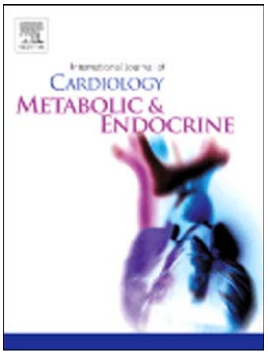

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Microvascular inflammation in atherosclerosis

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Abbreviations: ADMA, dimethylarginine; ECs, endothelial cells; ESAM, endothelial cell-selective adhesion molecule; ICAM-1, intercellular adhesion molecule 1; JAM, junction adhesion molecule; LDL, low density lipoprotein; MAdCAM-1, mucosal addressin cell adhesion molecule 1; NO, nitric oxide; NOS, NO synthase; PSGL-1, P-selectin glycoprotein ligand 1; ROS, reactive oxygen species; SOD, superoxide dismutase; V-CAM-1, vascular cell adhesion molecule 1; VSMC, vascular smooth muscle cell.

Abstract

Atherogenesis is the pathogenetic process leading to formation of the atheroma lesion. It is associated to a chronic inflammatory state initially stimulated by an aberrant accumulation of lipid molecules beyond the endothelial barrier. This event triggers a cascade of deleterious events mainly through immune cells stimulation with the consequent liberation of potent pro-inflammatory and tissue damaging mediators. The atherogenetic process implies marked modifications of endothelial cell functions and a radical change in the endothelial-leukocyte interaction pattern. Moreover, accumulating evidence shows an important link between microvascular and inflammatory responses and major cardiovascular risk factors. This review illustrates the current knowledge on the effects of obesity, hypercholesterolemia and diabetes on microcirculation; their pathophysiological implications will be discussed.

Anatomy of microcirculation

In most organs, microcirculation is composed of three anatomically and functionally distinct segments: arterioles, capillaries and venules. Arterioles are well innervated and anatomically characterized by endothelium surrounded by a smooth muscle cell containing wall and a diameter ranging from 10 to 100 μm. Typically, arterioles display a divergent branching pattern so that blood flows from one arteriole into two branches at each bifurcation. Arterioles are highly responsive to sympathetic vasoconstriction and represent a major player in the regulation of systemic vascular resistance. Because of these characteristics, the regulation of blood flow and ultimately oxygen delivery represent the primary function of arterioles. In addition arterioles, by participating to the regulation of capillary hydrostatic pressure, influence capillary fluid exchange [1, 2].

Blood flows from arterioles into capillaries, 5-10 μM calibre single endothelial cell layer vessels surrounded by basement membrane and devoid of smooth muscle cell wall and innervation. In some organs, however, a circular band of smooth muscle at the entrance of the capillary (precapillary sphincter) can regulate the number of perfused capillaries [1, 2].

Capillaries ensure large surface area and relatively high permeability to favour the exchange of fluids, gases, electrolytes and macromolecules. In different organs, the endothelial wall differs for its structural organization and permeability. In the liver, spleen and bone marrow gaps in both the endothelial layers and the basement membrane result in very high permeability of capillaries. Fenestrated capillaries are present in the intestinal mucosa, renal glomeruli and exocrine glands where endothelial cells display wide intercellular clefts surrounded by a continuous basement membrane. Continuous capillaries are found in the central nervous system, skin, muscle and the lung where intercellular gaps are tight and basement membrane is continuous. These capillaries display the lowest permeability [1-3].

Venules are small exchange vessels but with a larger caliber than the corresponding arterioles (10-50 μM caliber), resulting in a lower flow velocity and wall shear stress that promote leukocyte margination and facilitate adhesion to the vessel wall. Venules are composed of an endothelial cells layer surrounded by a basement membrane. Smooth muscle cells are present in larger venules but not in small postcapillary venules. The presence of smooth muscle and symphatetic

innervation in larger venules allows the regulation of venules tone and therefore capillary hydrostatic pressure [2, 3]. Due to their anatomical architecture resulting in relatively low permeability, fluid and macromolecules exchange occurs predominantly at venular junctions. Leukocyte margination is favoured in venules because of erythrocytes tendency to aggregate at low shear stress forces occurring in the central portion of venules. Erythrocyte aggregation in turn pushes leukocytes toward the vessel wall. In addition, the flow discharged by a capillary into a venule at site of confluent junctions is located to a region close to the venule wall thus promoting leukocytes to take contact with the endothelium. Finally, the most important factor determining the prevalence of leukocyte adhesion to venule walls during inflammation is the selective expression of adhesion molecules by venule but not arteriole or capillary endothelial cells [1, 2, 4].

