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Forum Review

Role of Redox Regulation and Lipid Rafts in Macrophages During Ox-LDL–Mediated Foam Cell Formation

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ABSTRACT

Hyperlipidemias and small dense LDLs in patients with high-triglyceride low-HDL syndromes lead to a prolonged half life of apoB-containing particles. This is associated with reactive oxygen species (ROS) activation and leads to formation of oxidized LDL (Ox-LDL). Generators of ROS in macrophages (MACs) include myeloperoxidase (MPO)-mediated respiratory burst and raft-associated NADPH-oxidase. The intracellular oxidant milieu is involved in cellular signaling pathways, like ion-transport systems, protein phosphorylation, and gene expression. Lipid oxidation through ROS can amplify foam cell formation through Ox-LDL uptake, leading to formation of ceramide (Cer)-rich lipid membrane microdomains, and is associated with expansion of the lysosomal compartment and an upregulation of ABCA1 and other genes of the AP3 secretory pathway. Ox-LDL may also affect cell-surface turnover of Cer-backbone sphingolipids and apoE-mediated uptake by LRP-family members. In contrast, HDL-mediated lipid efflux causes disruption of lipid membrane microdomains and prevents foam cell formation. Oxidation of HDL through MPO leads to a failure of lipid efflux and enhancement of MAC loading. Therefore, lipid rafts and oxidation processes are important in regulation of MAC foam cell formation and atherosclerosis, and the balance between oxidant and antioxidant intracellular systems is critically important for efficient MAC function. *Antioxid. Redox Signal.* 9, 1499–1518.

INTRODUCTION

ATHEROSCLEROSIS IS A CHRONIC INFLAMMATORY DISEASE that connects hyperlipidemia and other risk factors with the development and progression of atherosclerotic lesions, plaque rupture, and vascular thrombosis. At the cellular level, especially phagocytic monocytes rapidly transform to macrophage foam cells, characterized by the excessive uptake of atherogenic lipoproteins like Ox-LDL by receptor-mediated endocytosis (111). This uptake of Ox-LDL maintains the generation of ROS and RNS (129), leading to cellular signals and posttranslational modifications of cellular proteins responsible for apoptosis or necrosis. The macrophage colony stimulating factor (M-CSF), which regulates the survival, proliferation, differentiation, and chemotaxis of MACs, also enhances ROS by stimulating the raft-associated NADPH-oxidase (129). In addition, ROS can also be generated by MPO-mediated respiratory bursts. Excess ROS levels, known as oxidative stress, lead to induction of lipid oxidation and can amplify foam cell formation through Ox-LDL uptake. In addition oxidation of HDL through MPO leads to a failure of lipid efflux and in turn also enhances foam cell formation. Oxidative stress has been implicated in several pathologic conditions including atherosclerosis (106). Lipid-rich microdomains (lipid rafts) of the cell membrane are central to the understanding of cellular lipid homeostasis and the consequences of lipid loading on cell function. They are characterized by the enrichment of cholesterol, sphingolipids, and saturated phospholipids, and in addition by their insolubility in different detergents such as Triton X-100 and Lubrol WX. Rafts are thought to function as platforms for the dynamic association of signaling molecules. Concentration of the receptors for interaction with ligands and effectors on both sides of the mem-

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brane can allow efficient and rapid coupling of activated receptors to the effector system (139).

Cer, which is induced by Ox-LDL through enhancement of neutral and acidic sphingomyelinase (SMase) activity (76), leads to coalescence of submicroscopic rafts into large Cer membrane macrodomains (61). These macrodomains may serve as platforms for protein concentration and oligomerization, transmitting signals across the plasma membrane. Cer then activates a variety of diverse protein kinase- and protein phosphatase–dependent signaling pathways, which in most cases suppress cell growth or promote programmed cell death or both (61). In addition, Cer is a ligand for apoE, which binds more avidly to Cer-rich microdomains on sphingomyelinase-treated liposomes (99). Together with the stimulation of heparan sulfate proteoglycan (HSPG) and LRP-mediated uptake by MACs through Cer and apoE, which is crucial for foam cell formation (98), a function of Cer-rich microdomains in apoE-dependent metabolism is suggested.

This review mainly focuses on the redox regulation and signaling of mononuclear phagocytes: MACs during foam cell formation with atherogenic lipoproteins and the involvement of lipid rafts.

The atherogenic process and effects of M-CSF (macrophage colony-stimulating factor) on macrophage differentiation

Blood monocytes and antigen-presenting cells originate from bone marrow progenitors and differentiate either to phagocytic MACs, osteoclasts, or antigen-presenting dendritic cells (APCs) within the vascular wall. MACs play a central role in the initiation and progression of atherosclerotic lesions, and peripheral blood monocytes extravasate into the vessel wall triggered by several activation mechanisms involving innate, humoral, classic, and alternative pathways (55). Multiple mediators affect the monocyte during its residence time in the blood circulation and prime its differentiation into MACs with pro- or anti-inflammatory properties based on the individual genetic background. As depicted in Fig. 1, the high-triglyceride/low-HDL syndromes, as well as hypercholesterolemias, are both linked with atherosclerosis. Several signals or metabolites originating either from different organs or the plasma compartment itself affect monocytes and drive their transformation toward foam cell MACs or osteoclasts in calcifying lesions.

M-CSF is an important factor regulating the survival, proliferation, differentiation, and chemotaxis of MACs. During early myeloid differentiation, M-CSF synergizes with other growth factors and interleukin-3 to produce mononuclear phagocyte progenitor cells. After this initial differentiation process, M-CSF by itself can regulate the proliferation and differentiation of mononuclear phagocyte progenitor cells to monocytes, MACs, and osteoclasts and also supports survival and activity of fully differentiated MACs. The receptor for macrophage colony-stimulating factor (M-CSF-1R) is expressed in mononuclear phagocytes and APCs, which can be regarded as a specialized adaptive state rather than a separate lineage. M-CSF was considered an alternative marker of MACs, whereas APCs differentiate through the action of granulocyte-macrophage-CSF (GM-CSF), IL-4, and prostaglandin E_2 (65). M-CSF cooperates with the receptor activator of NF-κB ligand (RANKL) to regulate the differentiation of mononuclear phagocytes toward osteoclasts (118). M-CSF also enhances cytotoxicity, ROS (*e.g.*, superoxideradical, peroxynitryl-, hydroxyl-radical and hydrogen-peroxide production), as well as phagocytosis, chemotaxis, and cytokine production in monocytes and MACs (25) (Fig. 2).

M-CSF–mediated signaling (Fig. 2) involves many cytoplasmic molecules like c-Src, which is linked with c-Cbl and targets the Vav family members of guanine nucleotide exchange factors (GEFs), which in turn activate Rac-1 as a constituent of activated NADPH-oxidase. Alternatively coregulatory signaling pathways like integrin signaling (*e.g.*, $\alpha v\beta 3$, $\alpha M\beta 2$) also target Vav.

Regulation of the raft-associated NADPH-oxidase system

M-CSF stimulates raft-associated NADPH-oxidase, resulting in ROS formation, which regulates Akt and p38/MAPK, and thus contributes to monocyte/MAC survival (157) (Fig. 2). Superoxide-producing phagocyte NADPH-oxidase consists of a membrane-bound flavocytochrome b558 complex with the subunits gp91^{phox} and p22^{phox}, and the cytosolic factors p47^{phox}, p67^{phox}, and the small GTPase Rac-1, which translocate to the membrane to assemble the active complex after cell activation (Fig. 2). Activated Rac-1 stabilizes the NADPH-oxidase complex and promotes the production of ROS, which is used for host defense as well as oxidation of LDL. ROS can also directly activate extracellular signal-regulated kinases (ERK), a member of mitogen-activated protein kinases (MAPKs), which regulate cell proliferation, differentiation, motility, and survival, and the PI3-kinase enzyme complex, creating a bridge between the MAPK and PI3-kinase pathways (33). In addition, M-CSF can directly induce PI3-kinase activation and phosphatidylinositolphosphate formation, resulting in NADPH-oxidase–mediated ROS production, which leads to induced Erk activation and monocyte survival (11).

The assembly and activation of the NADPH-oxidase enzyme complex is also regulated by extracellular calcium influx and calcium release from intracellular stores through IP_3 from PI3-kinase modulation. PKCα-dependent phosphorylation of $cPLA_2$, followed by arachidonic acid (AA) release from cytosolic membrane phosphatidylcholine (PC) (21), also activates NADPH-oxidase by regulating the translocation of p47^{phox} and p67^{phox} to the membrane after phosphorylation mediated by PKCδ. In neutrophils and monocytes, the S100A8/A9 (MRP-8/MRP-14) protein complex functions as an AA-binding protein, which is mobilized on activation. In addition, this complex might serve as a scaffold protein for the cytosolic factors of NADPH-oxidase activation and help them to migrate to the membrane-bound flavocytochrome b, as shown for activated neutrophils. In this manner, the S100 A8/A9 complex could be considered a component of NADPH-oxidase activation (103). It could also be demonstrated that the S100 A8/A9 AA complex directly interacts with the fatty acid transporter FAT/CD36 in raft-membrane microdomains, thereby accelerating the dissociation of the complex and facilitating the uptake of fatty acids by endothelial cells (ECs) (75).

