Role of Monocytes in Atherogenesis

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Østerud, Bjarne, and Eirik Bjørklid. Role of Monocytes in Atherogenesis. Physiol Rev 83: 1069–1112, 2003; 10.1152/physrev.00005.2003.—This review focuses on the role of monocytes in the early phase of atherogenesis, before foam cell formation. An emerging consensus underscores the importance of the cellular inflammatory system in atherogenesis. Initiation of the process apparently hinges on accumulating low-density lipoproteins (LDL) undergoing oxidation and glycation, providing stimuli for the release of monocyte attracting chemokines and for the upregulation of endothelial adhesive molecules. These conditions favor monocyte transmigration to the intima, where chemically modified, aggregated, or proteoglycan- or antibody-complexed LDL may be endocytotically internalized via scavenger receptors present on the emergent macrophage surface. The differentiating monocytes in concert with T lymphocytes exert a modulating effect on lipoproteins. These events propagate a series of reactions entailing generation of lipid peroxides and expression of chemokines, adhesion molecules, cytokines, and growth factors, thereby sustaining an ongoing inflammatory process leading ultimately to lesion formation. New data emerging from studies using transgenic animals, notably mice, have provided novel insights into many of the cellular interactions and signaling mechanisms involving monocytes/macrophages in the atherogenic processes. A number of these studies, focusing on mechanisms for monocyte activation and the roles of adhesive molecules, chemokines, cytokines and growth factors, are addressed in this review.
I. INTRODUCTION

Three cellular components of the circulation, monocytes, platelets, and T lymphocytes, together with two cell types of the artery wall, endothelial and smooth muscle cells (SMC), interact in multiple ways in concert with low-density lipoprotein (LDL)-cholesterol in generating atherosclerotic lesions. The major objective of this review is to focus on the early phase of the development of atherosclerosis, i.e., the formation of fatty streaks (lesions) in the vessel wall, with an emphasis on the roles of monocytes and lipid oxidation.

Recruitment of monocytes and lymphocytes from the peripheral blood to the intima of the vessel wall is a primordial event in atherogenesis (see sect. III), an event that appears to depend on the local presence of high amounts of LDL. As the LDL accumulates, their lipids and proteins undergo oxidation and glycation. Cells in the vessel wall seem to interpret the change as a danger sign, and they call for reinforcement from the body’s defense system. These events appear to promote upregulation of adhesion molecules on the endothelial cells (EC), particularly vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). Thus monocyte and lymphocyte recruitment is initiated, leading to enhanced transmigration of monocytes, upregulated exposure of adhesion molecules on a variety of cells (see sect. ivB), and chemoattractant production and release (see sect. ivC). These are all essential elements of the transfer of monocytes to the intima and the concurrent differentiation of these cells into macrophages. Available LDL is a prerequisite for the further conversion of macrophages into lipid-loaded macrophages, major residents of the fatty streak formed just underneath the endothelium of the vessel wall. LDL and its modified forms (oxidized, acetylated, etc.; see sects. iv, A and B) are of particular interest, since the modification of LDL is associated with inflammatory reactions (see sect. ivC), amplifying proinflammatory events already initiated due to the adherence and transmigration of monocytes and lymphocytes into the intima.

The emerging notion that chronic infections may unleash atherogenic trigger mechanisms (see sect. viii) is suggestive of a very important role of monocytes in lesion formation by way of their proficiency in generating proinflammatory products. This warrants focus on the cellular signal transduction network of monocyte activation, including the early phase entailing phospholipase activation and release of arachidonic acid (see sect. vii). Transgenic mice have opened access to a substantial body of information regarding the roles of the various signal-systems of importance in atherosclerosis (see sects. vii, A and B, ivC, ivB, viiiC, and vii, B–D). Much of the work has focused on the enzymes involved in the conversion of arachidonic acid to leukotrienes and hydroxyfatty acids (lipoxigenases; see sect. vii, C and D) and prostaglandins (cyclooxygenases; see sect. viiiC), lending support to the notion that lipoxigenases may have a vital role in lesion formation (see sect. viiD), whereas the cyclooxygenases appear less important. According to recent evidence, the thromboxane A2 (TXA2) receptor may have an important function in promoting lesion formation (see sect. viiiG), unrelated to the binding of its major ligand, as demonstrated by the noninterference of acetyl salicylic acid (ASA).

In order that monocytes may promote atherosclerosis, a number of unfavorable factors or conditions may have to merge. Polymorphism of the gene of the monocyte CD14 receptor (see also sect. viiD) may be part of the explanation why there are major risk factors unrelated to high cholesterol. This receptor mediates not only the toxic effects of bacteria (lipopolysaccharide; LPS) in chronic infections (e.g., Chlamydia pneumonia), but also the cytokine-like effect of heat shock proteins, which is associated with coronary heart disease (CHD) (see sect. vii). Platelet activating factor (PAF) (see sect. viiF) and cytokines, particularly tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6 (see sect. viiC), and growth hormones, promote the inflammatory reactions associated with atherogenesis. Another significant regulator of monocyte/macrophage function is the peroxisome proliferator-activated receptor-γ (PPARγ), studies on which might shed further light on processes central to atherogenesis (see sect. viiH).

It has not been the intention in this article to cover all aspects of atherosclerosis. For example, changes in the endothelium during atherogenesis, the role of smooth muscle cells and their interactions with macrophages, T lymphocytes, and fibroblasts and their activation products, prominent in the later phase of atherogenesis, have not been presented. Furthermore, the events leading to plaque rupture and thrombosis have not been included.

II. INITIATION OF ATHEROSCLEROSIS

Atherogenic lesions have been observed to arise at regions of the vessel wall exhibiting endothelial activation. Possible causes of such activation comprise elevated and modified LDL, free radicals arising during oxidative stress and cigarette smoking, hypertesion, diabetes mellitus, genetic alterations, hyperactive monocytes/platelets, chronic infections such as by microorganisms like herpes viruses, Chlamydia pneumonia or others, and obviously combinations of these or as yet unrecognized factors (for a review, see Ref. 444). LDL, which may be modified by oxidation, glycation, and aggregation, or incorporated into immune complexes (254, 255, 370, 499), is a major culprit, initiating the signals which promote the recruitment and accumulation of monocytes and T lymphocytes (186, 346, 370). Neutrophils may also have a role in the very early immune response (143).
Such recruitment of leukocytes continues for as long as hypercholesterolemic conditions prevail (362), without any apparent impairment of the vascular endothelium, the latter contributing to leukocyte recruitment by upregulated expression of specific leukocyte adhesion molecules, notably VCAM-1 and ICAM-1 (see sect. iii). In baboons, monocyte margination or attachment observed over normal vessel areas or over plaques was not associated with morphological evidence of endothelial injury, whether under normal or hypercholesterolemic conditions. Migration of these cells through continuous aortic endothelium occurred primarily at junctional sites, between endothelial cells (464). Furthermore, strong interaction between monocytes and components of the extracellular matrix (ECM), e.g., collagen and proteoglycans, is probably a prerequisite for the formation of lipid-laden macrophages (foam cells) (238).

LDL particles trapped in the artery are prone to advancing oxidation, rendering them recognizable by scavenger receptors present on the surface of macrophages and thus targets for internalization by these cells (186, 201, 255, 346, 370, 499), a process ultimately leading to the formation of foam cells. The extent of LDL modification and hence the vigor of this rampant process may vary greatly (123, 186, 370). LDL modified and taken up by the macrophages activates the nascent foam cells, inducing release of inflammatory mediators such as TNF-α, IL-1β, IL-8, and macrophage colony stimulating factor (MCSF). This release in turn leads to increased transcription of the LDL-receptor gene and consequently enhanced binding of LDL to the endothelium and smooth muscle cells (194, 503), feeding the process even further.

Part of the proinflammatory effect of modified LDL on endothelial cells indirectly derives from its chemotactic effect on monocytes (105), and furthermore, it can upregulate the endothelial cell genes for MCSF and monocyte chemotactic protein-1 (MCP-1) (478). Either mechanism elicits an expanding number of monocyte-derived macrophages populating the artery wall.

The arterial wall may be subject to immunological injury leading to conditions favoring atherosclerosis development (81). Recent studies have implicated several immune complexes as proatherogenic. The fatty streak type of lesion typical of the earliest stage, frequently occurring in infants and young children (367), is of a purely inflammatory process involving only monocyte-derived macrophages and T lymphocytes (497). Thus the attachment of monocytes and T lymphocytes to the endothelium followed by their migration into the intima is one of the first and most crucial steps in lesion development. Immune complex-bound antigens taken up by phagocytes via Fc receptors are processed and presented to T cells in the context of major histocompatibility complex (MHC) class I or II molecules. Such antigen processing and presentation greatly enhance T-cell stimulation and the subsequent immune responses (1). This scenario is in accordance with the observation that atherosclerotic lesions are rich in immunoglobulins, which can be found in proximity to macrophages even in very early fatty streaks (390). These immunoglobulins comprise specific autoantibodies to neoepitopes arising during oxidative or other types of injury, some of which have been identified in immune complexes incorporating oxidized LDL (583).

The emerging lesson from transgenic mouse models is that monocytes and macrophages partake crucially in the development of atherosclerotic lesions, whereas results pertaining to T and B cells are more ambiguous and model dependent, suggesting that under certain conditions these cell types may also promote lesion formation (430, 491).

The enrichment of white blood cells in lesion-prone areas has been shown to be of primarily mononuclear origin (98, 439, 483, 549, 587). The prevalence of such changes has been affirmed in a normocholesterolemic rabbit model, where adhered white blood cells were detected in the lesion-prone flow divider regions of the large abdominal branches (320). Interestingly, lesion-rich aortic and coronary artery segments had significantly greater numbers of mast cells in the adventitia compared with those with a normal intima (19). In normal aortic segments, higher numbers of mast cells were located in the lesion-prone than in the lesion-deficient regions. These observations seem to suggest that part of the proatherogenic potential latent in a vessel wall derives from mast cells and their released products, such as histamine and tryptase.

The model of atherogenesis initiation outlined above is mainly based on experimental animal models with high rate of developing lesions, i.e., fat-fed and genetically hyperlipidemic animals. One may therefore question the validity of such animal models regarding the uptake of oxidized LDL and the effects exerted by oxidized LDL. In line with this skepticism is the proposal of a concept of lesions arising in preexisting intimal masses (465): “Given the evidence for a concordance of the distribution of lesions in adult humans, it seems likely that some property of the intimal cells accounts for localization of lesions.” Perhaps the simplest hypothesis, as put forward by Williams and Tabasas (565), is that cells making up the intimal masses have special properties and contribute to lipid accumulation at focal sites. Whether these intimal cells are part of the intimal cellular infiltration and connective tissue described in the coronary arteries of male children, adolescents and young adults remains to be clarified. Interestingly, black people and women have less than average cellular infiltration and connective tissue in their arteries. Whatever factors that are favoring LDL oxidation and macrophage lipid accumulation and retention, even at normal plasma LDL levels, might explain...
the emergence of the initial cluster of macrophage foam cells (336).

III. EXPERIMENTAL EVIDENCE THAT MONOCYTE RECRUITMENT TO THE INTIMA IS LINKED TO ATHEROGENESIS

A. Early Monocyte Recruitment to the Intima

An initial event in atherogenesis is endothelial cell activation, probably mediated by atherogenic lipoproteins such as remnants, modified LDL or oxidized LDL, particularly potent forms of which are those modified by oxidation or glycation (in diabetes) or trapped in immune complexes. Such activation of the endothelium causes upregulation of endothelial cell adhesion molecules and selectins, promotion of oxygen radical formation, increased apoptosis, and reduced endothelium-dependent relaxation.

The multistep process of localized accumulation of leukocytes, which is regulated by the expression of specific adhesion molecules, may leave the endothelium unperturbed. Thus focal recruitment and attachment of monocytes, whether over plaques or normal areas, was not associated with evidence of endothelial injury as shown in normo- and hypercholesterolemic baboons (464). In addition to monocyte recruitment, lymphocyte recruitment is one of the earliest detectable cellular responses in the process leading to the formation of atherosclerotic lesions.