Inflammatory state of microcirculation associated to atherogenesis.

Under normal conditions, vascular endothelial cells subserve several tasks that constitute the “endothelial function” including the regulation of blood flow, blood fluidity, vessel wall permeability and interactions with circulating leukocytes.

Blood flow fluidity is modulated through the regulation of the tone of surrounding vascular smooth muscle cells, that in turn, depends on the balance between vasoconstriction and vasorelaxant signals. Endothelial cells-dependent vasorelaxation of SMC is achieved through the production of nitric oxide. Nitric oxide is highly reactive (having a lifetime of a few seconds), yet diffuses freely across membranes. NO acts through the stimulation of the heterodimeric enzyme soluble guanylate cyclase, with subsequent formation of cyclic GMP. Cyclic GMP activates protein kinase G, which causes phosphorylation of myosin light chain phosphatase, and therefore inactivation of myosin light-chain kinase, causing smooth muscle relaxation through the dephosphorylation of the myosin light chain [5].

Endothelial cells actively inhibit blood coagulation ensuring blood fluidity through several mechanisms such as the expression of coagulation cascade inhibitors including heparane sulphate

proteoglycans, inhibitors of tissue factor pathways, and thrombomodulin. In addition ECs inhibit platelet activation by releasing NO and prostacyclin [6, 7].

In physiological conditions, plasmatic proteins are contained inside vascular lumen of continuous capillaries, the structure of junctions between adjacent ECs that include tight and adherens junctions. The permeability properties of capillaries depend on the structural organization and the presence of intercellular junction structures that varies considerably in different anatomical locations. Junctional structures are tighter in the capillaries of the central nervous system, in the liver and spleen sinusoidal capillaries and are characterized by discontinuous intercellular junctions that enable blood contact with underlying tissue. However, in most tissues, capillary ECs normally block extravasation of plasmatic proteins as small as albumin. On the other hand, they operate active transport of proteins from capillary lumen to tissue via vesicular transport [8].

In normal conditions, endothelial cells have rare interactions with circulating leukocytes, thus representing a barrier between tissues and inflammatory cells. In non-inflamed vasculature leukocyte-ECs, interaction is limited because of the very low constitutive expression of surface adhesion molecules such as, ICAM-1, VCAM-1 and E-selectin as well as the compartimentalization of P-selectin and chemokines into endothelial intracellular vescicles named Weibel-Palade bodies [2, 9]. In addition, resting ECs stimulated by shear stress express NO that inhibits proinflammatory genes transcription, release of Wiebel Palade bodies content and leukocyte activation (discussed below).

Atherosclerosis involves the formation of lesions in the arteries characterized by lipid accumulation, inflammation, cell death and fibrosis. These lesions known as atherosclerotic plaques grow and evolve over time with the consequent decrease of the vasal section. A limited and often insufficient blood flow accompanied by stenosis due to modification of blood vessel plasticity, leads to clinical complications. However, the most serious complications arise from lesions rupture and sudden exposure of thrombotic substances to circulating blood leading to abrupt formation of blood clots and occlusion. The atherogenic process involves three major steps: an early and persisting inflammatory component, a proliferative response and, ultimately, a mural thrombosis that is potentially responsible for vascular occlusion.

