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The vicious circle between oxidative stress and inflammation in atherosclerosis

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Abstract

The initial event in atherogenesis is the increased transcytosis of low density lipoprotein, and its subsequent deposition, retention and modification in the subendothelium. It is followed by the infiltration of activated inflammatory cells from the coronary circulation into the arterial wall. There they secrete reactive oxygen species (ROS) and produce oxidized lipoproteins capable of inducing endothelial cell apoptosis, and thereby plaque erosion. Activated T lymphocytes, macrophages and mast cells, accumulate in the eroded plaque where they secrete a variety of proteases capable of inducing degradation of extracellular proteins, thereby rendering the plaques more prone to rupture. This review summarizes the recent advancements in the understanding of the roles of ROS and oxidized lipoproteins in the activation of inflammatory cells and inducing signalling pathways related to cell death and apoptosis. In addition, it presents evidence that this vicious circle between oxidative stress and inflammation does not only occur in the diseased arterial wall, but also in adipose tissues. There, oxidative stress and inflammation impair adipocyte maturation resulting in defective insulin action and adipocytokine signalling. The latter is associated with increased infiltration of inflammatory cells, loss of anti-oxidant protection and cell death in the arterial wall.

Keywords: atherosclerosis • metabolic syndrome • diabetes • inflammation • oxidative stress • adipose tissue • vascular tissue • oxidized LDL

Introduction

One of the emerging cardiovascular risk factors is subclinical chronic low-grade inflammation [1]. Population studies showed a strong correlation between pro-inflammatory biomarkers (such as C-reactive protein, interleukin-6 [IL-6] and tumour necrosis factor-α [TNF-α]) and perturbations in glucose homeostasis, obesity and atherosclerosis [2, 3]. Another emerging risk factor is oxidized low-density lipoprotein (ox-LDL) that activates circulating monocytes, thereby increasing their ability to infiltrate the vascular wall. This increased infiltration is a key event in atherogenesis [4].

The metabolic syndrome clusters several cardiovascular risk factors including obesity, dyslipidaemia, hypertension and insulin resistance (IR) [5–8]. Increased inflammation [9, 10] and oxidative stress [11, 12] were found to be associated with the metabolic syndrome. It is a primary risk factor for diabetes and cardiovascular diseases [6, 13–20]. Recent data suggest that increased oxidative stress in adipose tissue is an early instigator of the metabolic syndrome and that the redox state in adipose tissue is a potentially useful therapeutic target for the obesity-associated metabolic syndrome [21]. Oxidative damage of adipose tissues is associated with impaired adipocyte maturation, production of pro-inflammatory adipocytokines by dysfunctional adipocytes and increased infiltration of macrophages into the adipose tissues of obese persons where they produce inflammatory chemokines [22, 23]. This enhanced infiltration is causatively related to the loss of insulin signalling [24]. The goal of this review is to give an overview of the molecular mechanisms in adipose and vascular tissues that can explain the vicious circle between oxidative stress and inflammation, in relation to atherosclerosis and cardiovascular diseases.

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Overview of molecular mechanisms explaining the association of inflammation and oxidative stress in the vessel wall