Perturbation of actin dynamics induces NF-κB activation in

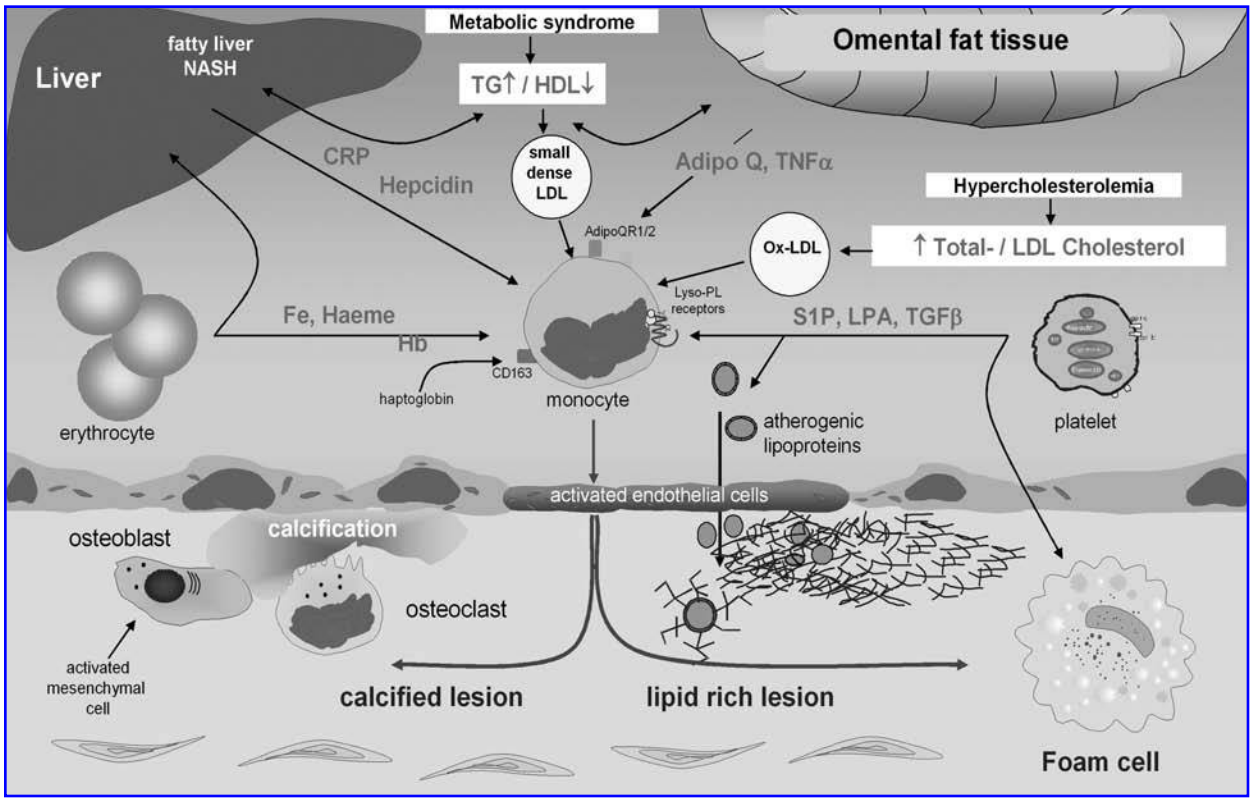

FIG. 1. The role of monocytes/MACs and the respective priming factors in the development of atherosclerosis. TG, triglyceride; CRP, C-reactive protein; TNF, tumor necrosis factor; Hb, haemoglobin; S1P, sphingosine-1-phosphate; LPA, lysophosphatidic acid; TGF, transforming growth factor.

myelomonocytic cells through an NADPH-oxidase–dependent pathway (83). NF-κB activation after actin cytoskeleton disruption could be physiologically relevant for monocyte activation or recruitment into injured tissues or both, where cellular attachment, migration, and phagocytosis result in cyclic shifts in cytoskeletal organization and disorganization. Several lines of evidence indicate that the intracellular redox status participates in modulating NF-κB activation. This is based on the following observations: (a) a broad range of antioxidants can block NF-κB activation; (b) most, if not all, agents activating NF-κB are known to trigger the formation of ROS; and (c) NF-κB activation can be induced by treatment with H_2O_2 (83).

Peripheral blood monocytes, recruited at sites of inflammation, acquire distinct MAC functions. The classic activation by interferon-γ, as well as the innate activation by TLR ligands, including lipopolysaccharide (LPS) lipoteichoic acid (LTA), and prostaglandins, induce the production of ROS, which is extensively reviewed by Shi C *et al.* (136). At least part of this system (*e.g.*, CD14, CD55, TLR2, and TLR4) is also associated with raft-membrane microdomains (117), and signaling leads to NF-κB activation.

NADPH-oxidase as well as the tyrosine kinases of the Src family (*e.g.*, Lck, Fyn, and Lyn), which are lipid-modified signaling proteins, GPI-linked proteins, and adaptor proteins are constituents of raft-membrane microdomains. On receptor binding, immune receptors become raft associated, and additional components of the signaling pathways are recruited to rafts to form signaling complexes. The entry of immune receptors into rafts can regulate cell activation, and raft integrity is crucial for the initiation and maintenance of intracellular signals (120). It has been shown that superoxide production by NADPH-oxidase is inhibited by cholesterol depletion because of impaired translocation of cytosolic pox protein subunits to the membrane (155). Formation of lipid rafts in the membrane of coronary ECs induces clustering and activation of reduced NADPH-oxidase, thereby forming a redox signaling platform on the cell membrane that mediates transmembrane signaling of death receptors, resulting in endothelial dysfunction (164). However, the inappropriate or excessive action of the NADPH-oxidase system results in chronic inflammatory disorders like atherosclerosis.

The sphingolipid Cer has been reported as one of the critical signaling molecules to mediate the activation of NADH/NADPH-oxidase in different cells. Rac-1, a regulatory component of NADH/NADPH-oxidase, which has a high affinity to lipid-binding oxidase, is involved in this process (162). The activation of the Cer-generating enzyme SMase through Ox-LDL could lead to Cer induction (32). Homocysteine (Hcys) stimulates *de novo* synthesis of Cer and consequently activates Rac GTPase, resulting in $O_2^{\cdot -}$ production *via* enhanced NADH/NADPH-oxidase activity in rat mesangial cells (162). Results from our laboratory demonstrated an induction of Cer rafts on Ox-LDL loading of human MACs (58), which could be involved in the activation of NADH/NADPH-oxidase.

Effects of cytokines on MACs

In addition to M-CSF, the cytokine TGF-β also acts as a differentiation factor for monocytes. It stimulates the production of various extracellular matrix proteins and inhibits their degradation, thereby contributing to tissue repair and suppression of inflammation. In injured tissue, stimuli that activate latent TGF-β include plasmin, thrombospondin, and ROS. TGF-β acts *via* autocrine and paracrine feedback loops to increase its production (16). From the cells associated with the blood compartment, platelets contain the highest amount of TGF-β, which is released on platelet activation and acts on ECs and monocytes at sites of endothelial damage and repair. TGF-β activity is regulated by clathrin- and caveolar/lipid-raft-mediated pathways (69).

Anti-inflammatory cytokines like IL-4, IL-10, IL-13, TGF-β, and glucocorticoids or the uptake of chemically modified lipoproteins lead to alternative activation or deactivation of MACs with induced phagocytosis and antigen presentation (136). IL-10, originally described as cytokine synthesis inhibitory factor (CSIF), is a pleiotropic molecule that displays both, immunoregulatory and immunostimulatory activities (65). IL-10 strongly inhibits the expression of cytokines, soluble mediators, and cell-surface molecules in cells of hematopoietic origin, with important consequences for their ability to activate or sustain immune and inflammatory responses. In addition, IL-10 mediates inhibition of free radical generation in MACs, resulting in a decrease of NF-κB activity, and hence decreases the expression of inflammatory cytokines (36).

ATHEROGENIC CONSTITUENTS AND RECEPTORS INVOLVED IN MACROPHAGE FOAM CELL FORMATION

Enhanced uptake of modified lipoproteins by MACs leads to foam cell formation. LDL particles must be chemically modified before they can significantly induce foam-cell formation through scavenger receptors (SRs) or opsonin receptors. The lipids taken up by MACs in lesions are believed to originate predominantly from Ox-LDL (127), enzymatically hydrolyzed LDL (E-LDL) (10), and LDL modified by advanced glycation end products (AGEs). E-LDL is generated by proteolytic cleavage of apoB and hydrolysis of core cholesteryl esters, leading to liposome-like particles similar to lesion-derived LDL, which are present at early stages in atherosclerotic lesions (10, 133). LDL oxidation activates a platelet-activating factor (PAF)-acetylhydrolase–like activity of the denatured apoB molecule with a PLA_2-like activity that strips PC from the Ox-LDL surface (112, 146). This leads to core aggregation of Ox-LDL particles, forming a polar surface with the remaining phospholipids on the aggregated particles. AGEs are a heterogeneous group of irreversible adducts from reactive derivatives of nonenzymatic glucose–protein condensation reactions, as well as lipids and nucleic acids exposed to reducing sugars. For a summary of atherogenic lipids/lipoproteins and their receptors, see Table 1.

Cellular uptake of these lipids and lipoproteins may be mediated by charge and motif receptors (scavenger receptors) directly recognizing chemically modified nonopsonized ligands. Alternatively, modified lipids and lipoproteins may be op-

FIG. 2. M-CSF signaling events leading to proliferation, apoptosis and cytoskeletal re-organization of MACs. Signals in blue, purple, red and green mediate proliferation, differentiation, apoptosis, and cytoskeletal reorganization, respectively, while yellow identifies those common to proliferation and differentiation. M-CSF activation leads to signaling through extracellular signal-regulated kinases (ERKs) and PI3K/Akt. M-CSF also activates NADPH-oxidase to enhance ROS production. NADPH-oxidase is also activated through intracellular $[Ca^{++}]_i$ from extracellular and intracellular pools. Rac-1 a constituent of NADPH-oxidase activates PI3K. Binding of M-CSF stimulates phosphorylation of Y807. Consequently, tyrosines 559, 697, and 721 are phosphorylated and become docking sites for proteins containing SH2 domains. C-Cbl contains docking sites for PI3K and Grb2 which is constitutively bound to Sos, which recruits the guanosine exchange factor Ras to the plasma membrane. This multimeric complex is predicted to be highly stable and hence generate prolonged signaling, with the subsequent Raf/MEK/ERK and PI3K/PDK1/Akt cascades acting as downstream effectors. c-Cbl also acts as an E3 ubiquitin ligase for a number of proteins. **Proliferation.** ERK 1/2 induce expression of D-type cyclins, which hyper-phosphorylate the pocket protein Rb, and the resulting changes in the upregulation and downregulation of multiple genes stimulate proliferation. Separately, enhanced Akt suppresses the levels of p27, an inhibitor of cyclin function, while increasing those of all three cyclin D isoforms. **Differentiation.** Prolonged ERK activation leads to a marked increase in the levels of several transcription factors, whose downstream targets include a numer of genes. C-Fos is a transcriptional activator of Fra 1 and then NFAT2. In parallel, ERKs phosphorylate the Mitf family of transcription factors. **Apoptosis.** M-CSF, acting through the PI3K/Akt axis, is a major mediator of survival signals. Via Akt M-CSF also targets the pro-apoptotic BCL-2 family member Bim and mTOR. **Cytoskeletal re-organization.** M-CSF, by activating their upstream GEFs, stimulates activation of small GTPases such as Rac and RhoA, which are remodelling the actin cytoskeleton. In addition GEFs can activate rac-1 of NADPH-oxidase. **Phosphatase regulation.** A number of phosphatases alter the strength of signals. SHP-2, recruited to Grb2 via the Gab docking proteins, inhibits the activity of RasGAP, a molecule inactivating RasGTP and thus increasing ERK signaling. The serine threonine phosphatases MKP-1 and PP2A, whose expression is induced by M-CSF, reverse the activity of ERKs and multiple substrates, respectively, once again limiting downstream signals. The polyinositide 5′ lipid phosphatase SHIP1, converts PIP3 3,4,5 to PIP2 3,4, a process that suppresses Akt activation. Integrin binding ($\alpha v\beta 3$) leads to recruitment of Syk to the β_3 cytoplasmic domain. Syk recruits c-Src, which becomes activated by phosphorylation on Y416. Activated c-Src phosphorylates and thus activates Syk, leading to activation of Vav3 GTPase from its GDP to GTP bound form, prompting organization of the actin cytoskeleton. (Modified from Ross F. and Teitelbaum S.; Immunological Reviews 2005; Vol. 208: 88-105 (126))

