes, including lymphocytes, to the endothelial lining. This concept would also be able to explain why oxLDL-induced leukocyte/endothelium interaction peaks at 15–30 min after intravenous injection while at this time point most of the injected oxLDL particles have been cleared form the circulation by endothelial cells in the liver [61]. Furthermore, the repeated observation that leukocytes tend to adhere in clusters over early atherosclerotic lesions suggests that (besides endothelial cells and smooth muscle cells) emigrated leukocytes and macrophages present in these lesions contribute to the chemotactic attraction, adhesion and emigration of further circulating leukocytes, thus entertaining a self-amplificatory circle of leukocyte recruitment.

All these experiments were performed using intravital microscopy on the skinfold chamber preparation. To document that these findings observed by intravital microscopy in the microcirculation represent a systemic event, we quantified the total circulating leukocyte count in the animals before and after injection of oxLDL: injection of oxLDL resulted in a significant drop in the total circulating leukocyte count as a result of leukocyte sequestration along the endothelial lining [50]. Indeed, inhibition of leukocyte adhesion with different inhibitor substances (superoxide dismutase, 5 lipoxygenase inhibitor, dietary fish oil, PAF-receptor antagonist) effectively prevented the drop in total circulating leukocyte count [54]. To document that the findings observed in the microcirculation also pertained to the endothelium of larger vessels, we used scanning electron microscopy and could show that vitamin C blocked leukocyte adhesion both in the microcirculation and on the aortic endothelium, while the lipid-soluble vitamin E or probucol did not affect leukocyte adhesion, either in the microcirculation or on the aortic endothelium.

6. Conclusion

Atherosclerosis is a disease that is generally thought to affect only middle-sized and large-caliber vessels. However, as this review has tried to demonstrate, the microcirculation is affected in different ways by atherogenesis. The microcirculation (i) bears a significant burden of the clinical manifestations of atherosclerotic lesions in larger vessels (downstream dysregulation), (ii) undergoes morphological changes itself during certain types of atherogenesis, such as transplantation-associated accelerated atherosclerosis, (iii) plays a crucial role as vaso vasorum, maintaining the increased metabolic demand of atherosclerotic modified vessel walls, and (iv) may serve as a valuable research tool for the study of pathomechanisms with relevance to atherogenesis in large-caliber vessels. In particular, the contribution of hypercholesterolemia and cell adhesion events in atherogenesis has lent itself to investigation at the microcirculatory level. Insights gained from such acute experiments (i.e., the inhibition of cell–cell interactions by pharmacological agents or simple dietary means, such as antioxidant vitamins) may not only help to understand phenomena that are otherwise difficult to model, but may also point toward novel treatment options that can then be tested in established chronic animal models of atherogenesis and eventually in clinical trials.

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