The initial event in atherogenesis is the increased transcytosis of LDL, and its subsequent deposition, retention and modification in the subendothelium within and outside the meshes of the hyperplasic basal lamina where LDL interacts with matrix proteins [25, 26]. It is followed by the recruitment of circulating monocytes into the vascular intima and their subsequent transformation into macrophage/foam cells are key elements of the initiation of atherosclerosis. This infiltration occurs preferentially at sites with disturbed flow dynamics where the endothelium becomes dysfunctional at the cellular and molecular level. Activated by the disturbed flow, the endothelium shows enhanced platelet adhesion, increased endothelial permeability to macromolecules such as LDL, and augmented endothelial gene expression of leucocyte adhesion molecules and chemotactic factors (Fig. 1). Activation of the leucocyte adhesion cascade ultimately leads to transmigration of monocytes into the subendothelial space of the intima and subsequent differentiation into macrophages. Adherence of circulating monocytes and T lymphocytes to the inflamed endothelium is mediated particularly by the vascular adhesion molecule-1 (VCAM-1), the intercellular adhesion molecule-1 (ICAM-1) and E- selectin and fibronectin. The monocyte chemoattractant protein-1 (MCP-1) and IL-8 play an active role as chemoattractants in the infiltration of leucocytes into the arterial wall. There, their activation accelerates the generation of reactive oxygen species (ROS). Activated macrophages express enzymes such as myeloperoxidase (MPO) and NADPH oxidase (NOX-1), which together generate a range of oxidants, including superoxide, hydrogen peroxide and hypochlorous acid [27, 28]. Each of them causes oxidative damage not only to invading micro-organisms, but also to host molecules in surrounding tissues. Among them are phospholipids in cell membranes and circulating lipoproteins which contain polyunsaturated fatty acid chains, such as the abundant phospholipid 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine (PAPC) that are particularly susceptible to oxidation by such mediators [29]. Products of PAPC oxidation have been shown to accumulate at sites of inflammation, and in cells treated with inflammatory stimulants such as IL-1β and TNF-α [30]. Oxidation of PAPC leads to the formation of a mixture of products, ranging from epoxyisoprostanes to truncated chain derivatives that are collectively termed ox-PAPC. Oxidation of phospholipids in LDL, that infiltrates into the injured vessel wall, by MPO and NOX-1, results in the accumulation of ox-PAPC in ox-LDL (Fig. 1).

The activation of macrophages is also characterized by increased expression of the scavenger receptor-A (SR-A), cluster of differentiation (CD)36, and the lectin-like ox-LDL receptor (LOX-1) which internalize ox-LDL [31]. This scavenger receptor mediated uptake is important for clearing ox-LDL. However, unregulated uptake of ox-LDL leads to production of lipid-loaded foam cells. The unlimited accumulation of lipids, also as a consequence of defective cholesterol and lipid efflux, leads to apoptosis and cell burst resulting in exposure of extracellular lipids. Both apoptotic cells and thrombogenic lipids induce the formation of microthrombi which further stimulate the adhesion and infiltration of inflammatory cells, and the secretion of inflammatory cytokines, and the production of ROS.

Besides its deleterious action in the production of foam cells, CD36 plays also a protective role as a receptor of thrombospondin (TSP-1). This interaction is important for activating TSP-1-mediated phagocytosis, thereby preventing inflammation and macrophage-induced elastin degradation by matrix metalloproteinases (MMPs), and thus vessel wall degeneration and plaque rupture [32]. By binding to TSP-1, ox-LDL does not only impair phagocytosis but also prevents TSP-1-dependent TGF-β activation, thereby increasing inflammation and atherogenesis [33].

Activated macrophages secrete a number of growth factors such as the macrophage colony-stimulating factor (M-CSF) that augments SR-A expression, and induces the production of cytokines and growth factors which stimulate intimal proliferation. Once resident in the arterial wall, the interaction between monocytes/macrophages and T cells results in a broad range of cellular and humoural responses that drive the progression of a relatively simple fatty streak to a more complex lesion.

Modified forms of LDL are immunogenic and activate both cell-mediated and humoural immune responses. Both types of responses are pro-inflammatory and are probably primary players in the perpetuation of the chronic inflammatory reaction characteristic of atherosclerosis. The immunologic response to modified LDL can be directed to major histocompatibilty complex class II (MHC-II)-associated peptides in the case of T helper cells, and to a variety of epitopes-modified lysine groups, modified phospholipids, proteins that become associated with ox-LDL (such as β_2 glycoprotein 1) – in the case of B cell responses [34]. At the other hand, the activation of regulatory T cells may be protective. Indeed, recent data showed that alum precipitates, containing antigens derived from ox-LDL, increased regulatory T cells activated by tolerogenic antigen-presenting cells presenting ox-LDL antigens [35]. Similarly macrophages, endothelial cells and smooth muscle cells (SMCs) appear to be activated, demonstrated by increased expression of MHC-II molecules, and of toll-like receptors (TLRs) of which the ligands induce the release of numerous inflammatory products, such as TNF-α, IL-6 and MCP-1. Recently, we found that ox-LDL can also induce inflammation by inducing TLR-2 and -4 and the interferon regulatory factor-1 [36] (Fig. 1).