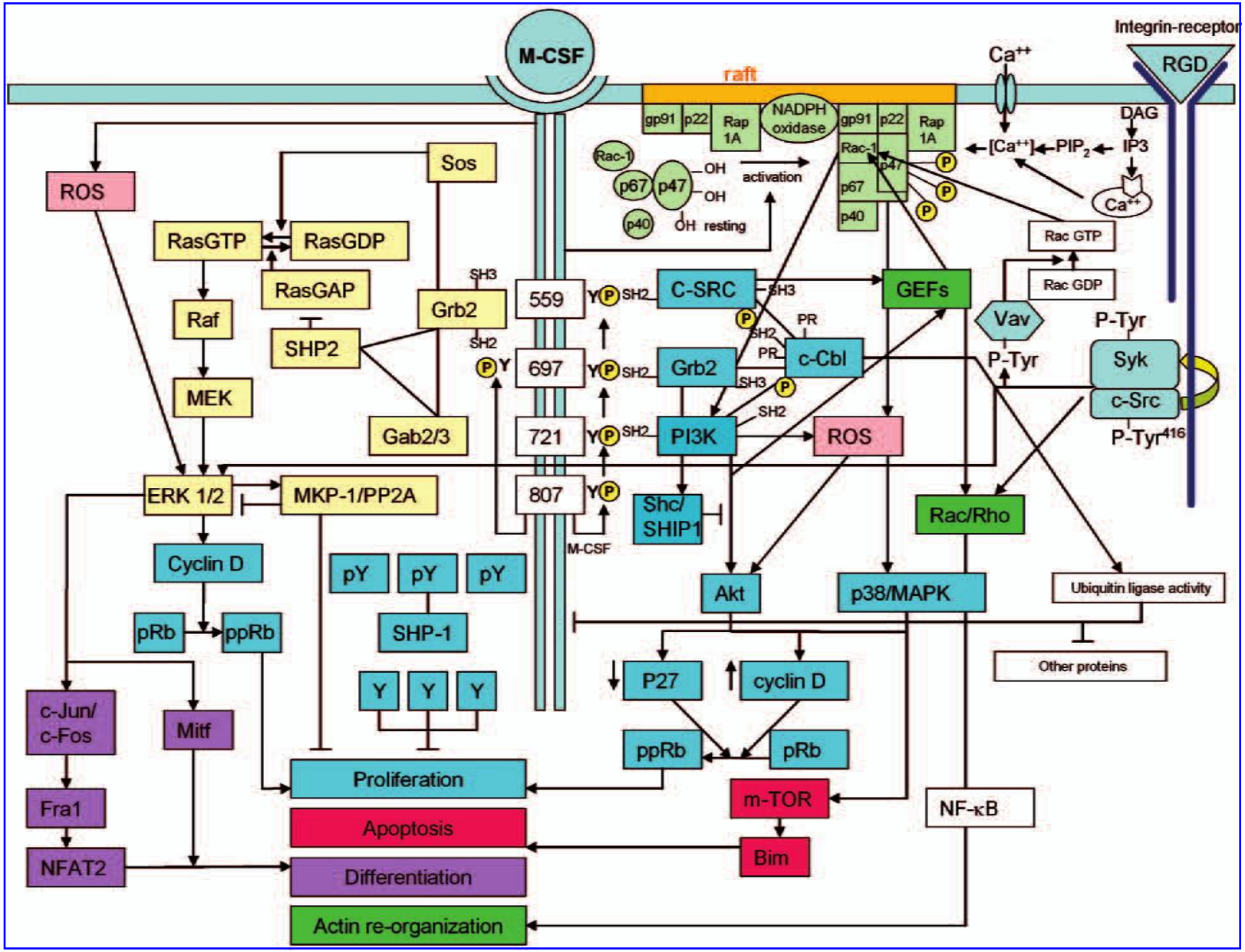
M-CSF
raft
Integrin-receptor
RGD
Ca++
DAG
IP3
PIP2
[Ca++]
gp91
p22
Rap 1A
NADPH oxidase
Rac-1
p67
p47
p40
OH
activation
resting
ROS
Sos
RasGTP
RasGDP
RasGAP
SHP2
Raf
MEK
ERK 1/2
MKP-1/PP2A
Grb2
Gab2/3
SH3
SH2
559
697
721
807
C-SRC
Grb2
PI3K
c-Cbl
PR
GEFs
Rac GTP
Rac GDP
Vav
P-Tyr
Syk
c-Src
P-Tyr416
ROS
Shc/ SHIP1
M-CSF
Rac/Rho
Akt
p38/MAPK
Ubiquitin ligase activity
Other proteins
Cyclin D
pRb
ppRb
pY
SHP-1
Y
P27
cyclin D
ppRb
pRb
m-TOR
Bim
NF-κB
c-Jun/ c-Fos
Mitf
Fra1
NFAT2
Proliferation
Apoptosis
Differentiation
Actin re-organization

FIG. 2.

Table 1. Atherogenic Constituents of Lipids and Lipoproteins and Their Receptors

Lipids/lipoproteins	*Atherogenic constituents*	*Receptors*
Oxidized LDL (Ox-LDL)	Hydroperoxy- and hydroxyl fatty acids, secondary aldehydic lipid peroxidation products, 7 β-hydroperoxycholesterol	SR-AI, SR-AII, MARCO, SR-B (CD36), Macrosialin (CD68), LOX-1, SR-PSOX
Enzymatically degraded LDL (E-LDL)	Opsonization of free cholesterol and lysophospholipids with	
	innate opsonins:	Opsonin receptors
	Collectins: complement components	CR1, CR2
		CR3 (CD11b/CD18)
	Pentraxins: CRP, SAP, SAA_2, PTX_3	CRP receptors (CD64, CD32)
	Galectins: e.g.: Gal-1, Gal-2, Gal-3	Lectin receptors
	Specific opsonins: immunoglobulins	Fcγ receptors (CD64/CD32/CD16)
LDL-proteoglycan complexes	Basic amino acid residues 3359-3369 in site B of apo-B100	Non-specific uptake by phagocytosis
Lipoproteins aggregated by PLA2, PLC	Lysophosphatidylcholine (LPC), lysophosphatidic acid	CD36 (LPC), CRP opsonization (CD64, CD32)
LDL hydrolyzed by SMase	Ceramide	ApoE (LRPs, CD1)
LDL hydrolyzed by phospholipase C	Phosphatidic acid, lysophosphatidic acid	G-protein coupled receptors (Edg2, 4, 7)
LDL modified by advanced glycation end products (AGEs)	ApoB modification of residues next to the putative LDL receptor binding site	RAGE, PSR, PSOx
Gangliosides (salic acid containing glycosphingolipids)	Sialic acid	Asialoglycoprotein receptor
HDL		SR-BI, ABCA1
LDL	ApoE, apoB	LDLR
	ApoE, chylomicron remnants, α2-macroglobulin, amyloid precursor protein (APP), tPA, protease/protese inhibitor complexes, lipoprotein lipase, PDGF, TGF-β	LRP1 (LRP)

sonized by either innate opsonins (collectins, pentraxins, galectins), or specific opsonins (immunoglobulins), or both, before cellular uptake through different opsonin receptors, including complement receptors, pentraxin family receptors, lectin receptors, and Fcγ-receptors.

Unlike the LDL receptor, SRs are not significantly downregulated during cholesterol uptake, and they induce cholesterol accumulation and foam cell formation. SRs are the most prominent receptors expressed on MACs and foam cell MACs in atherosclerotic lesions that bind to chemically modified lipoproteins, such as Ox-LDL, and remove these lipoproteins from the lesion. These receptors are divided into classes A, B, C, D, E, F, and G, and different types of receptors are members of each class.

Class A receptors include the type I, type II, and type III MAC scavenger receptors (MSR-A), and MARCO (MAC receptor with collagenous structure) (78, 80). MSR-A I and II are expressed on the cell membranes of MACs, whereas MSR-A III is localized in cytoplasmic vesicles and is unable to bind to extracellular ligands (57). CD36 and SR-BI (scavenger receptor type BI) belong to class B of SRs. CD36 is an 88-kDa raft-associated glycoprotein expressed on monocytes/MACs, platelets, ECs, and adipocytes. It binds to various ligands such as fatty acids, anionic phospholipids, S100A8/A9 protein, collagen, thrombospondin, Ox-LDL, and apoptotic cell membranes (42). In atherosclerotic lesions of human aorta, the expression of CD36 predominates in MAC-derived foam cells compared with nonloaded MACs. CD36 also plays a role in MAC fatty acid metabolism, adhesion, and phagocytosis of apoptotic cells (42). Binding of Ox-LDL to CD36 leads to endocytosis through a raft-membrane microdomain pathway that is distinct from clathrin-mediated or caveolin-dependent internalization (163). SR-BI is regarded as an HDL receptor, mediates the selective uptake of HDL cholesterol by hepatocytes and steroidogenic cells (2), and can also function as an LDL receptor (binding and selective uptake). SRB-I and CD36 are fatty acylated proteins that cluster in caveola-like cholesterol-rich lipid domains in cultured epithelial cells (42) and are present in MAC rafts. CD68/macrosialin, a class D receptor, is a member of the LAMP (lysosomal-associated membrane protein) family expressed on endolysosomal compartments and partly on the cell surfaces of MACs (121). CD68/macrosialin is expressed in MACs and MAC-derived foam cells in human and murine atherosclerotic lesions and binds Ox-LDL *in vitro* (122). MARCO, CD36, and macrosialin/CD68 are involved in the uptake of Ox-LDL by MACs and in the transformation of MACs into foam cells during atherogenesis.