Although atherosclerotic lesions occur in large arteries, the upregulated expression of adhesion molecules characteristic of EC activation, the reduced endothelium-dependent vasodilatation as well as oxidative stress are not confined to lesion-prone arteries where factors other than EC activation (e.g. elevated shear stress) might act to determine atheroma formation. Atherosclerosis is associated with a systemic inflammatory state characterized by endothelial and blood cells activation as well as increased plasmatic concentration of inflammatory factors and endothelial dysfunction consisting in reduced endothelium-dependent vasodilation [10]. Parameters such as the circulating concentrations of proinflammatory factors or soluble isoforms of adhesion molecules have been proposed as biomarkers for the assessment of cardiovascular risk. Enhanced production of inflammatory mediators such as cytokines, chemokines and reactive oxygen species occur in the atherosclerotic lesions determining and sustaining local intramural inflammation. However, inflammatory mediators can also be released in the circulation determining systemic inflammation with the involvement of the microcirculation. Alternatively, cardiovascular risk factors as for example elevated blood cholesterol levels, hypertension, diabetes, obesity and cigarette smoke might directly stimulate microvascular endothelial cells activation with consequent release of inflammatory mediators and soluble isoforms of adhesion molecules, thus determining microvascular dysfunction and the atherosclerosis-associated systemic inflammatory state [11]. In support of this hypothesis, many observations have indicated that the presence of cardiovascular risk factors such as hypercholesterolemia, obesity, hypertension and diabetes induce microvascular responses consistent with the induction of an inflammatory phenotype [12]. In both scenarios, due to its preponderant surface area, microcirculation would quantitatively represent the major source of circulating inflammatory mediators.

Accumulating evidence suggests that oxidative stress represents a main pathogenic mechanism underlying the development and the progression of atherosclerosis. Oxidative stress is defined as an imbalance between oxidants and antioxidants in favour of the former. Reactive oxygen species (ROS), including superoxide anions (O_2^-), hydrogen peroxide, and hydroxyl radicals are produced by a variety of cell types including endothelial cells, phagocytes and smooth muscle cells. Free radicals such as O_2^- and hydroxyl radicals are highly reactive and hold exalted oxidizing activity.

The activity of enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase, as well as the mitochondrial redox cycle can generate ROS.

Endothelial cells, vascular smooth muscle cells, and monocytes/macrophages cells contain potent defence systems against ROS, including the enzymes superoxide dismutase, catalase, and glutathione peroxidase, and non-enzymatic antioxidants. Nonetheless, excessive ROS production may occur and cause endothelial dysfunction, inflammation of the arterial wall, and atherosclerosis. Endothelial dysfunction is a very early marker of endothelial suffering. Being located between the blood and the vessel wall, the vascular endothelium has a strategic position in the cardiovascular system. NO produced by the endothelium has potent vasorelaxant activity, but it is also highly reactive with O_2. NO oxidation generates peroxynitrite, which is devoid of vasodilatory properties. Reduced NO bioavailability is therefore associated with impaired response to all vasodilators that act primarily by stimulating the endothelial release of NO. Notably, increased oxidative stress and endothelial dysfunction have been demonstrated in subjects with cardiovascular risk factors such as diabetes, hypercholesterolemia, hypertension, and in cigarette smokers. NO also influences other functions of the vascular endothelium as for example the regulation of blood coagulation and the recruitment of circulating leukocytes. By downregulating the surface expression of adhesion molecules, NO contributes to limit endothelial cell-leukocyte interaction thus dampening inflammation. In addition, NO exerts antithrombogenic and antiproliferative effects [13]. Reduced NO levels would therefore negatively impact vascular physiology. In addition, increased ROS concentrations translates into augmented oxidation of low-density lipoproteins, which are taken up by macrophages, cause foam cell formation and induce vascular inflammation [14-16]. A relevant quota of superoxide comes from the transfer of electrons from NADPH to oxygen catalyzed by membrane-bound NADPH oxidases expressed by endothelial cells, vascular smooth muscle cells, fibroblasts, and phagocytes. Atherosclerosis is associated with increased expression of NADPH oxidases. Thus, it is not surprising that the activity of such enzymes represents a major target of pharmacological treatment aimed at reducing atherosclerosis progression. In this context, it is worthy to point out that the NADPH-oxidase–mediated O_2- production is upregulated by angiotensin II (Ang II), therefore Ang II type 1 (AT1)-receptor blockade can effectively reduce

oxidative stress and inflammation in patients with hypertension, established coronary heart disease, or metabolic syndrome [17]. Moreover, hydroxymethylglutarylcoenzyme A reductase inhibitors (statins) inhibit cholesterol synthesis, as well as the formation of the of NADPH oxidases, thereby attenuating oxidative stress. As a result, statins and renin-angiotensin system blockers improve endothelium-dependent vasodilation and reduce circulating inflammatory molecules [18].