Moreover, activated macrophages produce pro-inflammatory secretory phospholipase A (2) (sPLA (2))-IIA. This enzyme lipolyzes the phospholipid monolayers of LDL, increasing its affinity to proteoglycans, rendering it more susceptible to aggregation, and enhancing its ability to insert cholesterol into cells [37]. This modification may promote scavenger receptor independent LDL uptake by macrophages leading to the formation of foam cells. In addition to sPLA2IIA that does not hydrolyse phosphatydylcholine (PC) into lyso-PC (LPC) [38], there are at least three sPLA2 that

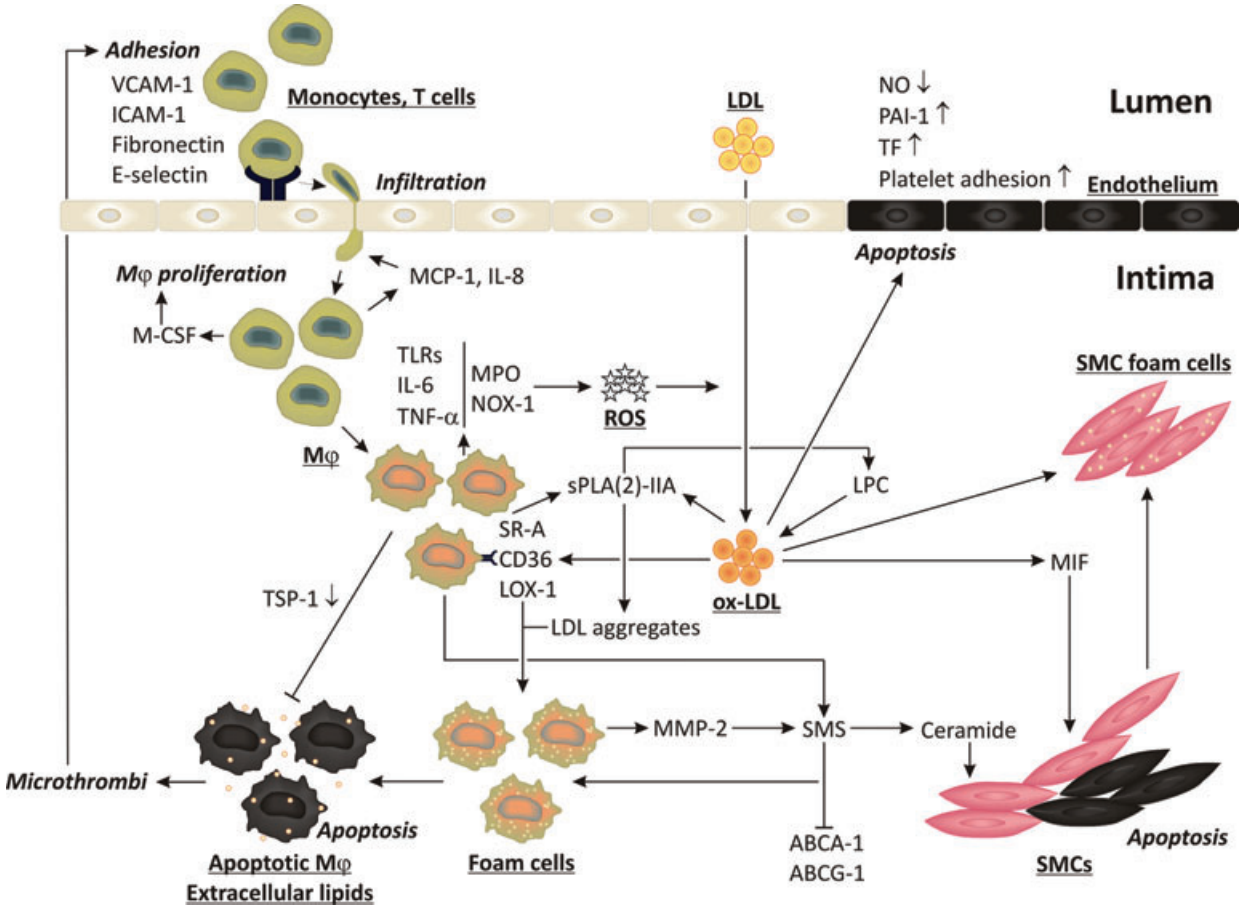

Fig. 1 Molecular mechanisms of inflammation and oxidative stress in atherosclerotic plaques. Endothelial dysfunction in relation to hypercholesterolemia, hypertension, type 2 diabetes, and smoking is associated with induction of adhesion molecules for inflammatory cells, ICAM-1, VCAM-1, E-selectin and fibronectin. The infiltration and activation of inflammatory cells are associated with the activation of the oxidant enzymes MPO and NOX-1, resulting in the production of ROS and the oxidation of phospholipids and protein in LDL, resulting in the accumulation of ox-LDL. It stimulates the endothelium to secrete MCP-1 and IL-8, which induce transmigration of leucocytes into the endothelial space. Macrophages secrete M-CSF, thereby stimulating macrophage proliferation and inducing the expression of scavenger receptors CD36, LOX-1 and SR-A. The scavenger receptor mediated uptake of ox-LDL by macrophages leads to massive cholesterol and lipid accumulation and formation of foam cells, finally resulting in apoptotic macrophages and exposure of thrombogenic lipids. Deficient TSP-1 expression is associated with a decreased phagocytosis of dead cells. Foam cells secrete MMPs and SMS resulting in the production of ceramide that induces smooth SMC apoptosis (black cells). Activation of SMS also blunts the action of ABCA-1 and ABCG-1 resulting in impaired cholesterol and lipid efflux from foam cells. Ox-LDL induces TLRs of which the ligands enhance the expression of inflammatory mediators IL-6 and TNF-α. Ox-LDL induces migration inhibitory factor that stimulates SMC migration. The uptake of ox-LDL by SMCs leads to the production of SMC foam cells and secretion of MMPs that degrade the extracellular