In a transgenic mouse model, two types of endothelial cell-derived adhesion molecules, VCAM-1 and ICAM-1, were shown to play an important role at sites where lesions eventually form (231, 362). Thus homozygous apolipoprotein E-deficient (Apo E−/−) mice that develop atherosclerotic lesions much like those found in humans were compared with normal control mice. Whereas in the latter specific VCAM-1 staining was weak and limited to sites of altered blood flow, in the Apo E−/− mice significant amounts of VCAM-1 appeared to be localized across the surface of EC at lesion-prone sites. The expression of VCAM-1 preceded lesion formation and correlated positively with the extent of exposure to plasma cholesterol. ICAM-1 too was expressed across the surface of EC and microvilli at lesion-prone sites, but at a rate insensitive to plasma cholesterol levels. Similarly, in a recent study, recruitment of circulating white blood cells by endothelial adhesion molecules was suggested to be more important during lesion initiation than during the late phase of rapid lesion growth (294). In rabbits, similar upregulation of endothelial adhesion molecules at lesion-prone as well as already atherosclerotic sites has been reported (231).

There is supporting evidence that many of these adhesion molecules are expressed by cells in human and experimental atherosclerotic lesions and that expression of adhesion molecules such as VCAM-1 is temporally related to lesion initiation and progression in animal models (for commentary, see Ref. 443).

The mechanism whereby LDL interacts with the endothelium is not quite understood. In one study it was shown that binding of native LDL (n-LDL) to the LDL receptor triggers a rise in intracellular calcium which acts as a second messenger in inducing VCAM-1 expression in human coronary aortic cells (9).

VCAM-1 expressed before leukocyte accumulation can initiate both monocyte and lymphocyte tethering and rolling (for leukocyte recruitment at the endothelium, see excellent review by Ley, Ref. 297) and can induce firm adhesion in the absence of chemoattractants. ICAM-1 exposed on microvilli at lesion-prone sites enhances firm adhesion of leukocytes that have reached the primary, transient adhesion step, whereas ICAM-1 more strongly expressed across the endothelial surface promotes cellular arrest where cell-cell contact has already been established. Finally, transendothelial leukocyte migration entailing subendothelial homing and formation of early fatty streaks is supported by the universally expressed platelet-endothelial cell adhesion molecule-1 (PECAM-1) and also possibly enhanced by locally expressed VCAM-1 (362).

A more detailed account of the evidence for the functional role of adhesion molecules and chemokines in disease models is given below.

B. Role of Adhesion Molecules

1. Selectins and VCAM-1

The most comprehensive body of evidence for an important role of adhesion molecules in atherogenesis has emerged from various studies using transgenic mice and a few transgenic rabbit models (286). Thus strong evidence that P-selectin is critically involved in initiation of the process was recently reported based on studies using P-selectin-deficient mice (P−/−) back-crossed onto a C57BL16 background (133). When subjected to a high-fat diet for 20 wk, these mice were significantly less prone to fatty streak formation than were the wild type. Fatty streaks are the first visible sign that foam cells accumulate within the intima, close to the surface of the vessel wall. Furthermore, in the same study, the hypercholesterolemia-prone offspring of P-selectin deficiency (P−/−) animals or a combination of P-and E-selectin deficiency (P/E−/−) animals interbred with mice lacking the LDL receptor (LDLR−/−), were fed an atherogenic diet for 8, 20–22, and 37 wk. At 8 wk, mice with combined P−/− and LDLR−/− had developed significantly smaller fatty streaks than was evident in their half-siblings expressing P-selectin. However, after a more prolonged exposure to the high-fat diet and progression of the lesions
to the fibrous plaque stage, the two forms of offspring were no longer discernibly different.

LDLR −/− offspring deficient also in P-selectin as well as E-selectin were even more profoundly protected against lesion development, even into the fibrous plaque stage (205).

Apo E-deficient mice spontaneously develop lesions when fed a normal Chow diet. They have very high cholesterol levels, on the order of that of hypercholesterolemic humans. The protective effect of P-selectin deficiency in Apo E −/− mice appeared to be more pronounced and long lasting than was the case in LDLR −/− mice (132). Even at 4 mo of age the size of fibrous plaque lesions in P-selectin −/−, Apo E −/− mice was one-fourth of that of Apo E −/− mice having wild-type P-selectin. Hartwell and Wagner (205) emphasize the critical role of P-selectin in atherogenesis suggested by these findings.

Thus the studies on Apo E deficiency mice indicate that P-selectin/VCAM-1-dependent leukocyte rolling is a mandatory step in the early development of atherosclerotic lesions. Further evidence to this end was obtained ex vivo using an isolated carotid artery preparation from 10- to 12-wk-old Apo E −/− and C57BL16 wild-type mice fed a Western-type diet (21% fat wt/wt) for 4–5 wk (424). Adherence and rolling of cells of the mononuclear U937 line on this surface were significantly impaired when P-selectin or its ligand P-selectin glycoprotein-1 ligand (PSGL-1) were blocked using specific antibodies. It was also shown that rolling velocities increased, corresponding to a weaker adhesion, when α4-integrin or VCAM-1 of the mononuclear cells had been blocked using specific antibodies. This was interpreted as evidence that the interaction between α4-integrin and VCAM-1 has a stabilizing effect on rolling interaction, prolonging the monocyte transit time.

In another study it was shown that endothelin-1 (ET-1), which is a potent vasoconstrictor and postulated to play a role in hypertension (502), ischemia-reperfusion injury (404), and atherosclerosis (148), can directly promote significant leukocyte adherence and rolling in a P-selectin-dependent reaction (453).

2. Integrins and ligands

In rats subjected to dietary-induced hypercholesterolemia, ICAM-1 expression was upregulated mainly in the lesion-prone areas of aorta during the early stages of atherogenesis. This was associated with a pronounced recruitment of monocytes and T lymphocytes to the intima (552). Prior injections of specific antibodies to ICAM-1/lymphocyte function antigen-1 (LFA-1) significantly reduced monocyte adherence and migration into the intima.

In another study, ApoE-deficient mice (apo E −/−) maintained on an atherogenic Western diet were subjected to arterial injury, and postransfer effects of the concurrent presence of additional ICAM-1 or P-selectin deficiencies were investigated (325). After 5 wk, a P-selectin deficiency linked 94% inhibition of neointima formation was found, whereas ICAM-1 deficiency gave no discernible protection against injury-induced plaque formation in these mice. It was concluded that absence of P-selectin but not of ICAM-1 reduces the plaque area and that P-selectin is critical for monocyte recruitment to sites of neointima formation after arterial injury.

Very late antigen 4 (VLA-4) is the ligand for VCAM-1 and fibronectin containing segment-1 (CS-1) (86, 138, 189, 555) (Table 1). Evidence has been found favoring the notion that VLA-4 has an important role in regulating leukocyte entry into early (479) as well as advanced (397) lesions. It was suggested that the VLA-4 integrin plays an important role in the initial phase of atherosclerotic lesion formation and lipid accumulation (479). According to the evidence currently available, it seems plausible that P-selectin and its ligand PSGL-1 together with VCAM-1 and its ligand VLA-4 may be the most important adhesion molecules involved in monocyte recruitment to atherosclerotic lesions (228).

3. Other factors

Von Willebrand factor (vWF) has been suggested to be potentially proatherogenic, due to its important role in platelet functioning (114, 164, 504) and in regulating factor VIII in blood (339, 343). Evidence in support of this notion was recently found in studies using transgenic mice deficient in the LDLR (LDLR −/−) as well as vWF (vWF −/−). When fed a Chow diet, these double deficiency mice had a 50% reduction in leukocyte rolling compared with rats with the LDLR −/− trait only, dropping to 20% when an atherogenic fat diet was given (342). The high reduction in leukocyte rolling when a Chow diet was administered was associated with a 50% size reduction of lesions in the aortic sinus. Furthermore, between the superior mesenteric artery and the renal artery, vWF −/− animals were markedly less liable to lesion formation than were wild-type ones.

A list of adhesion molecules and ligands and their leukocyte and endothelial cell targets is shown in Table 1.

C. Role of Chemoattractants

Certain adhesion molecules are currently seen as essential for the recruitment of monocytes to the intima. However, according to several studies it appears that a number of additional factors including several chemoattractants are needed in the orchestration of the process (for reviews, see Refs. 27, 315, 428). The most relevant chemoattractants pertaining to atherogenesis will be discussed in the following sections.
1. MCP-1

Chemokines or chemotactic cytokines belong to an expanding family of structurally related small protein molecules, allocated to subgroups (C, CC, CXC, CX3C) according to the number of and spacing of cysteine residues in the NH₂-terminal region. They are critically involved in directing leukocyte trafficking and activation. MCP-1 emerges as probably having some subordinate role in relation to atherosclerosis. Thus high expression of MCP-1 was found in human atherosclerotic lesions (428). Transgenic mice overexpressing MCP-1 had threefold increased oxidized lipid and revealed increased immunostaining for macrophage cell surface markers unique for the activated state (6). It was concluded that MCP-1 expression mediates enhanced atherogenesis, by increasing macrophage numbers as well as accumulation of oxidized lipid. Corroborative evidence for this notion was found using transgenic C57BL/6 mice deficient in apolipoprotein apo (a). When fed a high-fat diet these mice overexpressed MCP-1 and in a correlating manner macrophages accumulated in their vasculature (431). Correspondingly, when subjecting LDLR-deficient mice to a high-cholesterol diet, the ensuing hypercholesterolemia rapidly triggered MCP-1 expression in resident macrophages. Additional numbers of macrophages expressing MCP-1 accumulated over time, indicating that MCP-1 may initiate as well as amplify monocyte recruitment to the artery wall during early atherogenesis (272). This is consistent with the 50% reduction in lesion formation found in MCP-1-deficient mice relative to wild type (181). Recently, it was shown that MCP-1 induces proliferation and IL-6 production in human smooth muscle cells by differential activation of nuclear factor κB (NFκB) and activator protein-1 (AP-1), which may suggest that some hitherto unrecognized mechanism may be involved in the proatherogenic effect of MCP-1 (542). However, overexpression of MCP-1 at the vessel wall was not sufficient to generate lesion formation in rabbits fed normal Chow, whereas such overexpression under hypercholesterolemic conditions induced infiltration of monocytes/macrophages and subsequent lesion formation in the vessel wall (366). On the other hand, hypercholesterolemia by itself did not cause lesion formation in rabbits with normal MCP-1 despite upregulation of VCAM-1 and ICAM-1. It was concluded that activation of other factors induced by hypercholesterolemia is required.

2. MCP-1 receptors

The function of MCP-1 is totally dependent on its receptors. In monocytes three different receptors, CCR1, CCR2, and CCR3, have been identified, the former two of which were the only ones present in granulocytes (for a review, see Refs. 56, 241, 428). It has been proposed that CCR2 serves as the principal MCP-1 receptor. It is upregulated by cytokines and by LDL (202). Mice deficient in Apo E as well as CCR2 (ApoE −/−, CCR2 −/−) showed a 50% reduction in macrophage recruitment compared with wild
3. Factors affecting the production of MCP-1 and other chemoattractants

Adhesion molecules that tether circulating leukocytes to endothelial cells may also serve as transducers or modulators of incoming signals of cellular activation. P-selectin upregulates the secretion of MCP-1 and TNF-α from monocytes stimulated with PAF (560). Furthermore, IL-8 and MCP-1 were induced in monocytes by thrombin-activated platelets exposing P-selectin (559). IL-8 is produced both in monocytes and granulocytes and has been thought to act predominantly on neutrophils. However, recently it was found that it brought on firm adhesion of rolling monocytes onto monolayers expressing E-selectin, in the same manner as did MCP-1 (175), a faculty not shared by related chemokines. The production of IL-8 in leukocytes is upregulated by epinephrine-stimulated platelets (142). The prevailing perception of IL-8 as rather peripheral involved in atherogenesis may have to be corrected in view of these later findings. It could turn out more important for proinflammatory reactions and atherogenesis than anticipated, perhaps via some unexpected role in monocyte recruitment.

Monocyte-derived macrophages in the intima of the vessel wall generate MCP-1 and other chemoattractants important for the transendothelial migration of monocytes to the intima. Lysophosphatidylcholine (lysoPC) formed by the oxidation of LDL particles is a potent chemoattractant (87, 356, 361, 420), potentially enhancing the extravasation process. Moreover, since lysoPC serves to upregulate a series of proinflammatory products in monocytes/macrophages, it could have an impact on proinflammatory reactions taking place in a vessel wall accumulating oxidized LDL (87, 356, 361).

Although it has been proposed that MCP-1 serves as a principal chemokine directing monocyte infiltration (45, 373, 515, 563, 582), it may be anticipated that this role is shared somehow with the several other chemokines expressed in atherosclerotic plaques. Prominent among the latter are the chemoattractants for monocytes, MIP-1α, MIP-1β, and RANTES (regulated upon activation, normal T-cell expressed) (532). Their common receptor CCR5 is progressively expressed during monocyte differentiation (531). Hence, in contrast to CCR2, the main function of which is in the recruitment of monocytes from the peripheral blood, CCR5 and its ligands appear to have their main role in affecting macrophages already established within the lesions (for a review, see Ref. 428). MIP-1α and -1β colocalize in atherosclerotic plaques (563) and are expressed by T cells in macrophage/foam cell-rich areas of the plaque. This may be signifying chemokine-mediated cross-talk between T cells and macrophages, effectively promoting migration of macrophages more closely to the inflammatory scene.