The SR cysteine-rich CD163 is a MAC-restricted endocytic receptor for hemoglobin–haptoglobin complexes (39) prevent-

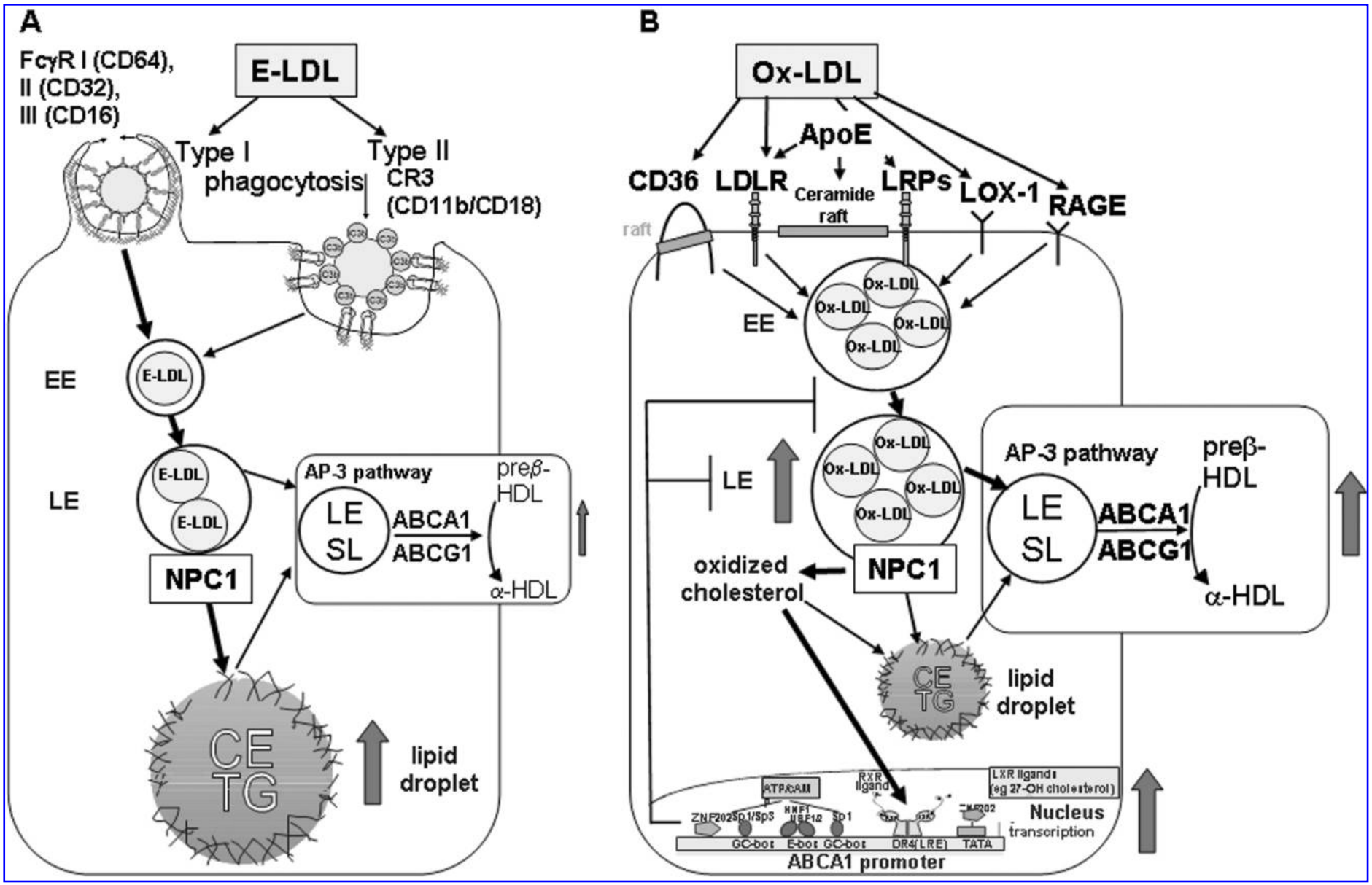

FIG. 3. Differential uptake mechanisms of E-LDL and Ox-LDL. A. Uptake of E-LDL through type I and type II phagocytosis leads to increased lipid droplet formation and moderate upregulation of ABCA1 and ABCG1. B. Uptake of Ox-LDL leads to a rapid expansion of the lysosomal compartment and a pronounced upregulation of ABCA1 and ABCG1. EE, early endosome; LE, late endosomes.

ing MACs from oxidative damage by decreasing heme/iron levels and ROS formation.

Lectin-like Ox-LDL receptor (LOX-1) and SR expressed by endothelial cells (SREC) are expressed on vascular ECs and are classified as class E and class F, respectively (101, 130). LOX-1 is also expressed on MACs in humans and mice (101). It mediates endocytic uptake and subsequent lysosomal degradation of Ox-LDL. Binding of Ox-LDL to LOX-1 induces several cellular events such as induction of NF-κB activation (27) and inhibits upregulation of MCP-1 (86) in ECs. In addition, two other distinct novel SRs, SR for phosphatidylserine (PS) and Ox-LDL (PSOX, SR-G class) and PS receptor (PSR), have been identified (40, 95). SR-PSOX recognizes PS and Ox-LDL and is predominantly expressed in lipid-laden MACs of human atherosclerotic lesions. This receptor does not share any structural homology with other Ox-LDL receptors. PSR is expressed not only in MACs but also in fibroblasts and epithelial cells and is involved in the phagocytosis of apoptotic cells (40).

Differential uptake mechanism of E-LDL and Ox-LDL in MACs

The resulting cholesterol-loaded "foam-cell" MACs after SR-mediated uptake of atherogenic lipoproteins are a hallmark of atherosclerotic lesions and contribute to lesion development. The large cholesterol-rich core of advanced lesions appears to be substantially derived from necrosis or apoptosis or both of foam cells, and cholesterol enrichment of MACs also affects the expression and secretion of several matrix proteins and hydrolases (14). Foam cells contain small but significant amounts of various lipid oxidation products, which are potent mediators of MAC function. E-LDLs are internalized predominantly by opsonin-driven type I and type II phagocytosis involving Fcγ and complement receptors, leading to rapid formation of lipid droplets associated with the cytosolic membrane of the ER (Fig. 3A).

In contrast, Ox-LDLs are recognized mostly by charge and motif receptors, such as CD36 and SR-A, and are often stored in endosomes because of impaired cholesteryl-ester hydrolysis before they induce lipid-droplet formation (73) (Fig. 3B). As a consequence, Ox-LDL loading is associated with a stronger upregulation of ABCA1, ABCG1, and other genes involved in the AP-3 secretory pathway (Fig. 3B). Ox-LDL may also affect cell-surface turnover of Cer-backbone sphingolipids and apoE-mediated uptake by LRP family members, which leads to cell-surface expansion of Cer rafts and activation of apoE/LRP1/CD1–mediated antigen presentation (152).

OX-LDL MODULATES REACTIVE OXYGEN/NITROGEN SPECIES (ROS/RNS) IN MACS

Prolonged half-life of LDL in hypercholesterolemia leads to MPO-mediated damage of Ox-LDL in the vicinity of the plasma membrane and in endocytic vesicles of MACs. A prerequisite for MAC uptake and cellular accumulation of cholesterol is the oxidative modification of LDL (159). The cellular uptake of Ox-LDL perpetuates the generation of ROS and RNS (129), leading to cellular signals and posttranslational modification of critical cellular proteins. In MACs, it is also described that Ox-LDL depletes PKCα and thereby attenuates ROS formation (79). However, PKC isoenzymes are recognized to enhance cellular ROS production through NADPH-oxidase activation (21), whereas adenosine and adenosine analogues target MPO and are potent inhibitors of the respiratory burst in monocyte/MACs (66). Excess ROS levels, known as oxidative stress, may be derived from the imbalance between cellular production of ROS and impaired antioxidant mechanisms. Oxidative stress is harmful to macromolecules such as proteins, lipids, carbohydrates, and nucleic acids, and has been implicated in several pathologic conditions, including atherosclerosis (106).

Constituents of Ox-LDL

Oxidative agents present in Ox-LDL that may be responsible for its proatherogenic effect are aldehyde end products of lipid peroxidation of polyunsaturated fatty acids like 4-hydroxynonenal (HNE), derived from phospholipids, mono-, di-, and triacylglycerols, or cholesteryl esters (CEs), as well as cholesterol oxidation products. These oxidized lipids contribute to lesion progression through their profibrogenic, proapoptotic, procoagulant, and proinflammatory effects. Oxysterols are generated endogenously through oxidation of the lipoprotein lipid moiety and through intracellular reactions like generation of lipoperoxides, which are transferred to LDL through the development of O_2-derived free radicals. Oxysterols may also enter the blood circulation as nutrients through the intestinal mucosa. Oxidation of LDL changes the composition of the particle. The effects depend on the type and concentration of the oxidant and the time of exposure (140). Ox-LDL can be prepared by copper oxidation (53), and especially minimally modified oxidized LDL can be generated by incubation with culture medium from 15-lipoxygenase–overexpressing fibroblasts (138). The unsaturated fatty acyl chains of phospholipids, cholesteryl ester (CE), and triglycerides are oxidized most readily, and a significant proportion of the unsaturated acyl chains are also oxidized to hydroperoxides, isoprostanes, and more AGEs (81). Cholesterol and saturated fatty acids react more slowly, and a small proportion of cholesterol is converted to oxysterols, initially 7-hydroperoxycholesterol. Oxysterols, 27-carbon products of cholesterol oxidation, are possible reactive mediators of structural and functional changes of the vascular wall, which are affected by the atherosclerotic process (85). ApoB, the dominant apoprotein of LDL, which is highly glycosylated, is subject to both direct oxidative modification and reaction with products of lipid oxidation.

Effects of oxysterols

One of the major targets of 4-HNE in ECs is the mitochondrion, where it selectively inactivates proteins containing reactive thiols, such as 2-oxoglutarate dehydrogenase and pyruvate dehydrogenase (71), and thereby can inhibit complex I–dependent (NADH-linked) respiration (72). Ox-LDL induce $O_2^{\cdot -}$ generation in mitochondria, which is converted into H_2O_2 (165). 4-HNEs cause ROS production and activate MAPK in ECs either by directly interacting with PKC or through activation of the EGF receptor (151). A strong HNE-dependent activation of

the transcription factor AP-1 exists for the regulation of a number of genes, namely those coding for TGF-β_1, procollagen type I, platelet-derived growth factor-AA (PDGF-AA), MCP-1, and cyclooxygenase-2 (COX-2) (84).

NF-κB activation relies also on the production of ROS (132). Oxidized LDLs exert a biphasic effect on NF-κB, which on activation upregulates proinflammatory gene expression, such as adhesion molecules, tissue factor, and LOX-1. In contrast, higher concentrations of Ox-LDL may inhibit NF-κB activation triggered by inflammatory agents, such as LPS, and may thereby exert an immunosuppressive effect (125).