matrix proteins rendering the plaque more prone to rupture. Ox-LDL stimulates platelet adhesion and aggregation by decreasing endothelial production of nitric oxide, and enhances the pro-coagulant activity of endothelium by inducing the release of tissue factor. Ox-LDL reduces the fibrinolytic activity of endothelium by increasing the release of plasminogen activator inhibitor-1. Finally, ox-LDL induces apoptosis in endothelial cells (black) contributing to plaque erosion and rupture.

degrade intact PC in human LDL leading to foam cells formation, including PLA2G10 [39] and PLA2GIII [40] and to lesser extent PLA2GV [41]. Moreover there is a specific lipoprotein-associated PLA2 (Lp-PLA2; PLA2G7) that degrades oxidized ceramide found in ox-LDL and that increases its inflammatory action [42, 43]. Interestingly, we showed that ox-LDL induced the phospholipase expression by monocytes and macrophages [42].

Furthermore, activation of inflammatory cells is associated with the activation of sphingomyelinases (SMS) in the *trans*-Golgi apparatus and in plasma membranes resulting in the synthesis of sphingomyelin (SM), which transfers the phosphorylcholine moiety from phosphatidylcholine onto ceramide [44]. The interaction between SM, cholesterol, and glycosphingolipid drives the formation of SM-enriched lipid rafts resulting in an inhibition of the

ATP-binding cassette transporter (ABC) A-1 and ABCG-1-mediated lipid efflux. This is important because macrophages cannot limit the uptake of cholesterol, and therefore depend on cholesterol and lipid efflux pathways to prevent their transformation into foam cells [45]. Ceramide production is associated with enhanced apoptosis of SMCs [46]. This may be beneficial because it prevents plaque growth. However, by preventing fibrous cap formation, it renders plaques more susceptible to rupture, and increases the risk of acute coronary syndromes. Interestingly, enhanced ceramide production was observed in association with the activation of MMP-2 by ox-LDL [47]. The association between ox-LDL, foam cell formation and MMP secretion is, however, not limited to macrophages but does also occur in SMCs [48]. Finally, ox-LDL induces the macrophage migration inhibitory factor that stimulates the migration of SMCs, contributing to intimal hyperplasia (Fig. 1).