Macrophages are the richest source of chemokines in atherosclerotic lesions (13, 45, 373, 429, 515, 550, 582). When monocytes are stimulated by the vast array of agonists present at the site of foam cell formation, they too start producing chemokines. The agonists comprise inflammatory cytokines, such as TNF, IL-1β, IL-6, IL-2, etc., produced by macrophages and T cells (304, 327), and modified LDL particles (105, 265, 511, 519). Thrombin-activated platelets induce the expression of chemokines MCP-1 and IL-8 in monocytes (559).

IV. ROLE OF LOW-DENSITY LIPOPROTEIN

A. Early, Low-Grade Events

Cholesterol is transported in the circulation by plasma lipoproteins. The principal cholesterol carrier LDL serves as an exogeneous source of cholesterol and other cellular nutrients for hepatic and various extrahepatic tissues, where it is taken up by receptor-mediated endocytosis. Alternatively, LDL may be entrapped extracellularly in arteries, thus being subjected to a milieu conducive to various kinds of enzymatic and chemical modification. Early stages of arterial lipoprotein modification, marked by the generation of bioactive lipid peroxidative products, may occur without any apparent change in cellular receptor recognition of the particles.

Monocyte-derived macrophages in arteries express cell surface receptors for LDL (LDL-R) as well as scavenger receptors for modified LDL (SR-A, CD36, CD68) (Table 2). Although minimally oxidized LDL particles appear physically indistinguishable from native plasma LDL, they bear a cargo of bioactive molecules. The latter have been shown to cause a 60% increase in arachidonic acid and a 100% increase in 12(S-HETE) release from EC, associated with induced binding of monocytes to EC (219).

Several studies have established that minimally modified LDL (MM-LDL) produces a distinct pattern of EC activation (47, 256, 394). Transcriptional activation of the macrophage-colony stimulating gene by MM-LDL was shown to be mediated by NFκB activation (423). This was further confirmed in a study assessing responses to MM-LDL by examining the expression of inflammatory genes involved in atherogenesis, including MCP-1 and MCSF, and the oxidative stress gene, heme oxygenase-1 (HO-1) (478). Furthermore, studies on EC isolated from the aorta of inbred mice with different susceptibilities to diet-induced atherosclerosis revealed that EC from the susceptible mouse strain C57BL/6J exhibited dramatic transcriptional induction of the inflammatory genes, whereas EC from the resistant strain showed little or no such induc-
endothelial cell activation by mildly oxidized LDL.

LPA receptor antagonists prevented platelet activation and stimulated endothelial stress-fiber formation (482). LPA receptor antagonists prevented platelet aggregation and endothelial cell activation by mildly oxidized LDL.

Altogether this suggests that genetic factors of the vessel wall are of importance in atherogenesis.

Lysosphosphatidic acid (LPA) has been identified as a bioactive compound formed in mildly oxidized LDL and minimally modified LDL. Thus LPA initiated platelet activation and stimulated endothelial stress-fiber formation and gap formation (482). LPA receptor antagonists prevented platelet aggregation and endothelial cell activation by mildly oxidized LDL.

Evidence from human surgical and autopsy material as well as from experimental animal specimens indicates that monocyte and T-lymphocyte adherence is preceded by the deposition of lipids underneath the endothelial cell layer and that monocyte and T-lymphocyte adherence is preceded by the deposition of lipids underneath the endothelial cell layer (478). Altogether this suggests that genetic factors of the vessel wall are of importance in atherogenesis.

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Modification of LDL via oxidative processes is believed to be a prerequisite for the development of atherosclerosis. Oxidation of the particle in the arterial wall is thought to be a complex reaction involving several cell types, including monocytes, macrophages, granulocytes, lymphocytes, endothelial cells, and SMC. Typically oxidation may take place in microenvironments that are exhausted in antioxidants such as vitamin E, carotenoids, ubiquinol, etc. (146). The importance of LDL oxidation in lesion formation was recently documented in ApoE-deficient mice consuming red wine for 2 mo (23) with a subsequent 40% reduction in basal LDL oxidation, and a similar decrement in LDL oxidizability and aggregation associated with a 35% reduction in lesion size. The resistance to oxidation was associated with an accumulation of flavonoids in the mouse macrophages whereby their capacity to oxidize LDL was reduced and they took up about 40% less LDL than macrophages from placebo-treated mice.

Polyunsaturated fatty acids present in LDL are oxidatively converted to lipid hydroperoxides, which are subsequently cleaved forming reactive aldehydes. The latter may covalently modify apolipoprotein B100 by forming Schiff’s bases with exposed lysine amino groups, thus masking the positive charge and in effect increasing the net negative particle charge, rendering the particles recognizable by macrophage scavenger receptors and subject to unrestrained accumulation by these cells.

Details of the oxidation process have been deduced mainly from the results of numerous in vitro studies. Several different reactive oxygen species including superoxide, hydrogen peroxide, hypochlorous acid, hydroxyl radicals, and peroxyxinitrite have been implicated in the initiation of lipid oxidation and peroxidation. Recently, it was shown that macrophage NAPDH oxidase, the principal vehicle for generating reactive oxygen species (ROS), does not contribute critically to the development of atherosclerosis (258), a somewhat surprising notion considering the likely exposure to many different ROS of the particle. Therefore, other unidentified sources of ROS must be considered, e.g., the macrophage and possibly the neutrophil.

B. Modulation of LDL

The spherical LDL particle has a cholesteryl ester-rich core and a surface dominated by free cholesterol, phospholipids, and apolipoprotein B100, the normal ligand for the LDL receptor. It is anticipated that under most circumstances LDL circulating in plasma is protected from oxidation by the presence of antioxidants. In contrast, the less protective milieu of the arterial wall renders the particle significantly more vulnerable and subject to oxidation.

### TABLE 2. Macrophage and endothelium scavenger receptors that bind lipoproteins

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Ligand</th>
<th>Expressing Cells</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scavenger receptor class A, type I and II (SR-A/I)</td>
<td>Oxidized LDL, Ac-LDL, long-chain fatty acids</td>
<td>Macrophages, SMC</td>
<td>115, 448, 509</td>
</tr>
<tr>
<td>CD36</td>
<td>Oxidized LDL, long-chain fatty acids</td>
<td>Macrophages, monocytes, platelets, SMC</td>
<td>363</td>
</tr>
<tr>
<td>CD68 (macrosialin in mice)</td>
<td>Oxidized LDL</td>
<td>Macrophages, neutrophils, mast cells</td>
<td>425</td>
</tr>
<tr>
<td>Scavenger receptor class B, type I (SR-BI)</td>
<td>HDL</td>
<td>Macrophages, epithelial cells</td>
<td>529</td>
</tr>
<tr>
<td>Lectin-like ox-LDL receptor-1 (LOX-1)</td>
<td>Oxidized LDL</td>
<td>Macrophages, SMC</td>
<td>278</td>
</tr>
<tr>
<td>SREC</td>
<td>Ac-LDL</td>
<td>EC</td>
<td>3</td>
</tr>
<tr>
<td>SR-PSOX</td>
<td>Oxidized LDL</td>
<td>Macrophages</td>
<td>218</td>
</tr>
</tbody>
</table>

LDL, low-density lipoprotein; SMC, smooth muscle cells; EC, endothelial cells.
lipoproteins abundantly accumulating at atherosclerotic sites. The finding is evidence for the presence of alternative systems contributing critically to lipoprotein oxidation. Prime candidates are the lipoxygenase enzymes (described later) catalyzing lipid alkoxy radical (LOO•) formation from esterified fatty acids. Such lipid peroxidation may bypass the generation of superoxide or any of its derived reactive products.

Although the mechanisms by which lipoproteins are oxidized in vivo are still debated, some of the products present in minimally modified/oxidized LDL (MM-LDL) have been identified, including 1-palmitoyl-2-(5,6)-epoxyisoprostan E$_2$ (PEIPC), 1-palmitoyl-2-glutaroyl-$sn$-glycerol-3-phosphorylcholine (PGPC), and 1-palmitoyl-2-oxovaleroyl-$sn$-glycerol-3-phosphorylcholine (POVPC), principal inflammatory mediators of reactions promoting atherogenesis. These phospholipid oxidation products are prominent in atherosclerotic lesions. Moreover, they have been shown to mediate the activation of monocytes and/or endothelial cells in vitro in the presence of PAF (290, 505). The administration of WEB 2086, a PAF analog and selective PAF receptor antagonist, to C57BL/6J LDLR (290, 505). The administration of WEB 2086, a PAF analog and selective PAF receptor antagonist, to C57BL/6J LDLR (290, 505) mice fed a Western diet, reduced the fatty streak formation by 62% (505). Altogether, these studies favor the notion of important roles for PAF and/or PAF-like phospholipid oxidation products in mediating atherosclerotic lesion development.

C. Proinflammatory Reactions Associated With LDL Oxidation

Although granulocytes are generally more prolific than monocytes/macrophages in generating oxidizing products, i.e., oxygen radicals essential in the defense system, it appears that also monocytes/macrophages contribute significantly to LDL oxidation. Furthermore, T-helper lymphocytes, generators of IL-4 and IL-13, have been shown to enhance LDL oxidation mediated by activated monocytes in a 15-oxypgenase (15-LO)-dependent fashion (155). Studies using 12-LO and 15-LO knock-out mice have confirmed the importance of the lipoxygenase pathway. Thus mice with a combination of ApoE $^{-/-}$ and 12- or 15-LO $^{-/-}$ traits were significantly less prone to lesion formation than were wild-type animals (107). When a specific inhibitor of 15-LO, PD 14167, was administered in a rabbit experimental model, atherosclerotic lesions had reduced monocyte/macrophage numbers, and fibro-foamy and fibrous plaque lesion development was diminished (53).

1. Impact of oxLDL

Once modified (e.g., oxidized or acylated) and taken up by macrophages, LDL activates the nascent foam cell. Modified LDL is chemotactic for monocytes and can up-regulate the expression of genes for macrophage colony stimulating factor (419, 422) and MCP-1 (293) (Fig. 1). Thus oxLDL may help expand the inflammatory response by stimulating the replication of monocyte-derived macrophages and the entry of new monocytes into lesions. Effects attributed to oxLDL pertaining to macrophage function include proinflammatory effects, such as increased proliferation (199, 330, 333, 450) and expression of inflammatory cytokines (222, 408), toxicity (300), increased expression of metalloproteinases (223), inhibited expression of inducible nitric oxide synthase (223), and effects on macrophage lipid metabolism and accumulation (179, 243, 500, 551). Released inflammatory mediators like IL-1β, TNF-$\alpha$, and MCSF enhance the binding of LDL to the endothelium and smooth muscle and increase the transcription of the LDLR gene (196, 503), thereby having an impact on the migratory pattern of LDL within the artery. Upon binding of modified LDL to scavenger receptors in vitro, various intracellular events are initiated, including the induction of IL-1β (170, 391, 392) and urokinase (147). Thus, given the generation of sufficient amounts of modified lipids and a progressing inflammation, unless there is some tip of the balance, atherosclerosis development is sustained by the mutual reinforcement of these processes.

V. MONOCYTE DIFFERENTIATION AND THE ROLE OF THE EXTRACELLULAR MATRIX INATHEROSGENESIS

A. Macrophage Formation

When circulating peripheral monocytes migrate from the vascular to the extravascular compartment, a process entailing maturation of the cells to macrophages is concomitantly launched. This differentiation process renders the cells ready for active participation in the inflammatory and immune responses. The process has been shown to depend on many transcription factors, and a novel role for NFκB has been suggested due to its accumulation in the cytoplasm of cells differentiated into macrophages (514). Part of the arsenal of the differentiated cells is an acquired acute responsiveness to, e.g., LPS, and enhanced capacity for TNF-$\alpha$ secretion.

Comparatively little is known about the functional properties of macrophages in vivo, but on average their overall reactivity appears to be substantially higher than that of circulating monocytes. Thus, although oxidized LDL may fail to induce monocyte activation in whole blood (65), an altogether different situation prevails in the intima, where a whole series of activation products may be anticipated after interaction of macrophages with oxidized LDL.