Oxidative stress and hypercholesterolemia

Microvascular inflammatory and thrombogenic response has been observed in several studies involving animal models of diet-induced hypercholesterolemia. This microvascular endothelial dysfunction is characterized by oxidative stress [19-21]. The resulting increased ROS generation and reduced NO bioavailability translate into impaired endothelium-dependent arteriolar vasodilation [22] and increased expression of adhesion molecules in the post capillary venules endothelium [23], the latter causing increased leukocyte and platelets recruitment. In turn, the increased interaction between EC and leukocytes sustains oxidative stress [19, 24].

Oxidative stress in post-capillary venules has been shown to occur early upon the onset of hypercholesterolemia [25, 26]. Increased ROS production also contributes to the generation of oxidized low-density lipoproteins that, in turn, are known mediators of inflammation and vascular dysfunction in atherosclerosis [27, 28].

Among ROS species, whose generation is elicited by hypercholesterolemia, superoxide is the best characterized and its metabolism can be taken as paradigmatic of other ROS species.

Superoxide formation can arise by reactions catalyzed by different enzymes including xanthine oxidase, lipoxygenase and NADPH oxidase. The latter is expressed by multiple cell types such as endothelial cells, smooth muscle cells, monocytes, T lymphocytes and platelets [29-31].

Because of its intrinsic toxicity, superoxide levels are controlled by a tight balance between production and degradation, the latter occurring primarily by the endogenous scavenger enzyme superoxide dismutase (SOD). Although SOD competes with NO for superoxide, superoxide-NO interaction is favoured and generates the toxic product peroxynitrite yet depleting NO.

Increased ROS production might lead to reduced NO bioavailability by an indirect mechanism also. For example, the activity of NO synthase (NOS) isoform expressed by ECs (eNOS) is pivotal for normal regulation of vascular tone and the maintenance of antithrombocgenic and anti-inflammatory endothelial cell phenotype. However, it requires sufficient levels of the cofactors such as tetrahydrobiopterin, that are impaired in the presence of elevated ROS production. Notably, in absence of adequate tetrahydrobiopterin concentration, eNOS turns to generate superoxide instead of NO, thus further contributing to the NO/ROS imbalance [32]. In addition, NOS activity can also be reduced as a result of the increased levels of asymmetric dimethylarginine (ADMA) occurring during hypercholesterolemia [33]. Interestingly, in hypercholesterolemic patients with impairment of the endothelium-dependent dilation of the epicardial coronary arteries, vascular function can be restored reducing serum cholesterol [34]. The mechanism underlying this dysfunction involves the reduced ability of substances such as acetylcholine or bradykinin that stimulate EC to release NO that, in turn, acts on VSMC. Stimulation with a NO donor, restores normal vasodilation thus indicating that VSMC responsiveness to NO is preserved [22, 23]. Two additional observations further support the hypothesis that the hypercholesterolemia-induced vascular dysfunction is, at least in part, mediated by ROS/NO imbalance: administration of L-arginine, substrate of NO synthase, or of superoxide dismutase reversed vascular function impairment in hypercholesterolemic animals [22, 35].

Oxidative stress to which microvascular endothelium is exposed is significantly sustained by EC interaction with inflammatory cells. Such interaction occurs primarily in post capillary venules but can affect the function of other microvascular segments. For example it has been shown that neutrophils adhesion to venular endothelium contributes to arteriolar dysfunction [22].

Leukocyte-endothelial cell interaction

Inflammatory state, as well as hypercholesterolemia, induces upregulated expression of adhesion molecules by EC and circulating leukocytes thus favouring blood cell recruitment.