Regulatory mechanisms of interactions between oxidative stress and inflammation

Recently, we obtained a mouse model of the metabolic syndrome that allowed the study of molecular mechanisms explaining the relations of the metabolic syndrome components with enhanced inflammation and oxidative stress. Indeed, we found that mice with combined leptin and LDL receptor deficiency (double knock-out [DKO] mice) are obese and show severe hypertriglyceridaemia, hypertension and IR and diabetes. This combination of metabolic syndrome factors was associated with accelerated atherosclerosis due to increased accumulation of macrophages in association with endothelial dysfunction demonstrated by increased expression of VCAM-1 and ICAM-1 in the aorta of DKO mice [49]. Increased macrophage accumulation was associated with elevated plaque ox-LDL. The latter could be partly attributed to increased MPO by plaque macrophages. In addition, impaired high-density lipoprotein associated anti-oxidant activity in the blood [50] was associated with more ox-LDL in the plaques. We then investigated the relation between metabolic syndrome components and the oxidation of LDL further by assessing the effect of weight loss. We selected this intervention because it had been demonstrated that the cardiovascular risk of insulin-resistant obese persons is higher than that of insulin-sensitive obese persons, and that weight loss reduces the risk of insulin-resistant obese persons [51].

Weight loss in obese mice was associated with a decrease of metabolic syndrome components, resulting in reduced inflammation and oxidative stress. Ultimately, these changes led to inhibition of atherosclerosis [52] due to decreased accumulation of macrophages and deposition of ox-LDL. We showed that weight loss was not only associated with a decrease of the volume of adipose tissues, but also with improved adipocyte maturation demonstrated by induction of peroxisome proliferator activated receptors (PPARs) resulting in improved glucose uptake and insulin signalling, and fatty acid metabolism. This improved metabolic profile was associated with increased anti-oxidant protection, supported by increased expression of superoxide dismutase (SOD), glutathione peroxidase (GPX) and nitric oxide synthase (NOS). The decreased inflammation was supported by decreased expression of ICAM-1, CD44 and CD68 [52]. Similar effects were obtained with rosuvastatin that is known to increase insulin sensitivity and inhibit the oxidation of LDL [53]. Cell experiments [54] indicated that PPAR-γ can inhibit the accumulation of ox-LDL by increasing the anti-oxidant defence by up-regulating SOD-1 and -3 and GPX (Fig. 2). PPAR-γ also increases CD36-mediated clearance of ox-LDL. The decrease of ox-LDL is associated with a reduction of the TLR-mediated inflammatory response. PPAR-γ also decreases expressions of monocyte-specific adhesion molecules and chemotactic factors, resulting in less macrophage accumulation. In addition, PPAR-γ increases NOS production associated with improved endothelial function, induces the efflux of cholesterol and lipids from foam cells by up-regulating the liver X receptors (LXR)-α and ABCA-1. Finally, PPAR-γ induces the expressions of the glucose transporter-4 and the insulin receptor substrate-2 (IRS-2), which activate glucose uptake and insulin action. The improved IRS-2-mediated insulin signalling is associated with increased SOD-2 expression, resulting in lower mitochondrial oxidative stress and ROS production. Finally, PPAR-γ activation was found to prime monocytes into alternative M2 macrophages with anti-inflammatory properties [55].

Overview of molecular mechanisms explaining the association of inflammation and oxidative stress in adipose tissues

Obesity is associated with reduced adipose tissue oxygenation and hypoxia, without the appropriate angiogenic response, due to defective activation of LXRs [56] and vascular endothelial growth factor (VEGF) [57], but with increased macrophage chemotaxis [23] (Fig. 3). Indeed very recent findings suggest that obese adipose tissue activates $CD8^+$ T cells, which, in turn, promote the recruitment and activation of macrophages in this tissue and support the notion that $CD8^+$ T cells have an essential role in the initiation and propagation of adipose inflammation [58, 59]. Finally, it was found that lean, but not obese, fat is enriched for a unique population of regulatory T cells that protects against the harmful effect of metabolic parameters [60].