Macrophages are key players in many aspects of
A whole spectrum of macrophage subpopulations with distinct and often uncharted functions may be formed, as directed by the microenvironments of the differentiating monocytes. Current knowledge about monocyte differentiation has largely been derived from in vitro studies, many aspects of which may not truly reflect the activation reactions associated with differentiation in vivo. Nevertheless, the upregulation of minimally modified LDL (MM-LDL), which upon further oxidation eventually becomes oxidized LDL. Recruitment of mononuclear cells (monocytes and T lymphocytes) as a specialized inflammatory response to modified LDL exposure characterizes the initiation phase of atherosclerotic lesion formation. Specific adhesion molecules such as von Willebrand factor, the selectins, and vascular cell adhesion molecule (VCAM)-1, expressed on the surface of activated vascular endothelial cells, mediate leukocyte adhesion. Once adherent, the mononuclear cells enter the artery wall directed by chemoattractant chemokines such as monocyte chemoattractant protein-1 (MCP-1). LDL particles trapped in the intima are prone to progressing oxidation, rendering them recognizable by macrophage scavenger receptors and thus targets for internalization by these cells. Upon extensive uptake of modified LDL via scavenger receptors (CD36 and SR-A), macrophages are ultimately turned into foam cells. This differentiation process may be accelerated by macrophage colony stimulating factor (MCSF), lipopolysaccharide (LPS) via the receptor CD14 in conjunction with toll-like receptor 4 (TLR4), by heat shock protein (HSP-60) via CD14, and by platelet activating factor (PAF) and cytokines released from macrophages in an autocrine loop. Peroxisome proliferator-activated receptor-γ (PPARγ) is activated by LDL lipids, leading to upregulation of CD36 and down-regulation of cytokine release. In the process of foam cell formation, cytokines released from macrophages and T lymphocytes are acting in concert on foam cells and on smooth muscle cells and endothelial cells (EC). T-cell mobilization and activation leads to secretion of the cytokine interferon-γ (IFN-γ), which primes the macrophages rendering them more susceptible to TLR-dependent activation. Activated T cells also express CD40 ligand (CD40L), which ligates its receptor CD40 on macrophages. Chemoattractants released from LDL, macrophages, and foam cells (MCP-1) promote further monocyte recruitment to the intima. Although the focus of this review is the role of monocytes/macrophages in the early phase of atherogenesis, it should be stressed that the accumulation of oxidized LDL in the intima and the resultant cellular activation products like chemoattractants, growth factors, and cytokines also promote smooth muscle cell proliferation, uptake of oxidized LDL, and eventually conversion to lipid-laden foam cells. Foam cells derived from smooth muscle cells together with those derived from macrophages generate the fatty lesion.
tion as well as downregulation of certain receptors is well documented. Thus alveolar macrophages showed much lower expression of α4-, α6-, and β2-integrins, CD11a, CD11b, L-selectin, Le (x), and sialyl Le (x) compared with monocytes (416). In one in vitro study it was shown that all adherent monocytes expressed CD14, CD36, and LDLR. In tissue macrophages these antigens were less consistently expressed and defined three cellular subpopulations: CD36+CD14−LDLR− (58 ± 12%), CD36+CD14+LDLR+ (18 ± 5%), and CD36−CD14−LDLR− (remaining cells) (567). Thus CD36 appeared to be present in three-fourths of the macrophages, a significant fraction considering the central role of CD36 as an oxLDL scavenger receptor. Another marker that is also induced during monocyte to macrophage differentiation is the class A macrophage scavenger receptor (SR-A). This protein mediated 80% of the uptake of acetylated LDL by human monocyte-derived macrophages (182). The upregulation of platelet-derived growth factor (PDGF) receptors on human monocyte-derived macrophages has been taken as evidence that PDGF has a role in atherogenesis, regulating the function of macrophages as well as SMC in the vascular wall (232).

B. Foam Cell Formation

A hallmark of the development of atherosclerotic plaques is the prior and concurrent accumulation in the arterial intima of lipoprotein particles subject to chemical modifications. This is associated with local inflammation in the vessel wall and further recruitment of monocytes from the circulation. By taking up such modified LDL (oxidized or acetylated), monocyte-derived macrophages are turned into fat-loaded macrophages residing in the vessel wall and furthering the local inflammatory response. The mechanisms underlying such foam cell generation has for several years been the focus of intensive research (46, 72, 90, 499, 568) (Fig. 1).

Macrophages are normally protected from the accumulation of toxic cholesterol loads by multiple mechanisms, notably the downregulation of surface LDL receptor molecules in response to replete intracellular cholesterol stores (72). However, oxidized or otherwise chemically modified LDL may be taken up by alternate “scavenger” or “oxidized LDL” receptors that are not similarly downregulated when the cholesterol load is in excess (46, 90, 499, 568), thereby evading regular homeostatic control mechanisms. A series of scavenger receptors have been identified and are listed in Table 2. These comprise several classes of transmembrane receptors, a common characteristic of which is an affinity for negatively charged macromolecules or particles like, e.g., modified LDL.

An important role of the SR-A receptor in atherogenesis was inferred from the first analysis of mice with a combined SR-A and ApoE deficiency. These mice had a 60% reduction in atherosclerotic lesion development compared with wild type (509). However, more recent studies have been less affirmative of such a proatherogenic role for SR-A. Thus a combined SR-A and LDL-R deficiency caused only 20% reduction in atherosclerosis (448). Furthermore, when subjecting apoEJ-Leiden mice (another proatherogenic mouse model) to the added deficiency of SR-A, no decrease in atherosclerosis was observed (118). This controversy has been interpreted as an indication that ApoE has a complex role in pathogenesis, related to the fact that ApoE not only acts in plasma lipoprotein metabolism, but it also has a stimulatory influence on the efflux of cholesterol from macrophages (209, 280). One would anticipate the latter effect to serve as a protective mechanism counteracting foam cell formation.

Recently, more solid support for a central role of the class B scavenger receptor CD36 was provided by the demonstration that crosses of ApoE deficiency mice with CD36 deficiency mice had a 70% reduction in lesion area compared with mice deficient in ApoE only, after 12 wk on a Western diet (150). CD36 has been identified as the receptor facilitating myeloperoxidase-modified (MPO-modified) LDL uptake. MPO-modified LDL has a proven capacity to induce foam cell formation in vitro and is likely to be highly proatherogenic. CD36 deficiency in mice reduced the uptake of MPO-modified LDL by almost 90% and the formation of foam cells by >50% (412). It has to be borne in mind, however, that one cannot automatically infer from this marker role of CD36 in knock-out mouse models, that it has a corresponding role in human pathophysiology. Several other scavenger receptors have also been shown to be important for the uptake of oxidized LDL and the pathogenesis of atherosclerosis (see Table 2). Among these scavengers are macrosialin/CD68, lectinlike oxidized LDL receptor (LOX-1) and SREC (for review, see Ref. 120).

The results emerging from transgenic mouse models should be interpreted with caution, considering such factors as control of genetic background, linkage problems, etc. Furthermore, to better mimic the human situation, more refined heterozygotic models assessing various markers and polymorphisms, alone or in combination, may be called for (for a review, see Ref. 266).

The prominent role of modified LDL in atherogenesis suggests that addressing the undesirable proliferation or action of LDL-related products might have considerable prophylactic or even curative potential. Conceivably the following approaches might prove useful: saturation with relevant antioxidants to prevent LDL oxidation, directly at the level of the particle itself or indirectly at the level of the cellular oxidative machinery, or conversion of already oxidized LDL to a nonatherogenic particle using HDL-associated paroxonase (PON-1) (249).
C. Roles of Extracellular Matrix and Metalloproteinases in Atherogenesis

Monocytes migrating into the subendothelial space probably interact with ECM components, notably collagen type I, a major constituent of the normal arterial wall (495), and the most prominent matrix component of atherosclerotic plaques (496). Strong interaction between monocytes and matrix is probably a prerequisite for the formation of lipid-laden macrophages, since lipid does not accumulate in monocytes that do not form stable interactions with tissues, even if they are allowed to differentiate into macrophages (558).

The ECM molecules are synthesized by resident cells of vascular tissue, such as endothelial cells, macrophages, and smooth muscle cells. Atherogenic lipoproteins gaining access to the subendothelial space are bound and retained through ionic interactions between positively charged residues on their apolipoproteins (apo B and apoE), and negatively charged sulfate and carboxylic acid residues on the glycosaminoglycan chains of extravascular or cell-associated vascular proteoglycans (for review, see Ref. 84). One of the tenets of the response to retention hypothesis is that prolonged residence time of lipoproteins in the intima leads to lipoprotein modification.

Recently, it was shown that oxidized LDL particles retained by ECM proteoglycans were taken up by macrophages, provided the ECM had been preincubated with lipoprotein lipase before adding ox-LDL (250).

Matrix metalloproteinases (MMP) are a family of enzymes comprising at least 16 zinc-dependent endopeptidases that are catalytically active against ECM components (for review, see Ref. 174). They are essential for cellular migration and tissue remodeling under both physiological and pathophysiological conditions (359). In vitro studies have demonstrated that the expression of MMP-1, MMP-3, and MMP-9 in macrophages and smooth muscle cells is enhanced by several mediators secreted from T lymphocytes and monocytes, e.g., TNF-α and IL-1β (165, 282), whilst it is suppressed by the general inhibitors of monocyte activation IL-4, INF-γ, and IL-10. The activities of matrix-degrading MMP are essential for many of the processes involved in atherosclerotic plaque formation, including infiltration of inflammatory cells as outlined above, SMC migration, and proliferation as well as angiogenesis. Probably the most serious consequences of MMP activities are the unfavorable effects on plaque stability and resistance to rupture, which may lead to unstable angina, myocardial infarction, and stroke (166).

MMP need to be activated, and urokinase (produced by macrophages) and the plasminogen system are known to play central roles in the processes where MMP are important (102). MMP are in fact tightly regulated by molecules controlling their activation and by specific inhibitors known as the tissue inhibitors of metalloproteinases (TIMPs) (149).

VI. MONOCYTES/MACROPHAGES AND THE ROLE OF THEIR ACTIVATION PRODUCTS

A. Regulation of Activation

Monocytes play a central role under several pathophysiological conditions, particularly when the progression of the disease stems from underlying inflammatory reactions, e.g., in diseases like rheumatoid arthritis, atherosclerosis, psoriasis, asthma, and inflammatory bowel disease. The proinflammatory potential of the monocytes can only be unlocked by their activation/differentiation and subsequent secretion of activation products, the main classes of which are summarized in Table 3. More than 100 different biologically active molecules are known to be secreted by monocytes/macrophages.

Monocytes are activated only according to what is dictated by their particular environment, notably by its content of agonists including primers. The various monocyte agonists associated with monocyte/macrophage activation during atherogenesis are listed in Table 4 according to their type or the group to which they belong. Most of these substances have so far been reported to have agonist properties in relation to monocytes, i.e., activa-

Table 3. Biological products of monocytes and macrophages

| Complement factors (produce all essential components of the complement system) (94) |
| Coagulation factors (the vitamin K-dependent FVII, FX, FV, prothrombin, fibrinogen and tissue factor: all factors needed to generate fibrin) (384, 386) |
| Prostaglandins (see review, Ref. 368) |
| Leukotrienes (451) |
| Growth factors [PDGF (211), TGF-β (547), MCSF and GCSF (75)] |
| Cytokines (TNF, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IL-18, IFN-γ) (78) |
| PAF (117), lyso-PC (429) |
| Chemokines (MCP-1, -2, -3, IL-8, RANTES, ELC, PARC, MIP-1α, β, eotaxin, MDC, TARC, LARC) (for review, see Ref. 27) |
| Oxygen radicals (42, 96) |
| Proteolytic enzymes (e.g., elastase, cathepsin G, metalloproteinases) (474) |

| PDGF, platelet-derived growth factor; TGF-β, transforming growth factor β; MCSF, monocyte/macrophage colony stimulating factor; GCSF, granulocyte colony stimulating factors; PAP, platelet activating factor; lyso-PC, lysophosphatidylcholine; MCP, monocyte chemoattractant protein; RANTES, regulated upon activation, normal T-cell expressed; ELC, EB11-ligand chemokine; PARC, pulmonary and activation regulated chemokine; MIP, macrophage inflammatory protein; MDC, macrophage-derived chemokine; TARC, thymus and activation-regulated chemokine; LARC, liver and activation-regulated chemokine; IL, interleukin; IFN, interferon; TNF, tumor necrosis factor. |
tion is induced/primed by their presence. However, the interpretation of these data has been complicated by the fact that certain poorly charted and hard to avoid low-grade monocyte activation phenomena may be inherent in the isolation and culturing processes per se. Prime suspected culprits are mechanical stress and trace contaminants going undetected or not even looked for in the system, the outcome being already preactivated and/or primed cells, quite commonly not acknowledged as such. Indeed, when monocytes adhere to plastic or to extracellular matrix proteins, signals are delivered that induce expression of immediate-early (IE) response genes (246). While mRNA accumulates in these adherent monocytes, the cytokine expression products including IL-1β and TNF-α are not secreted unless there is a “second stimulus” such as bacterial LPS (206).