Degradation of PC to LPC, as well as the generation of oxidized nonesterified bioactive fatty acid moieties, occurs through oxidative modification of LDL by lipoprotein-associated PLA_2 or PAF-acetylhydrolase (89). LPC is a potent chemoattractant for MACs (82) and neutrophils acting *via* G2A receptors (93), which activate innate immunity and suppress specific immunity at the level of T cells. LPC also upregulates the expression of vascular cell-adhesion molecules such as ICAM-1, which is present within the endothelium and increases monocyte adhesion. LPC can induce mitochondrial ROS production through a Ca^{2+}-dependent process that leads to the selective activation of the ERK/MAPK pathway. The mechanism remains to be defined in detail but appears to involve the interplay between the Ca^{2+}-dependent mitochondrial dehydrogenases and complex I (166).

Although modest levels of Ox-LDL may stimulate MAC survival and proliferation, high doses cause death, probably through oxysterols (26). Ox-LDL is directly chemotactic for monocytes/APCs and T cells (119), mitogenic for MACs and smooth muscle cells, and stimulates the release of MCP-1 and M-CSF from ECs and the production of many inflammatory mediators (*e.g.*, endothelin-1) from other vascular cells (41). Ox-LDL also activates NOS and increases production of NO as a key regulator of vasodilatation. Multiple interactions of $NO^{\cdot}$ with oxidizing lipids could lead to either vascular protection or potentiation of inflammatory vascular injury (107). Ox-LDL increases $O_2^{\cdot -}$ production in ECs and decreases the bioavailability of NO through a process involving LOX-1 (27). Low levels of $NO^{\cdot}$ generated by eNOS can terminate lipid radicals and inhibit lipoxygenases, which would be protective (107). But elevated levels of $NO^{\cdot}$, for example, after inducible nitric oxide synthase (iNOS) expression in inflammation, can be converted to prooxidant species like peroxynitrite ($ONOO^-$) and NO_2 (107). In the presence of available O_2 radicals, Ox-LDL may contribute to $ONNO^-$ formation, which can potentiate inflammatory injury of vascular cells. $ONOO^-$ is more than two orders of magnitude more potent than H_2O_2 in catalyzing lipid oxidation *in vitro* and, in contrast to transition metal catalysts, mediates LDL oxidation even in the presence of lipophilic antioxidants (107, 114). A role for $ONOO^-$ in initiating lipid oxidation in atherosclerosis has been suggested (19). Further, the reactions of $NO^{\cdot}$ and $NO^{\cdot}$-derived species (*e.g.*, $NO^{\cdot}$ or $ONOO^-$) with oxidizing lipids leads to generation of novel nitrated lipid derivatives. If formed in atherosclerotic lesions, these species will either act as novel $NO^{\cdot}$ donors or possess distinct signaling properties similar to eicosanoids (108).

According to the response to retention hypothesis (158), all atherogenic lipoproteins, once deposited in the intima, may exert direct or indirect proinflammatory effects. The endothelial cell and subendothelial cell matrix provide a stage for several interactions that mediate lipoprotein transport, retention, and modification during atherogenesis. ROS are important intracellular signaling molecules that regulate vascular function by modulating vascular cell contraction/dilation, migration, growth/apoptosis, and extracellular matrix protein turnover, which contribute to vascular remodelling. The atherogenicity of lipoproteins is dependent on type and extent of chemical alterations and their diversity of biologic effects to induce chronic inflammatory reactions during lesion formation. They can augment the production of cytokines by vascular cells, and through autocrine and paracrine mechanisms, the inflammatory reaction may lead to a vicious cycle resulting in perpetuation of repair processes and lesion progression.

Influences of Ox-LDL on peroxisome proliferating activating receptors (PPARs)

Fatty acids and their oxidation products activate the nuclear orphan receptors PPARs. These are ligand-activated transcription factors that play an important role in obesity-related metabolic diseases such as high-triglyceride/low-HDL syndromes, insulin resistance, and coronary artery disease (22). PPARs bind, on heterodimerization with RXR, to specific peroxisome proliferator response elements (PPREs) in the promoter of target genes, thus regulating the transcription of these genes. PPARs consist of isoforms α, γ, and δ with distinct expression patterns and biologic activities. PPARs are expressed in atherosclerotic lesions and have been shown to affect transcription of genes in vascular endothelial cells, smooth muscle cells, monocytes, and monocyte-derived MACs. PPAR-α induces an increase in ROS, which leads to induction of NADPH-oxidase activity in MACs and results in the generation of LDL species with PPAR-α activation properties (145). PPAR-γ expression is significantly increased on monocyte-to-MAC differentiation, and PPAR-γ protein is present at high levels in monocytes and MAC-derived foam cells of atherosclerotic lesions (123, 147, 148) and in circulating human monocytes, where its activation increases the expression of MAC-specific markers, such as CD14 and CD11b, which are constituents of lipid rafts.

Treatment of MACs with Ox-LDL *in vitro* induces mRNA expression of PPAR-γ and LXR-α, a direct transcriptional target of PPAR-γ. Internalization of Ox-LDL provides the cell with activators of PPAR-γ, such as the oxidized fatty acids 9- and 13-hydroxyoctadecadienoic acid (9- and 13-HODE), as well as with activators of LXRs such as 27- and 25-hydroxycholesterol (47, 104). PPAR-γ ligands can also be produced locally in atherosclerotic lesions through the oxidation of fatty acids by 12/15 lipoxygenase (68). Arachidonic acid metabolites derived from the cyclooxygenase and lipoxygenase pathways [*e.g.*, 15-deoxy-Δ-12, 14-prostaglandin J2 (PGJ2), and 15-hydroxyeicosatetraenoic acid (15-HETE) (77, 104)] activate also PPAR-γ. Synthetic PPAR-γ ligands are the antidiabetic glitazones like thiazolidones (TZDs) and the nonsteroidal anti-inflammatory drugs (NSAIDs) like indomethacin and ibuprofen. They also affect Ox-LDL processing like the endogenous ligands. It has been suggested that Ox-LDL not only elicits an MPO-dependent oxidative burst on first contact, but also promotes desensitization of MACs [*i.e.*, by reducing ROS pro-

duction *via* activation of PPAR-γ (44)]. PPAR-γ activators inhibit the expression of MMP-9 in human MACs (92) and vascular smooth muscle cells (91) and the production of the inflammatory cytokines TNF-α, IL-6, and IL-1β by activated monocytes (74). The transcription of MCP-1 is decreased, and the expression of intracellular adhesion molecules ICAM-1 and VCAM-1 is inhibited by troglitazone in human ECs (113). The induction of the scavenger receptor CLA-1/SR-BI is inhibited by PPAR-γ in human MACs (24). Activation of PPAR-γ has been shown to enhance CD36 expression of MACs, which may indicate that PPAR-γ could stimulate uptake of Ox-LDL and contribute to foam cell formation (104, 148). These CD36 effects may be compensated through the activation of LXRα, which promotes cholesterol efflux by modulating expression of ABCA1 and apoE (154).

Oxidative stress and the heme/haptoglobin/CD163 pathway

Heme/iron oxidative damage can be promoted by increased heme/iron levels released into the plasma from damaged red blood cells that are removed by binding to hemopexin and haptoglobin and subsequent cellular uptake *via* CD163 cysteine-rich SRs into monocytes and MACs (see Fig. 1). Heme is oxidized and rapidly converted into hemin. One portion is removed by hemopexin, but the rest interacts with cell membranes (5) and with circulating LDL and HDL (150). In EC membranes, heme releases iron and catalyzes EC damage by oxidants such as H_2O_2 (5) but also promotes regeneration of LDL oxidation (59). Accumulation of hemin, however, triggers an oxidative-stress response that promotes heme degradation by HO-1 into bilirubin, iron, and CO. Because overexpression of HO-1 has been found to protect animals from atherosclerotic lesions, it is suggested that hemin is a risk factor for atherogenesis and that HO-1 can protect cells from oxidative damage (150). Hemoglobin promotes formation of ROS and catalyzes LDL oxidation and covalent cross-linking of the LDL protein apoB through the globin radical (94). Haptoglobin binds to hemoglobin and prevents it from catalyzing oxidative reactions. In moderate quantities and bound to haptoglobin, hemoglobin forms a ligand for the hemoglobin scavenger receptor CD163, which is highly expressed in monocytes and MACs. However, when released in larger amounts, heme products become toxic by mediating oxidative stress and inflammation. Proinflammatory stimuli like LPS or IFN-γ suppress CD163 expression; anti-inflammatory molecules like glucocorticoids or IL-10 strongly induce CD163 on monocytes and MACs (18). In addition, the PPAR-γ agonist 15-deoxy-PGJ2, which inhibits the activation of monocytes and promotes phagocytosis, induces a downregulation of CD163 and an upregulation of CD36 (18). CD163-positive MACs are found *in vivo* during the healing phase of acute inflammation (149), in chronic inflammation (35), and in wound-healing tissue (54). In freshly isolated monocytes, the $CD14^{bright}/CD16^{+}$ monocyte subset revealed the highest expression of CD163 (124). Transcription of CD163 was upregulated during M-CSF–dependent phagocytic differentiation, whereas during dendritic differentiation in the presence of GM-CSF and IL-4, CD163 mRNA and protein levels were suppressed (124).

The peptide hormone hepcidin is the principal regulator of systemic iron homeostasis (50). Hepcidin acts by inhibiting the efflux of cellular iron through ferroportin, the sole known iron exporter that is expressed in the small intestine, in hepatocytes and MACs (50). Hepcidin is elevated during infections and inflammation, and its synthesis is homeostatically increased by iron loading and decreased by anemia and hypoxia. Non–protein-bound iron seriously damages biologic systems, mostly by catalyzing the production of ROS and RNS. They can interact with IRP1 and IRP2, which bind to iron-responsive elements (IREs) of several mRNAs coding for proteins involved in the maintenance of iron and energy homeostasis (63, 97). Iron also serves as a cofactor for certain enzymes of the electron transport chain in mitochondria and is involved in the assembly of iron–sulfur clusters and iron–porphyrin (heme) complexes in the mitochondrion. If excess iron exists in the mitochondria, protective mechanisms are overwhelmed, and mitochondrial damage ensues, leading to acute oxidative stress with structural damage and functional impairment (70). Long-term damage to the mitochondrial genome also can occur. The heme/haptoglobin/CD163 pathway seems to be significantly involved in rapid calcified lesion progression and plaque rupture of metabolic syndrome patients, especially in conjunction with end-stage renal disease. The mechanism behind this involves alternative targeting of monocytes toward either phagocytic MACs (foam cells) or osteoclastic MACs (lesion calcification) (see Fig. 1) by mechanisms that are not yet fully understood.