Infiltrated inflammatory cells produce and secrete TNF-α and IL-6 contributing to the pathogenesis of IR, together with factors produced by dysfunctional adipocytes such as leptin and

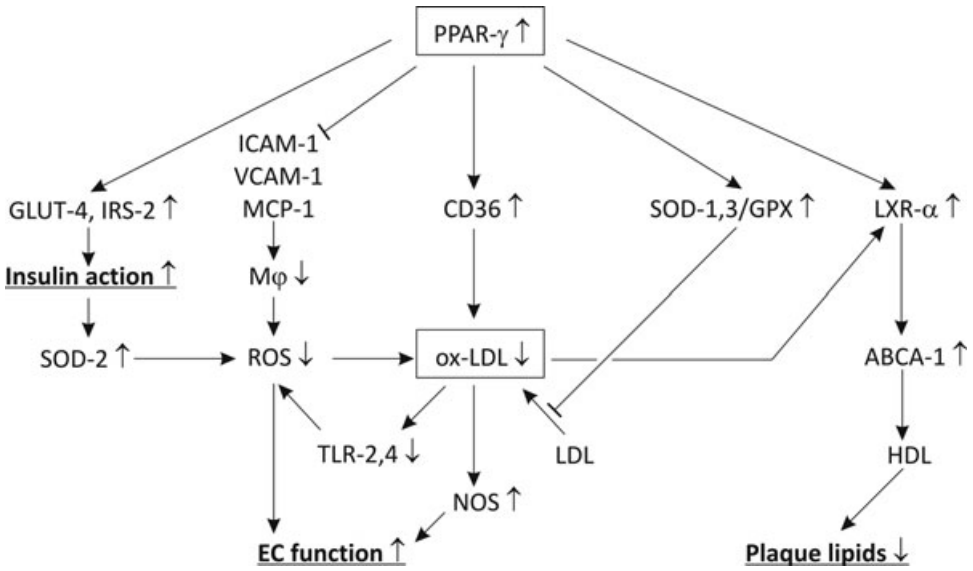

Fig. 2 Regulatory mechanisms of interactions between oxidative stress and inflammation. PPAR-γ reduces the expressions of ICAM-1, VCAM-1 and MCP-1, resulting in reduced macrophage accumulation, ROS production and ox-LDL deposit. In addition, PPAR-γ increases expressions of CD36 and anti-oxidant enzymes SOD and GPX, resulting in a further reduction of ox-LDL. Higher PPAR-γ is also associated with increased LXR-α and ABCA-1 expression, resulting in a decrease of cholesterol and lipids. The reduction in ox-LDL is associated with a decrease of TLR-mediated inflammation, and thereby reduced ROS production. In addition this reduction is associated with higher NOS production resulting in improved endothelial vasoreactivity, blood pressure regulation and left ventricle function. Reduction of ox-LDL results in restoring PPAR-γ expression that is associated with increased IRS-2 and glucose transporter-4 expressions, which are important regulators of insulin sensitivity and glucose uptake. Improved insulin action results in decreased mitochondrial oxidative stress, increased SOD-2 expression and reduced ROS production.

resistin. Conversely, insulin-sensitizing adiponectin is down-regulated during obesity [61]. A range of mouse models with loss-of-function mutations in genes important in macrophage recruitment (MCP-1 and chemokine (C-C motif) receptor 2), inflammatory cytokine production (TNF-α) and pro-inflammatory activation (NF-κB [nuclear factor of κ light polypeptide gene enhancer in B-cells] and IκB kinase [IKK]β) have demonstrated to be protected against high-fat diet-induced IR [62–64]. Interestingly, weight loss was associated with a reduction in the macrophage infiltration of adipose tissues, associated with an improvement of the inflammatory and oxidant profile of adipocytes and monocytes in adipose tissue and the circulation, respectively. Furthermore, weight loss resulted in enhanced insulin sensitivity and a decrease of the cardiovascular risk of insulin-resistant, obese persons [51].