In contrast, in the native environment of whole blood ex vivo, LPS appears to be the sole monocyte activator. Cytokines, growth factors, adhesion molecules, etc., reported as stimulants or agonists in cell culture studies, may in the native environment of whole blood only upregulate ongoing activation (383). Thus activation effects assigned to particular agonists cannot be seen in isolation from the overall experimental setting, and the relevance for the in vivo situation should be interpreted with caution.

Many of the products generated by activated monocytes/macrophages require similar transcription factors for their synthesis. Thus active nuclear factors NFκB and AP-1 are mandatory for the production of cytokines, tissue factor, and growth factors, strongly suggesting that they are crucial for monocyte reactivity (Fig. 2) (122). Evidence to support this hypothesis has been obtained from in vivo studies using a mouse strain subjected to an atherogenic diet. The susceptibility to aortic atherosclerotic lesion formation turned out to be associated with accumulation of lipid peroxidation products, induction of inflammatory genes, and the activation of NFκB-like transcription factors (301).

Further evidence that NFκB-dependent gene transcription has a role in atherosclerosis was obtained by subjecting C57B16 mice to intravenous administration of mildly oxidized LDL. This led to induced expression in the liver of the same set of genes as when the mice were fed an atherogenic diet. An inbred strain of these mice susceptible to fatty streak formation was shown to have particularly profuse expression in liver of genes associated with inflammation, and this trait cosegregated with a propensity for activation of a NFκB-like transcription factor and with the level of oxidized lipids in the same organ (302).

Most of the products listed in Table 4 are probably more effective in activating monocyte-derived macrophages in the intima than they are in activating monocytes. In relation to atherogenesis, it has been established that various cytokines, immunostimulatory agents, as well as growth factors are potent activators of macrophages (Table 4). Furthermore, platelet-derived activation products, lipids (e.g., ox-LDL), and certain eicosanoids have similar effects. Recently, it was also shown that monocyte activation is upregulated by fibrinogen, indicating a proinflammatory role of this protein (314). Since physiologically unperturbed macrophages are virtually impossible to obtain in isolation, it remains difficult to infer from such studies which of the agonists listed in Table 4 are playing the most important roles in atherogenesis.

### B. Autocrine- and Paracrine-Mediated Activation Reactions in Monocytes

Autocrine activation (effects of a substance secreted by a cell on that cell itself) and paracrine activation (action of substances produced by cells and acting at short range on neighboring cells) are important regulatory processes of monocyte activation. Probably one of the most important autocrine activators is TNF-α, produced in monocytes and known to upregulate the synthesis of cytokines (410) and tissue factor in monocytes (137). Furthermore, TNF-α may induce the production of

<table>
<thead>
<tr>
<th>TABLE 4. Agents that induce or enhance monocyte/macrophage activation and that have been associated with the development of atherosclerosis</th>
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<tbody>
<tr>
<td>Immune stimulatory agents</td>
</tr>
<tr>
<td>Lipopolysaccharide (190)</td>
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<tr>
<td>Immune complexes (15)</td>
</tr>
<tr>
<td>Complement factors C3a and C5a (393)</td>
</tr>
<tr>
<td>Lectins (427)</td>
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<tr>
<td>Virus (129)</td>
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<tr>
<td>Growth factors</td>
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<tr>
<td>Platelet-derived growth factor (144, 232)</td>
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<tr>
<td>Monocyte/macrophage colony stimulating factor (145)</td>
</tr>
<tr>
<td>Granulocyte colony stimulating factor (52)</td>
</tr>
<tr>
<td>Cytokines</td>
</tr>
<tr>
<td>IL-1β, TNF-α, IL-6, IL-2, IL-8 (78)</td>
</tr>
<tr>
<td>Lipids</td>
</tr>
<tr>
<td>Oxidized LDL (245)</td>
</tr>
<tr>
<td>Lipoproteins (70)</td>
</tr>
<tr>
<td>Platelet-derived activation products</td>
</tr>
<tr>
<td>P-selectin (for review, see Ref. 162)</td>
</tr>
<tr>
<td>Platelet microparticles (36)</td>
</tr>
<tr>
<td>Platelet factor 4 (141)</td>
</tr>
<tr>
<td>Eicosanoids</td>
</tr>
<tr>
<td>Leukotriene B4 (516)</td>
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<tr>
<td>Proteins</td>
</tr>
<tr>
<td>Fibrinogen (314)</td>
</tr>
<tr>
<td>Proteoglycans (421)</td>
</tr>
<tr>
<td>Proteases (304, 573)</td>
</tr>
<tr>
<td>Oxygen radicals (for review, see Ref. 40)</td>
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The recognition of lipopolysaccharide (LPS) from Gram-negative bacteria is mediated via the three membrane proteins CD14, MD2, and TLR4. Although CD14 has numerous ligands, TLR4 and MD-2 provide greater specificity for LPS. Activation of TLR4 triggers several subsequent steps including the recruitment of intracellular scaffold proteins (such as MyD88, TRAP, and Tollip), autophosphorylation of IRAK and ubiquitination of TRAF6. Ubiquitination of TRAF6 triggers its oligomerization and the recruitment and activation of kinase complexes that mediate phosphorylation of the inactive NF-κB complex, liberation of the transcription factor NF-κB, the phosphorylation and activation of the mitogen-activated protein kinase (MAPK) pathways. Together these events lead to the transcription of diverse proinflammatory genes as well as activation of proinflammatory enzymes such as cytosolic phospholipase A2 (cPLA2). Increases in Ca^{2+} levels induce cPLA2 and lipoxygenase translocation from the cytoplasm to the perinuclear region and nuclear envelope, where arachidonic acid is liberated from membrane phospholipids by cPLA2 and further metabolized to prostaglandins, thromboxanes, and leukotrienes by cyclooxygenase (COX)-1, COX-2, and lipoxygenases. The downstream signaling pathways used by the interleukin (IL)-1 receptor and the tumor necrosis factor (TNF) receptor 1 are also depicted. Inflammatory reactions by heat shock protein and saturated fatty acids are also induced through the TLR4 receptor. AA, arachidonic acid; CM, cellular membrane; ER, endoplasmic reticulum; FLAP, 5-lipoxygenase activating protein; HETEs, hydroxyeicosatetraenoic acids; IKK, Ik B kinase; IL-1R, IL-1 receptor; IRAK, interleukin-1 receptor-associated kinase; LBP, lipopolysaccharide-binding protein; 5-LO, 5-lipoxygenase; kinase; NF-κB, nuclear factor-κB; PLC, phospholipase C; PPAR, peroxisome proliferator-activated receptor; RIP, receptor-interacting protein; sPLA2, secretory PLA2; TLR4, toll-like receptor 4; TNFR1, TNF receptor type 1 (55-kDa TNF receptor); TRADD, TNFR1-associated death domain protein; TRAF 2/6, TNF-associated factor 2/6; TXA2, thromboxane A2.

IL-1β in monocytes, which in turn may promote a series of inflammatory reactions (273). The autocrine effect of TNF-α is mediated through the activation of NFκB (77).

The activation product PAF, produced mainly in granulocytes, has also proven to be a very potent proinflammatory substance and capable of activating macrophages (57) or enhancing the expression of monocyte activation products, e.g., tissue factor (TF) (381), the most potent trigger of thrombin generation. The observation that PAF antagonism was one of three major routes for inhibition of LPS-induced monocyte TF and TNF-α synthesis in a whole blood system (135) adds to the evidence.
that autocrine/paracrine activation plays a pivotal role in the activation of monocytes.

C. Roles of Cytokines Produced by Monocytes/Macrophages

Leukocyte generation of cytokines plays a pivotal role in the progression of inflammatory states such as rheumatoid arthritis, psoriasis, inflammatory bowel diseases, asthma, and also in atherogenesis (444). Unlike classical hormones, cytokines are produced locally by a variety of cells widespread throughout the body. In this review we focus solely on monocyte/macrophage cytokines that may have a role in atherogenesis.

1. TNF-α

Monocytes and macrophages produce a variety of cytokines, all of which are important regulators or modulators of inflammation and hence likely to be key players during atherogenesis. Among these the 17-kDa protein TNF-α has a master-type of role, initiating the release of a whole cascade of cytokines involved in inflammatory responses. This central role of TNF-α has been documented in studies where rheumatoid arthritis patients (139) and patients with Crohn’s disease were successfully treated (493).

Activation of a series of surface receptors, including CD11a, CD18, CD45, CD44, CD58, and P-selectin, have been reported to mediate the induction of TNF-α in monocytes cultured in vitro (210, 268, 475). However, these findings are probably highly system dependent and hinging on poorly defined low-grade preactivation events inherent in the isolation and culturing processes to which the cells are subjected (383).

TNF-α exerts many of its effects by binding to a trimeric receptor of two membrane receptors, TNFR1 (55 kDa) or TNFR2 (75 kDa) (230) (now named TNFRSF1A and TNFRSF1B, respectively; Ref. 311). These receptors both belong to the so-called TNF receptor superfamily. Other members of this superfamily comprise FAS, CD40, CD27, and RANK. These receptors are distinct from the IL-1 and LPS receptors (Fig. 2).

Cell stimulation by TNF-α involves the downstream activation of transcription factor NFκB, a mechanism central to several agonists, including IL-1β and LPS (Fig. 2). Recent studies have shown that both cytosolic and secretory phospholipase A2 may be involved in the TNF signal transduction pathway, ultimately leading to nuclear translocation of NFκB and ensuing activated gene expression (524).

The primary event of monocyte activation has been extensively explored, particularly when LPS serves as the agonist. Taking into account the emerging notion that chronic infectious diseases may unleash mechanisms that favor atherogenesis, the cellular signaling events leading to the systemic inflammatory response to LPS are summarized in Figure 2. In plasma LPS binds to LPS-binding protein, forming a complex which may then interact with CD14, a transmembrane domainless receptor on monocytes that by itself is unable to mediate activation of signal transduction (207). Recently, evidence has emerged that once formed the LPS-CD14 complex may bind to a Toll-like receptor (TLR4) serving as the actual mediator for signaling into the cells via a number of transducers recruited to the receptor, eventually leading to NFκB activation (for details, see Ref. 207). The latter factor is involved in regulating numerous genes critically for inflammatory responses, including IL-8, IL-6, VCAM-1, and E-selectin (113).

The role of TNF-α in the early phase of atherogenesis has been searched for. Surprisingly, it has been shown that C57BL/6 mice lacking p66 (TNFRSF1A) and fed an atherogenic diet developed significantly larger lesions than did receptor-positive mice, thus generating larger numbers of foam cells (461). In contrast, lack of TNF-α did not alter lesion development, whereas loss of lymphotixin-α (LT-α, a proinflammatory TNF-α homologous cytokine which also elicits responses though the p55 and p75 receptors) was associated with a 62% reduction in lesion size (462). These findings suggest that TNF-α may also be interacting with cells through receptors different from p55 and p75.

Other TNF receptor and ligand superfamily members have been shown to be new players in the emerging perception of atherogenesis. Thus TNF receptor superfamily 14 has been suggested to be involved in atherogenesis by inducing TNF-α and IL-8 as well as the matrix metalloproteinases MMP-1, MMP-9, MMP-13, and tissue inhibitors of MMP-1 and MMP-2 (285). Furthermore, TNFR5 (CD40) has also been implicated in atherosclerosis and is expressed by EC, SMC, T lymphocytes, and macrophages in lesions (317). Preventing CD40 ligation with anti CD40L (TNFSF5) antibodies in hyperlipidemic mice (LDLR−/−) reduced the size of developing aortic atherosclerosis lesions (316).

For further details on the variety of biological responses mediated by TNF-α, the reader is referred to an excellent review (4).

2. IL-1β

IL-1β in its mature form is a 17-kDa, 159-amino acid residues protein, with an isoelectric point of 5.5. During inflammation, transcription of the protein is stimulated by immune complexes, certain coagulation and complement proteins, substance P, and viral or bacterial products, notably LPS (93). It is also induced or enhanced by certain cytokines of lymphocyte origin, such as the granulocyte-macrophage colony stimulating factor (GM-CSF) (485)
and interferon-γ (IFN-γ). TNF has been reported to stimulate the production of IL-1β by monocytes (128), EC (372), and fibroblasts (281). Similarly, administration of IL-1β and TNF-α in vivo induced the generation of detectable circulating levels of IL-1β (127).