Adhesion molecules, with some exception, belong to one of three major families: selectins, integrins and immunoglobulins. Selectins bind to sialyl-Lewis X-like carbohydrate ligands

presented by sialomucin-like surface moleculses including P-selectin glycoprotein ligand 1 (PSGL-1) [36].

P and E-selectin expressions are generally upregulated on endothelium in most inflammatory processes and represent important determinants for leukocyte recruitment. L-selectin on leukocyte membrane can also bind PSGL-1 expressed on other leukocyte thus enabling leukocyte capture by intravascular adherent leukocytes. In the context of inflammation, P selectin plays a pivotal role among molecules expressed by endothelial cells. P selectin can be rapidly translocated to the plasmatic membrane of endothelial cells from the Weibel-Palade bodies upon exposure to inflammatory stimuli. Once on the surface, P selectin supports both leukocyte-EC and platelets-EC interaction. P selectin deficiency partially protects from atherosclerosis and neointima formation after vascular injury [37, 38].

The adhesion properties of selectins and their ligands are regulated by post-translational modifications, topographical distribution and shedding, all of which are influenced by inflammation.

Integrins are transmembrane $\alpha\beta$ heterodimers that bind many extracellular matrix proteins and certain immunoglobulin-like adhesion molecules on other cells [39, 40]. The most relevant integrins for leukocyte recruitment are the heterodimer $\alpha 1\beta 2$ lymphocyte function-associated antigen 1 (LFA-1), $\alpha 4\beta 1$ very late antigen 4 (VLA-4) and $\alpha 4\beta 7$. Integrins bind a variety of surface molecules of the immunoglobulin superfamily including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), both important ligands for VLA-4 and mucosal addressin cell adhesion molecule 1 (MAdCAM-1) representing the main ligand for $\alpha 4\beta 7$. Other relevant members of the immunoglobulin-like adhesion molecules group include and PECAM-1 (CD31) [41], junction adhesion molecule (JAM)-A, JAM-B and JAM-C [11, 42] and endothelial cell-selective adhesion molecule (ESAM) [43].

The current paradigm of the leukocyte recruitment describes a multi-step process named leukocyte adhesion cascade. The number of molecules implicated in each step and the multiplicity of the regulatory mechanisms involved afford high degree of specificity and a wide variety of potential therapeutic targets. The leukocyte adhesion cascade begins with first contact between leukocyte and endothelium in the “tethering” step. During the second step named “rolling” cell activation

occurs through the action of chemokines and chemokine-independent mechanisms. During the rolling step, L-selectin and P-selectin glycoprotein ligand-1 (PSGL-1) on the leukocyte, interact with sugars and P-selectin, respectively, on the endothelial cells. Ligation of immunoglobulins, such as intercellular adhesion molecule-1 (ICAM-1), on the endothelium by integrins CD11b/CD18 on the leukocyte participates to strengthen cell-cell interaction and turns some of the rolling leukocytes into firmly adherent cells [44, 45]. In the final stage, migration into tissue occurs in the presence of appropriate stimuli for both EC and leukocytes.

Increased leukocyte adhesion is widely diffused in the venular segments of microcirculation in prolonged hypercholesterolemic conditions, while the large vessels display distinct foci where leukocyte recruitment occurs [19-21, 23]. Microcirculation response to atherosclerosis-fostering conditions, such as hypercholesterolemia, includes upregulation of P-selectin and ICAM-1 in postcapillary venules. Leukocyte recruitment in the post-capillary venules has been found to be markedly reduced by P-selectin neutralization [20]. Enhanced leukocyte adherence to the endothelium of post capillary venules has been shown in animal models of diet-induced hypercholesterolemia.

Animal studies have shown that early upon the establishment of hypercholesterolemia, the predominant leukocyte subset to be recruited in post-capillary venules is represented by neutrophils [46]. A key role for oxidative stress has been demonstrated in the recruitment of neutrophils induced by elevated cholesterol levels. Mice overexpressing superoxide scavenger SOD did not display increased leukocyte adhesion in the post-capillary venules. In keeping, mice deficient for the p47phox component of the NADPH oxidase did not display cholesterol induced neutrophil recruitment.