In addition to their roles in inflammation, macrophages also promote vascular remodelling of adipose tissues [65] by producing MMPs. This occurs preferentially at sites of adipocyte death where macrophages form a crown-like structure that envelopes and ingests the moribund adipocyte and its potentially cytotoxic remnant lipid droplets [66]. As a consequence of lipid scavenging, macrophages within crown-like structures become lipid-loaded foam cells that fuse to multinucleate giant cells that have lost their capacity to remove dead adipocytes. During resolution, specific ω-3 polyunsaturated fatty-acid-derived mediators, including resolvin E1 and protectin D1, promote phagocyte removal during acute inflammation by inhibiting leucocyte infiltration and increasing macrophage ingestion of apoptotic cells [67]. This resolution is impaired in obesity leading to defective protection against oxidative stress-initiated inflammation by glutathione conjugates of lipid oxidation-derived aldehydes, such as 4-hydroxy-trans-2-nonenal present in ox-LDL [68].

A possible explanation for the relation between ox-LDL and obesity is that ox-LDL increases the volume of adipose tissues either directly by inducing adipocyte proliferation [69] or indirectly by increasing the infiltration of inflammatory monocytes/macrophages which increase adipogenesis [70]. The increase in adipose tissue mass may also be explained by a cellular hypertrophy due to an increased lipid accumulation in the pre-existing adipocytes rather than an increase in cell number or differentiation. Indeed, ox-LDL increases triglyceride production by inducing the expression of lipoprotein lipase [71], and the accumulation of fatty acids in adipocytes [72]. Interestingly, fatty acids stimulate the accumulation of ceramide that contributes to inflammation that, as discussed above, is associated with adipose hyperplasia. Ox-LDL was also found to decrease the production of adiponectin that in contrast with other adipocytokines is reduced in obese persons, and suppresses excess ROS production under high-glucose conditions, an effect that has implications for vascular protection in diabetes [73]. In addition, the decrease of adiponectin results in a loss of its capacity to protect cells against inactivation of the growth arrest specific 6 mediated survival pathway by TNF-α [74]. The observed relations between obesity and ox-LDL are important to understand the relation of obesity with IR [27] and the metabolic syndrome [75]. All these processes are summarized in Fig. 3.