ATHEROGENIC LDL AND LIPID MEMBRANE MICRODOMAINS

Lipid rafts are originally defined as sphingolipid- and cholesterol-rich microdomains in the plasma membrane that play a role in a number of signaling processes involving specific receptors (139). Lipid rafts can be disrupted by cholesterol depletion, whereas cholesterol enrichment stabilizes the formation of lipid rafts (139). E-LDL preferentially increases cellular cholesterol, whereas Ox-LDL increases cellular Cer content because of a higher mRNA expression of acid and neutral sphingomyelinase (SMase), neutral SMase activation–associated factor, and glucosylceramidase during Ox-LDL loading (unpublished observation). Moreover, a higher cell-surface expression of Cer, lactosylceramide (CDw17), globotriaosyl-ceramide (CD77), dodecasaccharide-ceramide (CD65s), and GM1 ganglioside on Ox-LDL loading could be observed. ApoE, in contrast to apoA-I, is bound mainly to Cer-enriched surfaces of Ox-LDL–loaded cells, which preferentially induce cholesterol/Cer-rich membrane microdomains, whereas E-LDL induces cholesterol/sphingomyelin (SM)-rich microdomains (unpublished observation). This is in accordance with the finding that the formation of Cer-enriched domains in lipid membranes enhances binding of apoE (99) and that surface SM reduces apoE-mediated binding and uptake of lipid particles (100), suggesting a higher affinity of apoE to Cer than to SM. These results imply that E-LDL and Ox-LDL differentially influence membrane-microdomain formation in human MACs and thereby differ in their regulation of MAC effector functions during atherogenesis. According to our results, Ox-LDL induces cellular Cer content by increasing acid SMase in human MACs and fibroblasts (32).

The Cer/sphingosine (Sph)/sphingosine-1-phosphate (S1P) rheostat

Cer also can be generated by diverse types of stresses (UVA light, irradiation, heat shock, and hypoxia) or biologic factors (TNF-α, IFN-γ, and Fas antibody) through stimulation of SMases, glucosidase, or Cer 1P-phosphatase, and Cer emerges as a lipid messenger of cell functions including differentiation and apoptosis (109) (Fig. 4).

Cer is degraded by ceramidase to the PKC-modulator sphingosine (Sph), which is then phosphorylated by sphingosine kinase (SphK) to form sphingosine-1-phosphate (S1P) (see Fig. 4). S1P promotes cell survival and growth, antagonizing Cer. This suggests that the metabolic conversion of Cer into S1P could switch cells from an apoptotic state to a survival or proliferative state *via* PI3 kinase/Akt activation. Coupling of multiple TNF- and TLR-family receptors to plasma membrane SMases (13) activates the Cer/Sph/S1P-rheostat on ligand binding (see Fig. 4). The balance between cellular concentrations of Cer and S1P at focal sites of the plasma membrane may trigger distinct downstream signals to determine the physiologic fate of the cell, which is also known as the "Cer/Sph/S1P-rheostat" (116). Its involvement in mechanisms related to membrane-microdomain dynamics in the cell membrane is also of upcoming interest (61).

Effects of Cer-membrane microdomains

Cer might induce coalescence of submicroscopic rafts into large Cer-membrane macrodomains because of its capacity to self-associate through hydrogen bonding (61). These larger structures may serve as platforms for protein concentration and oligomerization, transmitting signals across the plasma membrane. Cer then activates a variety of diverse protein kinase– and protein phosphatase–dependent signaling pathways, which in most cases suppress cell growth or promote programmed cell death or both. Sudden increases of Cer within the plasma membrane have been shown to be involved in the regulation of apoptosis. Stimulation of the CD95/Fas receptor triggers the translocation of acid SMase from intracellular stores to the extracellular leaflet of the cell membrane. Surface-bound acid SMase releases Cer from SM that rapidly forms Cer-enriched platforms in the cell membrane, which might serve as signaling platforms for the transmission of the apoptotic signal (60). Another example for Cer-induced signaling is the finding that the generation of cell-surface Cer through the action of acid SMase is a prerequisite for ligand-induced clustering of the FcγRII (CD32) E-LDL receptor within rafts, which then triggers receptor tyrosine phosphorylation and signaling (1). This Cer-dependent organization of signaling domains in the membrane may relate to progression and treatment of diseases like cancer, degenerative disorders, pathogenic infections, or cardiovascular disease.

Glycosphingolipids, as constituents of lipid rafts and especially Cer, are ligands for apoE, and apoE binds more avidly to Cer-rich microdomains on sphingomyelinase-treated liposomes (99). This indicates a function of Cer-rich microdomains in apoE-dependent metabolism (Fig. 5). The generation of Cer in the plasma membrane by SMases may stimulate HSPG and low-density lipoprotein receptor–related protein (LRP)-mediated uptake by MACs, which is catalyzed by apoE, and plays a crucial role in tissue remodeling and foam cell formation (see Fig. 5) (98). ApoE is also able to bind Cer-backbone lipid antigens and delivers them through CD1 receptor-mediated uptake into endosomal compartments in APCs for antigen presentation (152). Concomitantly, apoE and also apoA-I induce their own internalization signals by binding to plasma membrane phagosome-associated ATP-synthase/ATPase complex (see Fig. 5) (7, 90).

Induction of different raft-associated receptor clusters with Cer and LPS

Cer shares some structural and functional similarities with the lipid A moiety of LPS, and it has been suggested that LPS and Cer recognize the same intracellular molecules (160). The

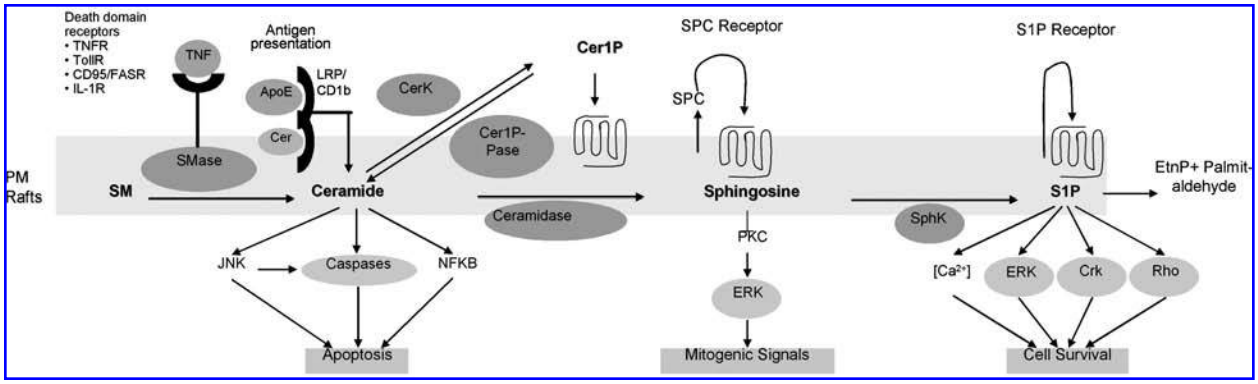

FIG. 4. The SM/Cer/Sph/S1P rheostat model. Cer can be either generated from sphingomyelin (SM) through SMase activation or from ceramide-1-phosphate (Cer1P) via ceramide-1-phosphate-phosphatase (Cer1P-Pase) or from glucosylceramide through glucosylceramidase (not shown in Fig. 4). The generated Cer is involved in antigen presentation via ApoE through LRP, CD1b as well as in apoptosis. Cer can then be further degraded by ceramide kinase (CerK) to Cer1P or through ceramidase to sphingosine. Sphingosine (Sph) is involved in mitogenic signaling via ERK and can be further converted by sphingosine kinase (SphK) to sphingosine-1-phosphate (S1P). S1P acts via ERK, Crk and Rho signaling pathways as a counterpart to Cer and induces cell survival.

cellular responses to LPS include changes in shape, metabolism, and gene expression but also induction of a variety of biologic effects that are involved in systemic inflammation and sepsis (143). The initiation of these effects is due to the activation of monocyte/MACs, leading to the secretion of proinflammatory cytokines such as TNFα, IL-1β, IL-6, and IL-8. Activation of Cer-activated protein kinase (CAPK) by both agonists leads to the activation of MAP kinase and the translocation of activated NF-κB. Although LPS and Cer may share many signaling components, the signaling pathways are not identical (160). The GPI-anchored raft-associated receptor CD14 plays a major role in the inflammatory response of monocytes to LPS, and the binding of Cer to CD14 induces clustering of CD14 to co-receptors in rafts. In resting monocytes, CD14 is associated with CD55, the Fcγ-receptors CD32 and CD64, as well as the pentaspan CD47. Cer exposure recruits complement receptor 3 (CD11b/CD18) and CD36 into the proximity of CD14. In contrast, LPS exposure induces co-clustering with Toll-like receptor 4 (TLR4), Fcγ-RIIIa (CD16a), and the tetraspanin CD81, whereas CD47 dissociates. The different receptor complexes may be linked to ligand-specific cellular responses through rafts initiated by CD14 (117). Thus, clustering of signaling competent receptors may provide an interesting mechanism by which different ligands induce distinct cellular processes in systemic inflammation (SIRS or sepsis) and cardiovascular disease. Host response to LPS occurs primarily through interaction of LPS with CD14 and subsequent activation of Toll-like receptors [for review, see (9)]. Basal TLR4 messenger RNA expression of human monocyte–derived MACs is markedly upregulated on *in vitro* loading by Ox-LDL (161).

Effects of Ox-LDL on lipid rafts and caveolae in ECs

Concerning the relation of atherogenic LDL and lipid rafts in human aortic ECs, Ox-LDL causes the disappearance of the lipid-raft marker GM1 from the plasma membrane (20). Exposure to Ox-LDL may result in the disruption or redistribution of lipid rafts, which in turn induces stiffening of the endothelium, an increase in endothelial force generation, and the potential for network formation (20).

A significant amount of eNOS, which generates NO in the endothelium, is found in specialized lipid rafts called caveolae. Caveolae are flask-shaped invaginations of the plasma membrane that are coated with the protein caveolin, which can function as a negative regulator of eNOS (43). They contain proportionately small amounts of phospholipids and large amounts of cholesterol, sphingomyelin, and glycosphingolipids as well as SR-BI and CD36. Ox-LDL causes an efflux of caveolae cholesterol out of the cell and onto Ox-LDL, leading to a redistribution of eNOS and caveolin to an intracellular membrane (12). This requires the presence of Ox-LDL binding to CD36 (23), whereas the absence of CD36 protects caveolae from cholesterol depletion and the translocation of eNOS out of caveolae and maintains the ability of acetylcholine to stimulate NO production. HDL binding to SR-BI induces the activation of Akt kinase, which subsequently phosphorylates eNOS and stimulates the enzyme to synthesize NO (96). The stimulation of eNOS activity by HDL SR-BI interaction could also be mediated by a Cer-dependent pathway independent of Akt (87). Caveolae-localized sphingomyelin may serve as the substrate for the generation of the Cer that stimulates eNOS. In contrast to Ox-LDL and CD36, which remove cholesterol from caveolae, HDL and SR-BI move cholesterol into them. This could serve as an indirect effect on eNOS function and helps to maintain the cholesterol level of caveolae, which allows eNOS to remain associated with caveolae (23). HDL can also induce the NO-dependent vasorelaxation *via* the lysophospholipid receptor $S1P_3$ (105).