Role of recruited leukocyte in microvascular inflammation

Bone marrow chimera experiments have shown that NADPH oxidase expressed by both endothelial cells and by leukocytes are important for determining the inflammatory phenotype associated of post-capillary venules [19]. This finding suggests that leukocyte recruitment reinforces inflammatory stimulation by locally increasing superoxide production. The enhanced

expression of superoxide due to leukocyte recruitment might contribute to inflammation by directly increasing circulating concentrations of oxidized LDL that represent another stimulus for increased leukocyte adhesion. In addition, superoxide by depleting NO bioavailability fosters the acquisition of an inflammatory phenotype by endothelial cells. In fact, it has been shown that NO, by inducing the inhibitor κB-α, an inhibitor of NFκB, counteracts cytokine-induced upregulated expression of adhesion molecules by the endothelium [47, 48]. A relevant source of cytokines reinforcing the acquisition of inflammatory phenotype by endothelial cells is represented by T lymphocytes. The role of T lymphocytes in the development of atherosclerostic lesions in the macrovasculature is well documented [10]. Before plaque development, a contribution of lymphocytes in determining the inflammatory phenotype of endothelial cells in the microvasculature has been shown in mice lacking B and T cells. However T lymphocytes play a prominent role. The two main T lymphocyte subsets CD4+ and CD8+ both contribute significantly to leukocyte adhesion to post-capillary venules occurring in diet-induced hypercholesterolemia. In fact, depletion of each subset leads to marked reduction of microvascular leukocyte recruitment, and the administration of lymphocytes to immunodeficient mice restored venular inflammation. In addition, the reconstitution of lymphocyte deficient animals with splenocytes from IFN-γ deficient mice failed to restore leukocyte adhesion indicating a non redundant function of such cytokine in the establishment of the microvascular inflammatory phenotype upon hypercholesterolemia [25]. Similarly, mice lacking the p35 or the p40 subunit of the heterodimeric cytokine IL-12 display reduced leukocyte adhesion to the microvasculature upon the establishment of diet-induced hypercholesterolemia [26]. As there is no obvious direct proinflammatory effect exerted by IL-12 on endothelial cells or leukocytes, it is likely that IL-12 effect on microvascular inflammation is indirect and possibly mediated by IFN-γ. Indeed IL-12 is pivotal in the polarization of T lymphocyte toward a type 1 phenotype characterized by IFN-γ expression and release upon activation [49]. T cell polarization occurs in secondary lymphoid tissues when antigens collected in periphery are presented to naïve T cells by professional antigen presenting cells (APC, e.g. dendritic cells) [50]. Sustained recognition of APC-borne MHC-bound antigen by T cell receptor along with appropriate costimulation are mandatory for naïve T cells activation. Interleukin-12 represents the key signal provided by APCs that induces epigenetic

modifications of gene expression in the T cell determining its commitment to the type 1 phenotype (IFN-γ but not IL-4 producing) [51]. Antigen presentation occurring in the absence of IL-12 favours the development of type 2 T cells producing IL-4 but not IFN-γ. It is worth noting that IL-12 shares the p40 subunit with IL-23, which in turn, plays a key role in the development of the IL-17-producing proinflammatory CD4+ T cell subset Th17 implicated in chronic inflammation [52]. Therefore, the observations made in p40-deficient mice might be due to the deficiency of both Il-12 and Il-23 and consequent blocked development of Th1 and Th17 subsets. Although the role of Th17 on microvascular inflammation associated to atherosclerosis has no been so far investigated, it has been shown that IL-17 synergized with IFN-γ to enhance IL-6, CXCL8, and CXCL10 secretion [53].

Interleukin-18 has also been implicated in the induction of IFN-γ in atherogenesis. Gerdes and coworkers showed IL-18 production by infiltrating macrophages and important levels of IL-18 receptor expression in human atheroma. IL-18 receptor was expressed by macrophages, ECs and smooth muscle cells [54]. Stimulation with IL-18 induced IL-6, IL-8, ICAM-1 and metalloproteinases expression. The study showed, for the first time, the ability of IL-18 alone or in combination with IL-12 to induce IFN-γ production not only in macrophages but also by smooth muscle cells.