The IL-1β receptor protein is a conserved member of the TLR family and is located at a site separately from that of LPS-CD14 (see Fig. 2). NFκB activation induced via this receptor is mediated by the same signal transduction system as that of LPS-CD14, since both pathways converge at the level TRAF6 activation (see Fig. 2). Along with TNF-α, IL-1β is one of the principal proinflammatory products generated in monocytes/macrophages. In fact, IL-1β may mimic activation signals typically induced by TNF-α. Furthermore, IL-1β acts as a chemoattractant for neutrophils (for review, see Ref. 312), induces release of neutrophils from the bone marrow into the circulation (11), and enhances leukocyte adherence to the endothelium. Like IL-6 it stimulates hepatocytes for secretion of other acute phase proteins. Also, IL-1β promotes endothelial cell proliferation and induces T-cell activation through increased IL-2 production and upregulation of the IL-2 receptor.

An IL-1 receptor antagonist, attenuating this receptor’s function, is present in circulating blood (313). In a recent study where transgenic mice (LDLR −/−) overexpressing IL-1 receptor antagonist were fed a high-cholesterol/high-fat diet containing cholate, a 40% decrease in lesion was observed compared with LDLR knock-out mice lacking the transgene (117). Further evidence for an important role of IL-1β in atherogenesis was provided by blocking of IL-1β in ApoE −/− mice, which impeded the development of atherosclerosis (136).

The IL-18 cytokine is a member of the IL-1 family, and its receptor and signal transduction system are analogous to those of IL-1β (7). IL-18 has potent IFN-γ-inducing activities. It is a proatherogenic cytokine by increasing lesion development through enhancement of an inflammatory response involving a IFN-γ-dependent mechanism (561). Thus IL-18, which by itself is generally a weak stimulator of IFN-γ release, functions synergistically with IL-12 to induce pronounced secretion of IFN-γ production by T cells, natural killer (NK) cells, and macrophages (353). Accordingly, it has been shown that administration of exogenous IL-12 to ApoE −/− mice will promote lesion development (284).

3. IL-4 and IL-13

IL-4 in its mature form is a glycoprotein of 129 amino acids and is produced by Th2-T lymphocytes and also secreted by mast cells and NK cells. It is a pleiotropic cytokine acting on T and B lymphocytes, monocytes, polymorphonuclear cells, fibroblasts, and EC. Its effects on B cells entail upregulation of MHC class II and Ig class switching. IL-4 blocks some of the effects of IL-12, whereas IFN-γ blocks some of the effects of IL-4 (32). The effects of IL-4 and IL-13 on human monocytes and endothelial cells are quite similar, since the receptors for these cytokines share a common predominant receptor signaling chain (IL-4Rα) (204). Thus both enhance the LDL oxidizing potential of human monocytes, whereas IFN-γ inhibits the cell-mediated oxidation. The up- and down-regulation of activated monocyte-mediated LDL oxidation correlates with the expression of 15-LO activity (155). In accordance with this scenario it is suggested that dietary lectins, which have been shown to trigger IL-4 and IL-13 release from basophils, may play a role in inducing so-called early IL-4 required to switch the immune response toward a Th2 response and type 1 allergy (63). The latter condition is probably mediated by 15-LO (192).

Recently, it was found that IL-4 deficiency decreases atherosclerotic lesion formation in a site-specific manner in female LDLR −/− mice (257). IL-4 is generally considered to be an anti-inflammatory cytokine, but several processes that are regulated by IL-4 could hypothetically increase lesion formation, including monocyte recruitment (566), monocyte adhesion (34), lipoprotein modification (97, 506), and macrophage metabolism of modified lipoproteins (99, 579). However, in another study, IL-4 deficiency did not affect early atherosclerosis in C57BL/6 mice fed a high-cholesterol diet (172). The reasons for the discrepancy between these two studies are unknown.

4. IL-6

IL-6 is a pleiotropic cytokine that has been implicated in the pathology of many diseases, including atherosclerosis, rheumatoid arthritis (216), multiple myeloma (38, 253), psoriasis (188), Kaposis sarcomas (344), sepsis (545), and osteoporosis (240). IL-6 belongs to a family of cytokines that includes IL-11, leukemic inhibitor family (LIF), oncostatin, cardiotoxin-1, and ciliary neutrophilic factor (159, 581) (for a review, see Ref. 484). IL-6 plays a central role in diverse host defense mechanisms, such as the general immune response, acute phase reactions, and hematopoiesis (for reviews, see Refs. 8, 260). IL-6 is also important for the activation of the coagulation system, since its elimination attenuated the activation of this system during experimental endotoxemia in chimpanzees (538).

The biological activity of IL-6 is mediated via two membrane receptor proteins, a unique low-affinity binding receptor (IL-6R) and the high-affinity transducing β-subunit gp130. gp130 is also a partner in receptor complexes that have LIF, OSM, CNTF, and IL-11 as ligands (159, 167, 233, 309, 510). The documentation that IL-6 serves as a proatherogenic cytokine remains rather limited. However, infusion of recombinant IL-6 in C57BL/6 mice (ApoE-deficient mice) exacerbates early lesions in...
these animals (227). The IL-6 injection was associated with a rise in IL-6, IL-1β, TNF-α, and fibrinogen, whereas total cholesterol levels were unchanged between recombinant IL-6 (rIL-6)-treated and nontreated groups. However, IL-6 may also be regarded as an anti-inflammatory cytokine as it attenuates the synthesis of proinflammatory cytokines through the induction of the synthesis of glucocorticoids and furthermore promotes the synthesis of IL-1 receptor antagonist and release of soluble TNF receptor (inhibitory of TNF function) in human volunteers (525).

5. **IL-12**

IL-12 is a heterodimeric protein in which the 35-kDa and 40-kDa subunits are linked by two disulfide bridges. This cytokine is produced by phagocytic cells, dendritic cells, skin Langerhans cells, and B cells. IL-12 induces IFN-γ in NK cells and T cells, thereby shifting T-cell differentiation toward a TH1 response. It stimulates growth of activated NK cells, CD8+ T cells, and CD4+ T cells (for a review, see Ref. 25). Furthermore, IL-12 increases production of TNF-α, which acts in synergy with IFN-γ, and it suppresses IL-4-induced IgE production. It has been suggested that IL-12 may play an active role in regulating the immune response during the early phase of atherosclerosis in apoE-deficient mice (284).

6. **IFN-γ**

The cytokine IFN-γ has an important role in inducing and modulating a whole array of immune responses. It is produced by Th1 T lymphocytes and by activated NK cells. IFN-γ produced in NK cells is important in acute inflammation, mainly because of the activating effect it has on the adhesion properties of the endothelium and on mediator production by mononuclear phagocytes. Thus it upregulates the expression of endothelial ICAM-1 and of monocyte IL-1, PAF, and H2O2. INF-γ has a suppressive effect on IL-8 expression but upregulates the production of LPS-induced NO as well as IL-12 in monocytes (for reviews, see Refs. 49, 517).

IFN-γ has been suggested to play a central role in atherogenesis. IFN-γ was shown to promote atherosclerosis in ApoE −/− mice when subjected to intraperitoneal administration of exogenous IFN-γ (562). Furthermore, mice lacking either INF-γ or the INF-γ receptor (191, 358) were less prone to atherosclerosis development than were their wild-type controls.

IFN-γ exerts its effects through a number of mechanisms that have been demonstrated in cultured cells. This entails effects on 15-lipoxygenase (97), lipoprotein modification (91), lipoprotein-cell recognition (169), ECM synthesis (468), lipoprotein metabolism (242), and apoptosis (171). Furthermore, IFN-γ has been found to upregulate CD38 expression, and it was suggested that CD38 may play a specific role in the activation and adhesion processes to which monocytes are subjected (357).

7. **IL-10**

IL-10 is an anti-inflammatory cytokine produced by activated monocytes and lymphocytes. IL-10 most likely exerts its anti-inflammatory effects on the vascular system through inhibition of leukocyte-EC interaction and inhibition of proinflammatory cytokine and chemokine production by monocytes/macrophages or lymphocytes (321, 322, 417). Studies have been presented showing evidence that IL-10 can inhibit MM-LDL-induced monocyte-endothelium interaction as well as atherosclerotic lesion formation in mice fed an atherosclerotic diet (411). In this study, IL-10 null mice were associated with increased lesion formation compared with either control or IL-10 transgenic mice. Similar results were obtained by another group (321). IL-10 exerts its biological effects on cells by interacting with a specific cell-surface receptor (see review in Ref. 518).

8. **Transforming growth factor-β**

Transforming growth factor-β (TGF-β) is found in highest concentrations in platelets (185). It has both proatherogenic as well as antiatherogenic effects since it stimulates macrophage secretion of various growth factors such as, e.g., PDGF, and secretion of extracellular matrix proteins, e.g., collagen and proteoglycans (88, 459). Furthermore, macrophage chemotaxis and TIMP secretion are primed by TGF-β. On the other hand, TGF-β inhibits production of reactive oxygen and nitrogen metabolites in activated macrophages (for reviews, see Refs. 55, 126). TGF-β1 downregulates cytokine-induced expression of E-selectin and VCAM-1 in endothelial cells (124, 395) as well as VCAM-1 in SMC (488). The effect of TGF-β1 on human umbilical vein EC (HUVEC) stimulated with TNF-α or IL-1β entails downregulation of MCP-1 expression through downmodulation of TNF receptors (219). IL-8 production by TNF-activated EC as well as inhibition reversal of IL-8-dependent migration of neutrophils through the activated endothelial monolayer are inhibited by TGF-β1 (488).

Although the proinflammatory effects of the substantial number of cytokines are well documented, their exact role in lesion formation in the early phase of atherogenesis is still unresolved. Furthermore, because most of the anti-inflammatory cytokines, except IL-1ra, have at least some proinflammatory properties as well, the importance of the various cytokines in the early phase of atherogenesis is still open to debate.
D. CD14 Polymorphism on the Gene of the CD14 Receptor

The leucine-rich 55-kDa membrane glycoprotein CD14, better known as the LPS receptor, is significantly expressed by mature monocytes, macrophages, and activated neutrophil granulocytes (183). Anchored at the cell surface (see Fig. 2), it facilitates cellular internalization of LPS (463). Other agonists, notably the heat shock protein HSP70, also activate monocytes by binding to CD14 (18). Two European groups reported for the first time a relationship between promoter polymorphism in the CD14 gene and increased risk of atherosclerosis (225, 533). One group found that T>C at position −159 was associated with increased risk of myocardial infarction, and the other reported C>T at position −260 was more frequent in myocardial infarction survivors than controls. Recently, two Japanese groups confirmed the occurrence of the C (−260)→T nucleotide change and an associated predisposition to increased risk of coronary artery disease (235, 480). The polymorphism was apparently associated with an enhanced risk for myocardial infarction (MI), particularly in patients who did not otherwise have any significant risk profile for atherosclerosis. Because the polymorphism is associated with an upregulation of CD14 receptors on monocytes, these observations corroborate the growing evidence that chronic infections of, e.g., Chlamydia pneumonia, Helicobacter pylori, Epstein Barr virus, etc., may be important risk factors for the development of atherosclerosis and consequently of MI. Whether CD14 polymorphism is the much sought for culprit underlying the prevalence of high responder monocytes in families with high incidence of MI (385) is an issue that would need further investigation.

A deviating pattern of differentiated subpopulations of monocytes has been documented in hypercholesterolemic compared with normocholesterolemic individuals (446). Thus, in a group of hypercholesterolemic patients, HDL-cholesterol levels correlated negatively with the population size of CD64−CD16− monocytes [monocytes that lack FcRI (CD64) and FcRIII (CD16)], whereas on the other hand, plasma LDL-cholesterol and lipoprotein (a) levels correlated positively with expression of the variant antigen CD45RA in peripheral blood monocytes. Cellular activation has been shown to increase the expression of CD45RA at the cell surface in vitro (71). On the other hand, cells lacking CD64 show lower expression of CD14 antigen but higher expression of MHC II antigen, i.e., they have a higher capacity for antigen presentation and a deviant pattern of stimulus-induced cytokine release response compared with the predominant monocyte phenotype of peripheral blood (184). This appears to fall in line with the failure to see enhanced monocyte reactivity in the blood of hypercholesterolemic patients (382, 385). Furthermore, a high number of healthy individuals in families with a high risk of atherosclerosis despite normal cholesterol levels appeared to have hyperactive monocytes (385). Accordingly, high reactivity of peripheral monocytes was proposed to be a risk factor for coronary artery disease.