ANTIOXIDANTS INVOLVED IN ROS DEFENSE

The thioredoxin/glutathione system

Oxidative stress is the result of an imbalance of the intracellular redox state, which is regulated by different redox couples. A redox couple is a molecule or enzyme that switches between reduced and oxidized forms. Two of the most important redox couples are thioredoxin and glutathione. Trx is a small protein carrying two cysteines that reversibly switches from dithiol to disulfide [$Trx(SH)_2$ to $TrxS_2$]. $TrxS_2$ is generally reduced by NADPH and flavoprotein thioredoxin reductase (TrxR) (Fig. 6). GSH is a cysteine-containing tripeptide that reversibly forms a homodimer, GSH disulfide (GSSG). By providing electrons, Trx and GSH maintain intracellular proteins in a reduced state. As part of the cellular defence against oxidative stress, expression of different genes of the GSH and Trx systems is increased when cells are exposed to ROS (106). ROS, RNS, and electrophilic lipids contribute to the posttranslational modification of protein thiols (protein-SH) to form *S*-nitrosothiols (SNOs). This is a prevalent posttranslational protein modification involved in redox-based cellular signaling. Under physiologic conditions, protein *S*-nitrosylation and SNOs provide protection preventing further cellular oxidative and nitrosative stress. Conversely, increased oxidative stress and the resultant dysregulation of NO are implicated in the pathogenesis of cardiovascular diseases (142).

The Trx system in MACs is important in the propagation of cell proliferation, differentiation, and the maintenance of specific MACs functions and influences the process of inflammation by modulating the expression and secretion of signaling compounds that are involved in the cellular crosstalk between MACs and T and B cells. In addition, the GSH/glutaredoxin (GR) system plays a critical role in protecting MACs from Ox-LDL–induced cell death, and the disturbance of this system renders MACs susceptible to oxidative stress-induced cell injury (156). Because the GSH/GR system is intimately involved in the recycling of dietary antioxidants such as vitamins E and C, a compromised GSH/GR system in MACs and foam cells in atherosclerotic lesions may at least in part explain why classic antioxidant therapy has not proven successful in combating atherosclerosis.

GSH and Trx are involved in cardiovascular disorders, and low serum concentration of GSH is associated with coronary artery disease (102), whereas elevated serum Trx levels are correlated with acute coronary syndrome (137). Ox-LDLs are able to modulate the intracellular GSH level through the induction

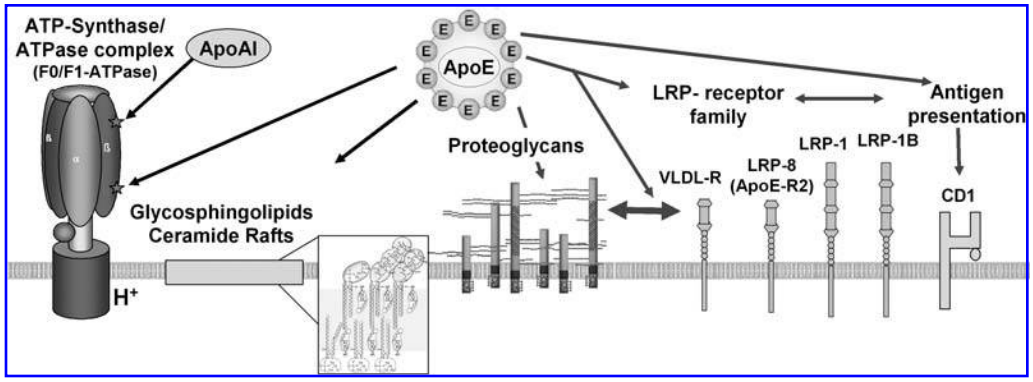

FIG. 5. ApoE is a multireceptor ligand that modulates low density lipoprotein receptor-related protein (LRP)-receptor family mediated turnover of plasma membrane proteins. In addition apoE and apoA-I induce internalization signals through binding to ATP-synthase/ATPase located in the plasma membrane. Glycosphingolipids especially Cer are ligands for apoE. The generation of Cer in lipoproteins by SMase may stimulate heparan sulfate proteoglycan (HSPG) and low density lipoprotein receptor-related protein (LRP)-mediated uptake by MACs, which could be intensified by apoE, and play a crucial role in foam cell formation. ApoE is also able to bind lipid antigens and delivers them by receptor-mediated uptake into endosomal compartments containing CD1 in antigen presenting cells.

of enzymes in GSH synthesis (135), and MACs treated with Ox-LDL have increased cellular content of GSH (29) and activate the antioxidative Trx and GSH systems and Trx, TrxR1, and GSH reductase (62). Pharmacologic depletion of cellular GSH enhanced Ox-LDL cytotoxicity to human monocytes and MACs (56), suggesting that GSH may protect MACs from Ox-LDL cell injury. In human MACs, Ox-LDL promotes thiol oxidative stress and cell injury by disrupting the GSH redox state (156). Three major pathways appear to contribute to this process: (a) depletion of GSH, (b) inhibition of GSH-reductase, and (c) oxidation of protein thiols (156). The convergence of these three pathways results in enhanced protein-*S*-glutathionylation, and the ensuing loss of protein functions leads to MAC death. Glutaredoxin (thioltransferase), which acts as

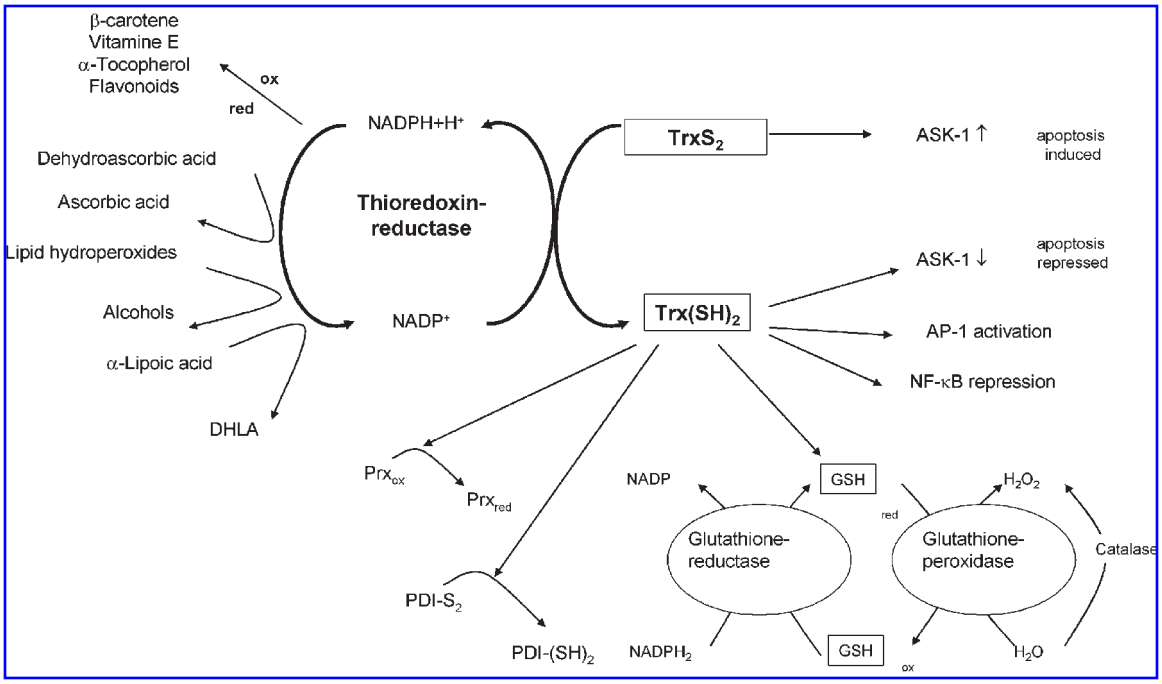

FIG. 6. Major physiological functions of the mammalian thioredoxin system. Reduction of hydrogen peroxide and regeneration of the active site in glutathione peroxidase are reactions that can also be directly catalyzed by Thioredoxinreductase (TrxR). Thioredoxin-dependent reduction of hydrogen peroxide is highly efficient in conjunction with the activities of peroxiredoxins and to some extent glutathione peroxidases, although the latter also function without involvement of the thioredoxin system (via GSH and GR). thioredoxin (Trx), dihydrolipoic acid (DHLA), apoptosis signal-regulated kinase 1 (ASK-1), peroxiredoxin (Prx), proteindisulfidisomerase (PDI), glutathione (GSH)

an electron carrier in the glutathione-dependent synthesis of deoxyribonucleotides by the enzyme ribonucleotide reductase, was found highly expressed in MACs invading atherosclerotic plaques (110). Many intracellular redox-sensitive processes, including synthesis of DNA precursors by ribonucleotide reductase, transcription factor regulation, and cellular growth (3), are modulated by the Trx system, composed of Trx, TrxR, and NADPH (3, 106) (see Fig. 6). Because the many antioxidant and regulatory roles of cytosolic Trx are dependent on the activity of cytosolic TrxR, this selenoenzyme together with Trx is increasingly being recognized as an essential component for cellular redox control and antioxidant defence (3, 106). The ubiquitous 55-kDa selenoprotein TrxR1 was found upregulated in human atherosclerotic plaques and expressed in foam cells (49). TrxR1 mRNA in human monocyte–derived MACs dose-dependently increases with Ox-LDLs, but not with native LDLs.

Another role for Trx in the processes of proliferation, differentiation, and apoptosis is the activation of transcription factors like NF-κB and AP-1 by reducing critical cysteines that are important for protein/protein interaction like dimerization and for protein/DNA interactions like the binding of zinc fingers to DNA response elements (67). The apoptosis signal-regulating kinase1 (ASK-1) is also regulated by Trx (128). Although reduced Trx prevents apoptosis *via* an inhibitory binding to ASK-1, this binding is lost when Trx is oxidized (106).