Many studies indicated IFN-γ as a cytokine participating to atherosclerotic plaques development [10, 55]. Several mechanisms might link IFN-γ to vascular dysfunction: IFN-γ stimulates macrophages thereby enhancing their production of nitric oxide, pro-inflammatory cytokines and pro-trombotic and vasoactive factors. Additionally, IFN-γ stimulates superoxide production in endothelial cells as well as NADPH activity in monocytes and granulocytes [56-58]. In keeping, IFN-γ-knockout mice display reduced oxidative stress in the microcirculation [26]. Notably, in conditions of reduced NO bioavailability, IFN-γ has been shown to promote LDL oxidation [59]. Furthermore, IFN-γ is a potent inducer of ICAM-1 expression on vascular endothelial cells [60] thereby increasing circulating leukocyte recruitment and might be implicated in the arteriolar dysfunction associated with hypercholesterolemia.

Microvascular inflammation and obesity

Inflammatory phenotype of microvessels is associated with cardiovascular risk factors that favour atherosclerosis. In obese subjects the expanded adipose tissue is also characterized by activated phenotype of adipocytes and infiltrating cells such as macrophages and T cells. Expansion and activation of adipose tissue in obesity translates into increased release of adipose tissue derived mediators, including inflammatory cytokines such as TNF-α, IL-1, IL-6 and adipokines [61]. In obese subjects as well as in animal models of obesity, increased local concentration of cytokines and adipokines in adipose tissue are able to induce evident signs of inflammation in the local microcirculation. In obese mice arterioles, capillaries and postcapillary venules of visceral adipose tissue undergo changes that are consistent with active inflammation [62]. Also, reduced blood flow in adipose tissue, due to arteriolar dysfunction with consequent induction of hypoxic state, has been observed. As a consequence, increased expression of HIF-1 alpha and capillary proliferation is also evident in obese adipose tissue [63]. In addition, leptin, resistin and other adipokines have been shown to inhibit endothelial dependent vasodilation [64]. Moreover, activation of capillary endothelium may contribute to the blood flow reduction due to increased leukocyte adhesion and platelet aggregation. In fact, in adipose tissue of obese animals, administration of neutralizing anti ICAM-1 antibodies improves capillary perfusion, likely by reducing vessel ploughing by leukocytes, but also reduces vascular permeability. This may indicate that leukocyte adhesion induces microvascular barrier dysfunction [62]. Similarly, adipose tissue venules display inflammatory phenotype with enhanced expression of P-selectin, E-selectin and ICAM-1 and increased leukocyte recruitment. Increased local levels of TNF-α with parallel decreased adiponectin concentrations as well as increased oxidative stress likely contribute to enhanced adhesion molecules by endothelial cells as well as to the increased P-selectin expression on platelets accompanied by augmented platelet-leukocyte aggregates [62, 65]. Along with increased formation of leukocyte-platelet aggregates, in both obese subjects and animal models of obesity, reduced fibrinolysis and increased thrombogenicity are also evident. Increased levels of the leptin enhance the risk of thrombosis in obese human subjects. Activation of leptin receptor by its natural ligand induces the activation of phospholipase C and protein kinase C via intracellular calcium

mobilization by PI3K Akt signaling pathway. Impaired platelet aggregation is observed in both leptin and leptin receptor deficient mice. The pro-thrombotic activity of leptin is counteracted by adiponectin as suggested by the increased thrombi formation in adiponectin-knockout mice [66, 67]. Adiponectin also inhibits inflammatory cells adhesion to the endothelium by counteracting TNF-α induced NFκB activation via cyclic AMP-dependent mechanism [68] and consequently inhibits endothelial adhesion molecules expression. [69]. In addition, adiponectin-dependent increased endothelial cell cytoskeleton stability reduces vascular permeability in response to TNF-α or angiotensin II via a cyclic AMP dependent pathway. Conversely, leptin promotes capillary vascular fenestration and permeability [70].