VII. CELLULAR SIGNALS INVOLVED IN MONOCYTE ACTIVATION

A. Role of Phospholipases

Phospholipase A₂ (PLA₂) catalyzes the hydrolysis of phospholipids, like phosphatidylcholine (PC), phosphatidylserine (PS), or phosphatidylethanolamine (PE) (489), at position sn-2, where arachidonate is most frequently located (350). The released free arachidonic acid in turn serves as substrate for two major pathways, the lipoxygenase (LO) pathway and the cyclooxygenase (CO) pathway, leading to the generation of leukotrienes and prostaglandins, respectively (Fig. 3). The other product derived from the PLA₂-dependent cleavage is lysophospholipid.

Two major PLA₂ are found in monocytes/macrophages, a secretory type (sPLA₂) and a cytosolic type (cPLA₂), respectively. sPLA₂ (14 kDa) is stored in granules for release to the extracellular milieu upon leukocyte activation (29, 526). This enzyme probably remains inactive within the granules, becoming functionally active only when exocytosed (for a review, see Refs. 328, 355). The substrates for sPLA₂ are phospholipids exposed at the outer surface of the plasma membrane (Fig. 2).

Recently, it was shown that when monocytes were treated with SB 203347, a specific inhibitor directed against the active site of sPLA₂, leukotriene and PAF formation were totally blocked, whereas the production of prostanoids remained unaffected (328). On the other hand, an inhibitor of cPLA₂ blocked the generation of prostanoids but not leukotriene C₄ (LTc₄) or PAF in zymosan-stimulated monocytes (328). Thus the evidence suggests that sPLA₂ provides substrate for monocyte leukotriene and PAF formation, whereas cPLA₂ plays a more significant role in prostaglandin generation. This is in agreement with an earlier report showing that depletion of human monocyte 85-kDa PLA₂ did not significantly affect the leukotriene formation (329).

cPLA₂ (85 kDa) is a monomeric enzyme located primarily in the cytosol of unstimulated cells, in segregation from its substrate. In activated cells, cPLA₂ is effectively regulated by the elevated cytosolic calcium levels, whereby binding of the enzyme via an amino-terminal C2 domain is facilitated (Fig. 2). The C2 domain is a conserved Ca²⁺-triggered membrane-docking module that targets numerous signaling proteins to membrane surfaces where they regulate diverse processes critical for cell signaling (365). Thus translocation of the enzyme to...
the nuclear envelope and the endoplasmic reticulum is induced. A transient calcium flux may cause only reversible and transient translocation of cPLA$_2$, insufficient for arachidonic acid release, whereas a more substantial and persistent flux may lead to prolonged perinuclear translocation and arachidonic acid liberation (215, 418). In intact cells, MAPK-dependent serine phosphorylation of cPLA$_2$ may also be required for arachidonic acid release (306).

As documented in vivo, cPLA$_2$ activity may also be indirectly regulated by inhibition at the level of of phosphatidylinositol 4,5-bisphosphate (PIP$_2$) generation, effectively reducing cPLA$_2$-mediated arachidonic acid metabolism (30). This may entail an alternative and calcium-independent mechanism of cPLA$_2$ translocation to and interaction with membranes.

The cartoon depicted in Figure 2 shows how cPLA$_2$ and sPLA$_2$ and their activities comprise part of the transduction mechanism systems for monocyte activation.

### B. Role of Phospholipases in Atherosclerosis

1. sPLA$_2$

   sPLA$_2$ has been detected in atherosclerotic lesions of the aorta, where it colocalized with macrophages and SMC and has also been found extracellularly in the lipid core (229, 291, 341, 456). This sPLA$_2$ was fully active and capable of hydrolysis of LDL phospholipids, promoting the release of proinflammatory lipid products at LDL deposition sites of the arterial wall. The activity was upregulated via binding to decorin, a small proteoglycan with chondroitin/dermatan sulfate glucosaminoglycans comprising an integral part of the collagen network in human arteries (237, 291, 454).

   Direct evidence that sPLA$_2$ may promote atherogenesis was obtained in transgenic mice expressing sPLA$_2$ (237, 291). These mice had a dramatically higher incidence of atherosclerotic lesion development than had...
regular mice, when maintained on a high-fat, high-cholesterol diet or even when given a low-fat Chow diet. Immunohistochemical staining revealed the presence of sPLA2 in the atherosclerotic lesions, and the mice had lower high-density lipoprotein (HDL) and higher LDL very-low-density lipoprotein (VLDL) levels than did wild-type mice, accompanied by significantly decreased paraoxonase activity in their plasma. Paraoxonase is a HDL-ester hydrolase with a proven capacity of hydrolyzing lipid peroxides in vitro. Thus it may destroy biologically active phospholipid peroxides by acting as a phospholipase hydrolyzing sn-2 ester bonds (553), and it is known to account in part for the inhibition of LDL oxidation by HDL. A profound inhibitory effect of paraoxonase on cell-mediated LDL oxidation in vitro has been demonstrated (479, 553). Para-oxonase in HDL may protect against the induction of inflammatory responses in cells of the artery wall, by destroying biologically active lipids present in mildly oxidized LDL (553) (Fig. 1). Recently, it was shown that polymorphisms in the paraoxonase gene are associated with carotid arterial wall thickness in subjects with familial hypercholesterolemia (205). However, the role of human paraoxonase is still debated, as some population-based studies have not found any association between polymorphisms in the gene that encodes paraoxonase and CHD (22).

Acute phase responses typical of chronic inflammatory conditions like, e.g., arthritis, frequently entail severalfold enhanced circulating sPLA2 as part of their profile. This has been suggested as a vehicle underlying the observed prevalence of coronary artery disease in conjunction with many chronic inflammatory diseases (237).

2. cPLA2

By inference from several studies cPLA2 is considered a principal mediator of LDL oxidation. In human monocytes activated by opsonized zymosan, both cPLA2 protein and enzymatic activity were induced. The cPLA2 activity was inhibited in a dose-dependent manner by the specific inhibitor AACOCF3, and there was a concomitant activity was inhibited in a dose-dependent manner by the specific inhibitor AACOCF3, and there was a concomitant significantly decreased paraoxonase activity in correlation with the severity of the inflammatory con-
diation. However, the role of COX-1 is overshadowed by that of the apparently more important COX-2 enzyme (541, 564). Inducible COX-2 has been found in fibroblasts (135), mucosa cells (441), macrophages as well as atheroma-associated cell types like SMCs and endothelial cells (16, 380, 438) in a variety of conditions.

The constitutive expression of COX-1 in nonatherosclerotic arteries as well as in atherosclerotic lesions (458) suggests that the enzyme has a role under physiological conditions in maintaining homeostasis, rather than having a role during inflammation (308). Selective studies on human carotid atheroma and healthy aorta revealed that atherosclerotic lesions contained both COX-1 and COX-2. The enzymes colocalized mainly within macrophages in the shoulder region of the atheroma and with the peripheral region of the lipid core, whereas in healthy SMC the levels of these enzymes were comparatively less pronounced. The enhanced COX-2 expression detected within such a focal site of chronic inflammation is in accordance with earlier studies reporting COX-2 expression in cells that typically reside in atheroma, including macrophages, SMC, and EC, in a manner dependent on stimulation with cytokines such as TNF-α and IL-1β (16, 380, 438).

The mechanisms whereby the various products from the cyclooxygenase pathway affect the atherogenic process remain only partly charted. Two classes of receptors may transduce signals in response to prostaglandin ligand binding: G-coupled membrane receptors (69) and the nuclear PPAR class (157). The two COX isoforms may in part exert different functions by way of their location in partially separated cellular compartments (347). Thus COX-2 is located in the perinuclear region, which is relatively low in COX-1 (Fig. 2). In a recent study it was found that COX-2 promotes early atherosclerotic lesion formation in LDLR-deficient mice (76).

2. Prostanoid and thromboxane products

Certain prostanoids, such as PGE2 and PGF2α, typically mediate anti-inflammatory signaling via cAMP increase (59, 368), in effect leading to a downregulation of monocyte/macrophage activation (59, 66). Furthermore, PGF2α also downregulates inflammation by way of its antiplatelet effect and by promoting vasodilation. On the other hand, TxA2 is known to have proinflammatory and prothrombotic effects (for review, see Refs. 131, 477), being a very potent agonist of platelet activation, and also serving as a vasoconstrictive PG product. Studies on TxA2 receptor deficiency mice revealed increased bleeding tendency and resistance to cardiovascular shock (523).

TxA2 has been identified as the dominant cyclooxygenase-dependent metabolite of monocytes, with lesser amounts of PGE2, PGF2α, and PGFβ being formed in declining order (398, 399). However, the powerhouse for TxA2 generation in blood is the platelets. When rabbits were given the TxA2 antagonist UK-38485 in conjunction with a high-cholesterol diet, lesion formation was reduced, in accordance with the proinflammatory potential of TxA2 (486). In low responder rabbits, treatment with UK-38485 significantly reduced the percentage of the thoracic aorta surface covered by lesions, an effect not seen when high responders were used. The area covered did not correlate with serum cholesterol levels.

3. Isoprostanes

Isoprostanes are prostaglandin isomers that are primarily generated by free radical oxidation from arachidonic acid (348, 376a). In addition to serving as useful indices of oxidant stress in vivo, isoprostane effects exerted on cells in vitro have been demonstrated. Thus 9α,11α,15S-trihydroxy-(8b)-prosta-5Z,13E-dien-1-oic acid (iPF3α-III) activates the receptor for TxA2 (the TP) (20). This latter compound and the analogous iPGE2-III modulate platelet function and adhesive interactions between platelets and endothelial cells (289, 580) and are also potent vasoconstrictors in vitro and in vivo (213, 512). Immunohistochemical studies have shown that foam cells adjacent to the lipid necrotic core of plaques were markedly positive for iPGE2β,III (415).

3. Cytochrome P-450-derived products

Several oxygenases have been implicated in the process of LDL oxidation in the arterial wall. Among these the cytochrome P-450, which is expressed in monocytes/macrophages (35), has been shown to induce LDL oxidation in vitro, where it also was demonstrated that hydrogen peroxide and superoxides were involved (24). The fact that some of the P-450 enzymes are present in liver and in arterial wall has prompted the suggestion that they are pathophysiologically relevant in conjunction with LDL oxidation and atherogenesis. Cytochrome P-450 enzymes may metabolize arachidonic acid, leading to many biologically active compounds (see Fig. 3), some of which are potent regulators of vascular tone (376).

4. Lipooxygenases and LO-dependent products

The lipooxygenases comprise a class of nonheme iron dioxygenases recognizing the 1.4-pentadienyl structure of polyunsaturated fatty acids, catalyzing single oxygen molecule incorporation at specific sites and the formation of dienic fatty acid hydroperoxides (574, 575). The classic substrate is arachidonic acid released from membranes by PLA2 (Fig. 3). Different types of lipooxygenases are specified by the numbering of the target carbon in their substrate, i.e., 5-LO catalyzes oxidation at C-5 (61), and 12-LO and 15-LO at C-12 (197) and C-15 (198), respec-
tively. Along with 8-LO (163, 276, 498), they are the only lipoxygenases identified in mammalian cells thus far.

There are two subtypes of 12-LO, one of which has been isolated from platelets. The other subtype, commonly referred to as leukocyte-type 12-LO, has been found in murine, porcine, bovine, and canine species. Leukocyte 12-LO is very different from platelet 12-LO and more closely related to mammalian 15-LO (577). The biochemical activity of 15-LO is quite distinct and differs from that of the other lipoxygenases in several ways. Whereas 5-LO and platelet 12-LO are rather substrate flexible in preferring free arachidonic acid, leading to the products hydroperoxiethanes typically formed by transcellular metabolism initiated by the sequential actions of 15- and 5-lipoxygenases or 5- and 12-lipoxygenases depending on the cellular context. Lipoxins have been shown to inhibit P-selectin mobilization and downregulate ICAM-1, E-selectin, and VCAM-1 via protein kinase C (PKC)-dependent pathways (151, 224).

Although 12/15-LO activity leads to the generation of several products anticipated to have important anti-inflammatory properties, a number of dioxygenation products have a documented proinflammatory activity. Thus, for example, 8,15-diHETE is a neutrophilic chemotactic factor. 12/15-LO is also essential for the generation of another important group of eicosanoids called lipoxins (see Fig. 3). These are trihydroxy metabolites of arachidonic acid, and the enzymes involved are either 12/15-LO pathways and the 5-LO pathway. Both 15-HETE and 12-HETE suppress 5-HETE and LTB4 production, and conversely, it has been shown that 5-HETE inhibits 15-HETE production in rabbit PMN (536, 537). Furthermore, 15-HETE, but not 15 HETE, downregulates Fc receptors on human T cells and monocytes (180). Also, 15-HETE downregulates important proinflammatory processes, such as LPS-induced TNF-α production in monocytes and TNF-α-induced expression of ICAM-1, E-selectin, and VCAM-1 via protein kinase C (PKC)-dependent pathways (151, 224).