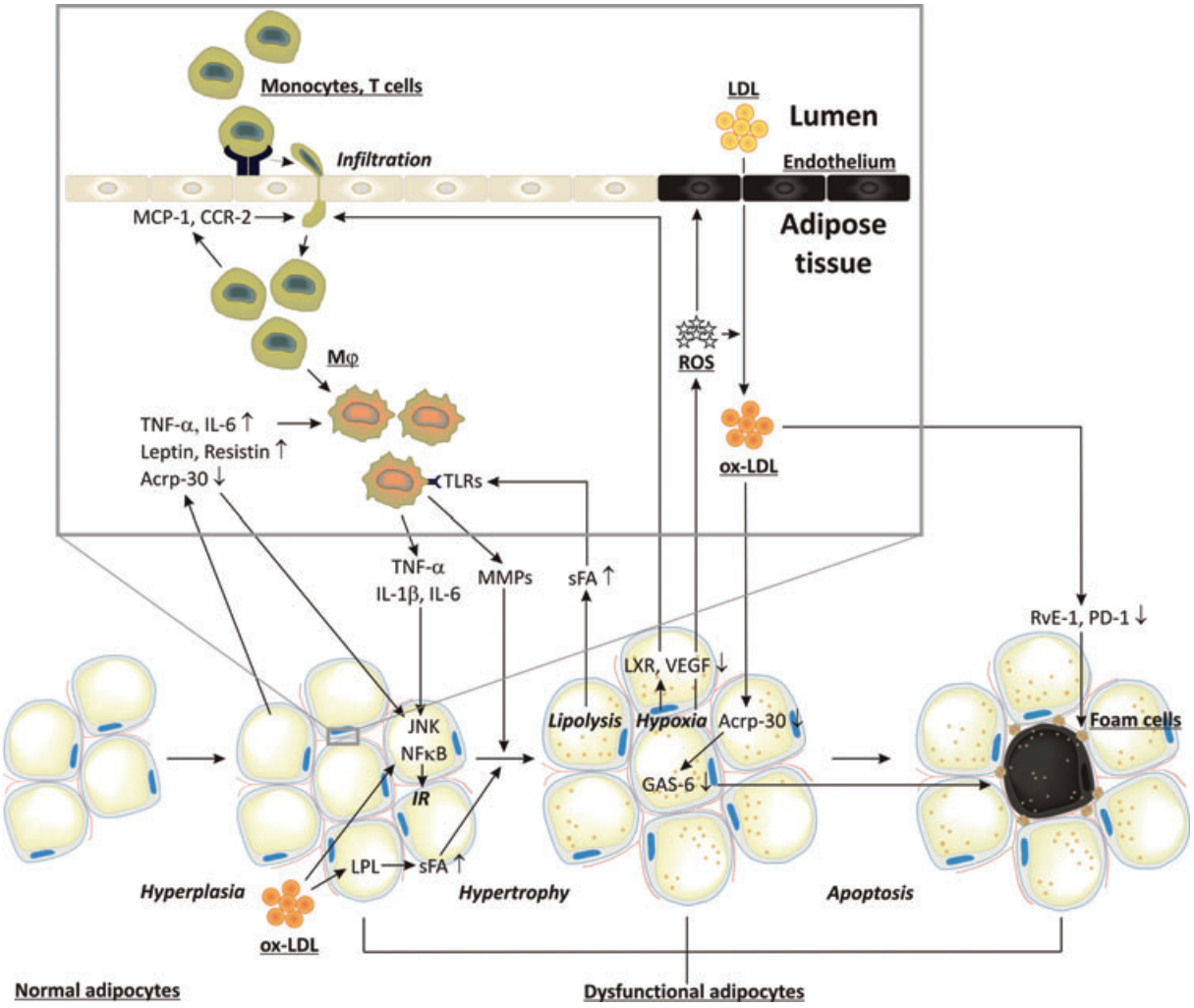

Fig. 3 Macrophage infiltration in adipose tissues, oxidative stress and IR. Circulating monocytes adhere to activated endothelial cells. Activated $CD8^+$ T cells and chemokines induce monocyte migration into adipose tissues where they differentiate into macrophages. Interaction of saturated fatty acids with TLRs leads to secretion of inflammatory cytokines/chemokines (IL-6 and TNF-α). Together with the adipocytokines leptin and resistin, they impair c-jun N-terminal kinase and NF-κB signalling, resulting in IR and reduced adiponectin (Acrp-30) secretion, and thereby loss of adipocyte maturation. Together they also induce adipocyte proliferation. Infiltration of inflammatory cells is associated with ROS and ox-LDL production, endothelial cell apoptosis, impaired LXR and VEGF signalling, and decreased blood flow leading to hypoxia and increased oxidative stress. Ox-LDL can further induce adipose tissue hyperplasia, and by inducing lipoprotein lipase it enhances lipid accumulation resulting in adipose tissue hypertrophy. The latter is also facilitated by MMPs secreted by macrophages. Hypoxia and increased oxidative stress induces apoptosis of adipocytes. Increased apoptosis also results from reduced Acrp-30 secretion and growth arrest specific 6 mediated survival pathway. Apoptotic adipocytes attract macrophages which normally remove apoptotic adipocytes. However, ox-LDL impairs phagocytosis of dead adipocytes by inhibiting resolvin E1 and protectin D1 production.

Conclusions

Experimental, clinical and population studies demonstrated active roles of oxidative stress and inflammation in the development of obesity-associated metabolic syndrome and cardiovascular diseases. Herein, we presented evidence of a vicious circle between inflammation and oxidative stress not only in vascular but also in adipose tissues. Inflammatory cells increase the production of ROS and ox-LDL by secreting oxidant enzymes. They impair the function of endothelial cells and SMCs in vascular, and of endothelial cells and adipocytes in adipose tissues. Especially, inhibiting the IRS-mediated insulin action is associated with a decrease in anti-oxidant enzymes, and an increase of mitochondrial oxidative stress in both tissue types. By inducing the production of foam cells, ox-LDL decreases the phagocytosis of apoptotic cells by macrophages, resulting in impaired insulin action. By attacking

endothelial cells, ox-LDL impairs blood flow and causes hypoxia and oxidative stress.

In addition, we showed that PPAR-γ and adiponectin protect against oxidative stress and inflammation. Loss of adiponectin-mediated signalling between adipose and arterial tissues is of particular importance in the development of obesity-associated atherosclerosis and cardiovascular diseases.

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