Specific protein disulfide targets for reduction by Trx are protein disulfidisomerases (PDI) and Trx is also a specific electron donor for many peroxiredoxins, which are important for the reduction of peroxides and have generated recent interest for their potential to regulate signaling pathways. In macrophage-derived foam cells on Ox-LDL stimulation, peroxiredoxin I (Prx I) plays a dual role. As an antioxidant, induction of Prx I during treatment with Ox-LDL led to improved cell survival with a decrease in ROS. Additionally, activation of p38/MAPK was dependent on the upregulation of Prx-I. Therefore, Prx I in macrophage-derived foam cells could be considered both an antioxidant and a regulator of oxidant-sensitive signal transduction (28).

Antioxidants and scavengers affecting MAC redox status

The LDL oxidative state is elevated by an increased ratio of poly/monounsaturated fatty acids and is reduced by elevation of LDL-associated antioxidants and scavengers such as ascorbic acid (vitamin C), α-tocopherol (vitamin E), β-carotene, lycopene, and polyphenolic flavonoids (4). Flavonoids, polyphenolic compounds of the human diet, are also radical scavengers that may suppress LDL oxidation and inflammatory progression in the artery wall. Certain flavonoids, such as morin, fisetin, quercetin, and gossypetin, were shown to inhibit the oxidative modification of LDL by MACs, at least partly by conserving the α-tocopherol content of the LDL particles (31). The positive effects of flavonoids have been attributed to competitive inhibition of cyclic nucleotide phosphodiesterase (PDE), an elevation in cAMP level, and subsequent activation of protein kinase A (cAMP-dependent protein kinase), which targets lipid droplet–associated lipases and activates rehydrolysis of triglycerides and cholesteryl esters (115). Thus, PDE_4 relates to lipid droplet formation, and protein kinase A regulates lipid droplet hydrolases for lipid droplet elimination. In addition, flavonoids can reduce MAC oxidative stress by inhibition of cellular oxygenases such as NADPH oxidase or by activating cellular antioxidants such as the GSH system (48). Reduction of the formation and release of ROS/RNS from MACs by flavonoids reduces cell-mediated oxidation of LDL and foam cell formation, leading to attenuation of the development of atherosclerosis.

OXIDIZED HDL AND MYELOPEROXIDASE

When cholesterol acceptors such as high-density lipoproteins (HDLs) are present, cholesterol efflux from MACs is accelerated, which prevents foam cell formation. The ATP-binding cassette transporters (ABCs) ABCA1 and ABCG1 facilitate transport of free cholesterol and cholesterol/phospholipid complexes (UC/PL) across cell membranes in cholesterol efflux pathways. During this process, ABCA1 promotes nascent discoidal pre-β-HDL particle formation from lipid poor apoA-I. In humans and mice, apoA-I is produced primarily in the liver and intestine. Extracellular sources of apoA-I have been shown to increase cholesterol efflux from MACs *in vitro* (64) and are considered to be necessary for the activation of cholesterol efflux through the ABCA1 pathway (144). The Rho family GTPase Cdc42 directly interacts with ABCA1 to control filopodia formation, actin organization, and intracellular lipid transport (34). Vesicular transport processes involving different interactive proteins like β2-syntrophin are involved in cellular lipid homeostasis controlled by ABCA1 (131). ABCA1 and Cdc42 were found partially localized in Lubrol- but not in Triton-X (TX) detergent-resistant membrane microdomains (rafts), and apoA-I preferentially depleted UC/PL from Lubrol rafts, whereas HDL_3 additionally decreased the cholesterol content of TX rafts (37).

Mutations in the ABCA1 transporter gene cause familial HDL-deficiency syndromes such as Tangier disease (TD), which lead to the development of either coronary artery disease or splenomegaly, dependent on specific ABCA1 mutations (17).

In summary, HDL particles or their major apolipoprotein apoAI mediate cholesterol efflux by (a) enhancing lipoprotein degradation and processing through the endosomal lysosomal route, and (b) retroendocytosis and (c) enhance hydrolysis of CE from lipid droplets, and (d) translocation of Chol/PL-complexes from the ER to the plasma membrane directly or (e) through Golgi- and AP-3–mediated secretory pathways.

If HDL precursors are unable to interact with ABCA1 because of oxidative damage, cholesterol clearance is impaired. HDL isolated from patients with cardiovascular disease contains elevated levels of 3-chlorotyrosine and 3-nitrotyrosine, which are two characteristic products of MPO, a heme enzyme secreted by MACs (153).

MPO-dependent oxidation of specific amino acids, mainly tyrosine and methionine residues of apoA-I, impairs its ability to remove excess cellular cholesterol *via* the ABCA1 pathway (134). MPO also generates hypochlorous acid (HOCL), which is also secreted by MACs like H_2O_2 and other ROS (141). Enzymatically active MPO was found in human atherosclerotic le-

sions (30), and lipoproteins that have been modified by HOCL have been detected in advanced human atherosclerotic plaques. Moreover, MPO converts NO_2^-, an oxidate of NO to nitrogen dioxide radical $NO_2^{\cdot -}$, a potent nitrating intermediate (38).

In plasma, HDL is the major carrier of lipid hydroperoxides, which are potentially atherogenic (15) and which are reduced to their corresponding hydroxides when methionine residues of apoA-I are converted to methionine sulfoxide (51, 52). Enzymes carried by HDL, including paraoxonase-1, paraoxonase-3, lecithin:cholesterol acyltransferase, lipoprotein-associated phospholipase A_2, and possibly glutathione phospholipids peroxidase have been proposed to degrade lipid oxidation products (6, 153). Therefore, the antioxidant effects of HDL may be important for its antiatherogenic and anti-inflammatory properties. HDLs inhibit monocyte transmigration in response to Ox-LDL. This property appears to be related to paraoxonase and PAF-acetyl hydrolase on HDL and is reduced in acute inflammatory states as a consequence of the HDL accumulating serum amyloid A (6). Oxidized forms of HDL may activate NF-κB and promote its nuclear translocation in a process that is linked to an increase in the generation of intracellular ROS. NF-κB is activated by ROS and maintained in an inactive state by low levels of NO. The ability of HDL to inhibit ROS generation and promote the synthesis of NO, and thus to inhibit the activation of NF-κB, may therefore also contribute to their inhibition of adhesion molecule expression (6).

Evidence suggests that oxidatively modified lipoproteins might retard the formation of atherosclerotic lesions in hypercholesterolemic apoE-deficient mice (88). Moreover, tyrosylated HDL is more potent than native HDL in removing cholesterol from lipid-laden fibroblasts and MACs *in vitro* (46). This process does not appear to involve passive cholesterol desorption from the cell-surface membranes (46), which suggests the possibility that tyrosylated HDL promotes reverse cholesterol transport by interacting with ABCA1 in MACs and perhaps other peripheral tissues more efficiently than native HDL (45, 88). This implicates a yin and a yang for oxidative events in the artery wall (8). Enzymatically active MPO and elevated levels of dityrosine, a marker for protein oxidation by tyrosyl radicals, have been detected in human atherosclerotic plaques (30). However, it is not yet known whether HDL is tyrosylated *in vivo*. Remarkably little is known about oxidative modifications of HDL that occur in the human artery wall.

CONCLUSION AND FUTURE PERSPECTIVES

More detailed work on membrane-microdomain dynamics and dysregulation is necessary to understand how the balance between redox control and antioxidative defence affects membrane-microdomain pathophysiology toward foam cell formation. This research will help to identify novel pharmaceutical targets that regulate raft dynamics and suppress raft-associated oxidative stress responses during LDL exposure in hyperlipidemias. For example, it could be possible to diminish the oxidative stress response by avoiding lipoprotein oxidation by MACs through inhibition of MPO or NADPH oxidase. As another opportunity to prevent oxidative stress, antioxidant mechanisms could be enhanced to protect LDL from modification, or the uptake receptors for atherogenic lipoproteins could be blocked by receptor antagonists.

ABBREVIATIONS

AA, arachidonic acid; ABC, ATP-binding cassette transporter; AGE, advanced glycation end product; APC, antigen-presenting cell; APP, amyloid precursor protein; ASK-1, apoptosis signal–regulated kinase 1; CAPK, ceramide-acitvated protein kinase; CE, cholesteryl ester; Cer, ceramide; CerK, ceramide kinase; Cer1P, ceramide-1-phosphate; Cer1P-Pase, ceramide-1-phosphate-phosphatase; COX-2, cyclooxygenase 2; CSIF, cytokine synthesis inhibitory factor; DHLA, dihydrolipoic acid; EC, endothelial cell; E-LDL, enzymatically degraded low-density lipoprotein; ERK, extracellular signal–regulated kinase; GEF, guanine nucleotide exchange factor; GM-CSF, granulocyte macrophage colony-stimulating factor; GR, glutaredoxin; GSH, glutathione; GSSG, glutathione disulfide; Hcys, homocysteine; HDL, high-density lipoprotein; HETE, hydroxyeicosatetraenoic acid; HNE, hydroxynonenal; HOCL, hypochlorous acid; HODE, hydroxyoctadecadienoic acid; HSPG, heparan sulfate proteoglycan; iNOS, inducible nitric oxide synthase; IRE, iron-responsive element; LAMP, lysosomal-associated membrane protein; LOX-1, lectin-like Ox-LDL receptor; LPC, lysophosphatidylcholine; LPS, lipopolysaccharide; LRP, low-density lipoprotein receptor–related protein; LTA, lipoteichoic acid; MAC, macrophage; MAPK, mitogen-activated protein kinase; MARCO, macrophage receptor with collagenous structure; M-CSF, macrophage colony-stimulating factor; MPO, myeloperoxidase, MSR-A, macrophage scavenger receptor class A; NSAIDs, nonsteroidal anti-inflammatory drugs; $ONOO^-$, peroxynitrite; Ox-LDL, oxidized low-density lipoprotein; PC, phosphatidylcholine; PDGF, platelet-derived growth factor; PDI, protein disulfidisomerase; PDE, phosphodiesterase; PGJ2, prostaglandin J2; PL, phospholipids; PPAR, peroxisome proliferating activating receptor; PPRE, peroxisome proliferators response element; Prx, peroxiredoxin; PS, phosphatidylserine; PSR, phosphatidylserine receptor; RANKL, receptor activator of NF-κB ligand; RNS, reactive nitrogen species; ROS, reactive oxygen species; SM, sphingomyelin; SMase, sphingomyelinase; Sph, sphingosine; SphK, sphingosine kinase; SR, scavenger receptor; SR-BI, scavenger receptor type BI; SREC, scavenger receptor expressed by endothelial cells; S1P, sphingosine-1-phosphate; TD, Tangier disease; TLR, toll-like receptor; Trx, thioredoxin; TrxR, thioredoxin reductase; TX, Triton-X; TZDs, thiazolidones; UC, unesterified cholesterol.

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