Notably, during adipogenesis, leptin expression is increased while adiponectin production is markedly reduced. This suggests that leptin/adiponectin imbalance participates in promoting the vascular inflammatory phenotype observed in adipose tissue of obese animals, in particular the increased coronary microvascular permeability observed at early stages of obesity. Beside the local effects of increased adipokines levels occurring in hypertrophic adipose tissue, the accumulation of such factors in plasma elicit prime endothelial cells for activation in response to low concentration of inflammatory stimuli, causing the low-grade systemic inflammation that is associated to obesity [71, 72].

Microvascular inflammation and diabetes

Hyperglycemia is an important trigger of oxidative stress. In insulin-independent tissues, local production of oxidants is enhanced along with the increase of cellular glucose uptake. Hyperglycemia stimulates superoxide production by glucose oxidase and NADPH oxidase. Such effect is mediated by protein kinase C activation [73-76].

Oxidative stress resulting from hyperglycemia has been implicated in the impaired vascular function that is associated with diabetes. Diabetes results in enhanced expression of the angiotensin 1 receptor as well as the p47 and gp91 subunits of the NADPH oxidase complex. In these conditions, AT1r antagonism or NADPH oxidase inhibition restores NO-dependent vascular function [77, 78].

Hyperglycemia and oxidative stress are recognized as two major determinants of the increased leukocyte-endothelial cell interaction occurring in diabetes. Using intravital microscopy, Booth and colleagues showed that local glucose levels increased endothelial P-selectin expression along with leukocyte rolling, firm adhesion and migration in post capillary venules [79]. Moreover, similar effects are mimicked in vitro in endothelial cell cultured exposed to sera of diabetic patients or advanced glycation endproduct-albumin and are significantly reduced in vivo by protein C inhibition or the administration of insulin or SOD. In addition, a possible role for the receptor for advanced glycosylated endproducts (RAGE) as endothelial adhesion molecule binding to beta 2 integrin on leukocyte surface, has been proposed [65, 79-82].

Oxidative stress, hyperglycemia and increased leukocyte interaction with the endothelium have been linked to increased vascular permeability that is observed in animal models of diabetes and in diabetic subjects. Diabetes-associated retinopathy and nephropathy represent an example of major complications due to the loss of the endothelial barrier function . In fact, oxidative stress, hyperglycemia and increased leukocyte recruitment cause loss of perycytes and endothelial cells, basement membrane thickening as well as capillary closure in the retina. Consequent hypoxia stimulates angiogenesis. The blockade of the renin-angiotensin system by ACE inhibitors or by antagonists of the angiotensin receptor reduces retinal angiogenesis and the expression of VEGF and VEGFR [83-85]. PPARgamma, VGEF, protein kinase C and the renin-angiotensin system are believed to play a relevant role in diabetes-induced vascular permeability [86-90].

Increased platelet activation is associated with diabetes. This is thought to be, at least in part, dependent on a reduced capacity of NO to inhibit platelet activation possibly as a result of the oxidative stress associated with diabetes and consequent reduction of NO bioavailability [91-99].

Summary

The evidence linking microvascular and inflammatory responses to cardiovascular risk factors indicates the existence of common events that initiate and promote these responses. Oxidative stress, reduced NO bioavailability, endothelial activation are common early features of microvascular responses to cardiovascular risk factors. Another important consequence of

exposure to such risk factors is the increased extent of tissue damage after ischemia reperfusion. For example, obese, hypercholesterolemic and diabetic animals display markedly increased microvascular oxidative stress and enhanced NADPH oxidase activation compared to animals without risk factors [100-104]. Furthermore, larger extent of leukocyte and platelets adhesion to endothelial cells in post-capillary venules is associated with hypercholesterolemia, obesity and diabetes. Increased leukocyte and platelet recruitment is associated to markedly enhanced vascular permeability after ischemia reperfusion. Finally, in the presence of risk factors the beneficial effects of ischemic preconditioning are severely attenuated [105].

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