D. Role of Lipoxygenases in Atherogenesis

Evidence that lipoxygenases are critically involved in atherogenesis has recently emerged from various transgenic mouse studies. Thus mice with ApoE deficiency (ApoE −/−) in combination with a 12-LO −/− trait had significantly reduced tendency to lesion formation compared with mice with the ApoE −/− trait only (107). This observation falls in line with the known prerequisite of 12-LO for monocyte chemotactic activity (371), and the recent report that 12-LO products, notably 12-HETE, play an important role modulating MM-LDL induction of
monocyte binding to endothelial cells (219). In the latter study, either of the LO inhibitors 5,8,11,14-eicosatetraenoyic acid (ETYA) and cinnamyl-3,4-dihydroxy-α-cynocinnamate (CDC), when used at concentrations claimed to specifically abolish 12-LO activity, also blocked monocyte binding to MM-LDL-treated endothelial cells.

15-LO was initially hypothesized to have a role in LDL oxidation, since it catalyzes the generation of available peroxyl radicals (535). This hypothesis was supported by studies showing that 15-LO inhibitors reduce EC- and macrophage-mediated oxidation of LDL (338, 396, 426). Isolated 15-LO actively exerts oxidative modifications of LDL (41). Cells transfected with 15-LO gene had a higher capacity for LDL oxidation than had cells transfected with a control gene (44). Despite such observations, the suggested role of this enzyme in initiating LDL oxidation remains controversial, the main issue being whether 15-LO could be the sole agent needed (41). However, atherosclerosis induced in rabbits by a dietary regimen was attenuated by the administration of a highly specific 15-LO inhibitor lacking significant antioxidant properties (53, 469). The finding in two animal models that benzothiopyranidole, another 15-LO inhibitor lacking significant antioxidant activity, could nonetheless eliminate lesion formation without significantly affecting plasma lipids (100), is evidence to the same effect. Furthermore, in a recent study using 12/15-LO-deficient (12/15-LO −/−) mice that had been crossed with ApoE-deficient (ApoE −/−) mice, it was found that urinary and plasma levels of the specific isoprostane 8,12-iso-IPF 2α-VI, as well as IgG autoantibodies against MDA-LDL, were significantly reduced in the double deficiency mice in parallel with decreased atherosclerosis, relative to ApoE −/− controls (106). It was concluded that 12/15-LO contributes significantly to the initiation and propagation of atherosclerotic lesion formation in mice. Furthermore, the strong correlations found between lesion size, isoprostane levels, and MDA-LDL autoantibodies is in vivo evidence for an enzymatic (12/15-LO) component to lipid peroxidation in atherogenesis.

In apparent contradiction to the above results, transgenic rabbits expressing 15-LO in a macrophage-specific manner and given a high-fat, high-cholesterol diet for 13.5 wk, developed less lesions than did nontransgenic rabbits (476). It was suggested that the antiatherogenic effect of 15-LO might be mediated via the conversion of linoleic acid into the platelet chemorepellent 13-HODE, inhibiting platelet adhesion to the endothelium and stimulating PGI2 production in endothelial cells (472) and decreasing platelet TXA2 production (73, 74, 193). Current knowledge falls short in resolving the contradictions inherent in the results issuing from the overexpression of 15-LO in rabbits versus those obtained in the other knock-out studies. The possibility of species-specific effects or secondary effects derived from the gene manipulation cannot be ruled out.

5-LO is the rate-limiting enzyme in leukotriene synthesis. When a 5-LO knock-out allele was bred into LDL receptor-null (LDLR −/−) mice, these mice showed a dramatic decrease in aortic lesion development (340). In line with this report is a very recent study which shows an important role for LTD4 (5), a major product in the 5-LO pathway (see below).

1. The lipooxygenase products LTB4, LTC4, and LTD4

The activation of monocytes and macrophages is associated with a significantly enhanced production of LTB4. Residential alveolar macrophages generate several-fold more LTB4 than do circulating monocytes (31, 48). Although LTD4 is of prime importance, it is but one of several lipoxygenase products generated upon cellular activation.

An array of functional properties is exhibited by LTB4, in conjunction with phenomena such as chemotaxis, modulation of cytokine production, IL-2 receptor and c-fos and c-jun expression, tumor cytotoxicity in monocytes and macrophages, as well as the production of IFN-γ, IL-2, IL-4, IL-5, and IL-10 in lymphocytes, probably rendering this the principal proinflammatory LO product in atherogenesis. Interacting with endothelial cells, it promotes leukocyte adherence to the endothelium (221), thus facilitating monocyte recruitment and LDL oxidation at the endothelial surface. Furthermore, interaction of LTB4 with receptors on monocytes and PMN (178, 275, 433) leads to an amplified activation of these cells, entailing induction of oxygen radical formation and augmented release of reactive oxygen metabolites, PAF (501), cytokines (440), and proteases (103).

By way of its role as a powerful mediator of inflammatory reactions, LTB4 promotes events that are likely to be of vital importance in atherogenesis. Such a role for LTB4 would be in accordance with the notion that chronic infectious diseases provide thriving conditions for the emergence of CHD, a subject discussed in more detail later in this review. LTB4 has been shown to depend for its functional activity on a LTB4 receptor, the recently cloned gene of which codes for a protein predicted to have seven membrane-spanning domains (585). Like other transmembrane proteins of this type, it is coupled to a heterotrimeric G protein, which varies between cells expressing the receptor, thereby allowing for a diversity in responses. Considering the above properties of LTB4 and its receptor, it appears sensible that a LTB4 receptor antagonist reduced lesion formation in LDLR −/− mice (5). Interestingly, it was also found that LTB4 antagonism had no significant effect on lesion size in mice possessing the null alleles for MCP-1 (MCP-1 −/− × LDLR −/−), suggesting that MCP-1 and LTB4 may either interact or exert their effects by a common mechanism.

Although both LTC4 and LTD4 were reported to stim-
ulate cultured endothelial cells so as to synthesize PAF and bind neutrophils (337), whether they are involved in atherogenesis remains an open question. However, LTD₄ has been shown to increase vascular smooth muscle cell (VSMC) proliferation and induce IL-1β in these cells (413). This may indeed be part of the inflammatory mechanism during atherogenesis.

E. Availability of Arachidonic Acid

Monocytes of whole blood appear to be activated through PLA₂-dependent pathways, generating products like interleukins, TNF, and other cytokines, as well as tissue factor (the trigger of blood coagulation) and growth factors (383). This explains the extraordinary efficacy of corticosteroids in bringing about inhibition of LPS-induced production of cytokines (544). Although it appears that under certain conditions cytokine and tissue factor synthesis may be induced through protein kinase C-dependent pathways, as seen from the several studies on monocytes in cell cultures, it should be emphasized that such studies are carried out under conditions that are unlikely to allow for true mimicking of the chain of events occurring in vivo (383). This was recently brought home by the finding that acetyl salicylic acid (ASA) enhanced LPS-induced tissue factor expression in human monocytes in vivo (403), as also found ex vivo in whole blood (379, 387). In contrast, in cultured monocytes, ASA had an inhibitory effect on LPS-induced tissue factor and cytokine generation (378). This discrepancy is most probably caused by the activation of monocytes and subsequent activation of protein kinase C system during the isolation of cells (383).

The central role of PLA₂ in atherogenesis is consistent with the above-mentioned ex vivo observations. Thus the generation of available arachidonic acid appears to be a limiting step regulating inflammatory reactions. More specifically, one may hypothesize that this is of importance for the activation of monocytes/macrophages when interacting with modified LDL in the intima, as well as for activation processes mediated by autocrine and paracrine mechanisms. The degree of phospholipase activation and subsequent arachidonic acid formation is strongly influenced by the lipid composition of the cell membrane. Dietary omega-3 fatty acid supplementation was reported to induce changes in the fluidity of human leukocyte membranes, associated with a 39% reduction in the release of arachidonic acid when the cells were exposed to calcium ionophore in vitro (283).

F. PAF

1. Production and mechanism of action

PAF and eicosanoids are synthesized from a common arachidonic acid precursor source (274, 589). PAF is synthesized by either of two pathways (not shown): the remodeling pathway or the de novo pathway, the former being the principal pathway in leukocytes and endothelial cells. PAF is secreted with the fluid phase from human monocytes and eosinophils, whereas when synthesized by the same pathway in endothelial cells it is translocated to the plasma membrane and retained on the cell surface (589). The remodeling pathway incorporates a PLA₂-catalyzed step, whereby 1-O-alkyl-2-acyl-sn-glycero-3-phosphocholine is converted into 1-O-alkyl-2-lyso-sn-glycero-3-phosphocholine (lyso-PAF), and this lyso intermediate is then acetylated in an acetyl CoA:lyso-PAF acetyl transferase (lyso-PAF AcT)-dependent reaction to form PAF. Cells do not produce PAF constitutively, but only when exposed to an appropriate agonist stimulus.

The receptor protein (PAFR) for which PAF serves as the cognate ligand belongs to the serpentine family of receptors, characteristically spanning the plasma membrane seven times. The intracellular signaling evoked by ligand binding is mediated by G proteins linking PAFR to downstream signaling events, such as turnover of phosphatidylinositol, intracellular calcium fluctuation, and activation of protein kinase C and other kinases (for a review, see Ref. 402).

PAFR gene expression is regulated by various cytokines and eicosanoids (440), apparently in much the same way in human monocytes, neutrophils, platelets, and B-lymphatic cell lines (351), entailing up- and downregulation. Thus IFN-γ caused PAFR upregulation in human monocytes, associated with enhanced response to PAF (388), whereas PGE₂-induced cAMP downregulated the expression of PAFR in human monocytes, associated with reduced responsiveness to PAF (522).

2. Biological and pathophysiological effects

PAF has a variety of biological functions (Table 5), and is implicated in many pathophysiological states.

<table>
<thead>
<tr>
<th>TABLE 5. Biological effects of PAF</th>
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<tbody>
<tr>
<td>Primes or activates platelets, neutrophils (200), monocytes (58, 382), macrophages (176), and SMC</td>
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<tr>
<td>Uregulates adhesion molecules in leukocytes and endothelial cells, promotes migration of monocytes across the endothelium (506)</td>
</tr>
<tr>
<td>Enhances the formation and action of oxidized LDL (554)</td>
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<tr>
<td>Enhances superoxide (O₂⁻) generation (252), CD11/CD18 expression, and elastase/cathepsin G in neutrophils (67)</td>
</tr>
<tr>
<td>Induces production of VEGF (369), NO, and collagenase (39)</td>
</tr>
<tr>
<td>In concert with oxidized LDL it induces TNF-α production (160)</td>
</tr>
<tr>
<td>PAF activation of LPS-primed macrophages triggers fast activation of cPLA₂ intracellularly (158)</td>
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SMC, smooth muscle cell; VEGF, vascular endothelial growth factor; NO, nitric oxide; PAF, platelet activating factor; TNF-α, tumor necrosis factor-α; LPS, lipopolysaccharide; PLA₂, phospholipase A₂.
Thus, along with certain cytokines (TNF-α, IL-1β, IL-6), it is probably one of the most important mediators of inflammatory responses, and it is also playing an essential role in endotoxin shock (43) and ischemia reperfusion injury (277).

A role of PAF in leukocyte adhesion in vivo has been suggested (337) and was recently explored using P-selectin deficiency mice. These mice were entirely protected from the usually deleterious effects of PAF injection. Thus the characteristic state of shock and intestinal necrosis never appeared, and there was no mortality associated with the injection. It was concluded that P-selectin plays an important role promoting PAF-induced injury in mice and that selectins and the integrin-ICAM-1 system work in concert in mediating the inflammatory response to PAF in vivo (507). It has been shown that P-selectin has a priming effect on monocytes, leading to enhanced PAF synthesis (140, 560). These studies have prompted the suggestion that P-selectin expressed on endothelial cells or platelets may serve a dual role in anchoring monocytes locally at sites of vascular inflammation or thrombosis and in priming these cells for responses augmenting inflammation.

The migration of monocytes across unactivated endothelium in response to macrophage inflammatory protein-1α (MIP-1α), RANTES, PAF, or MCP-1 was completely inhibited by monoclonal antibodies to the CD18 component of the CD11b/CD18 (Mac-1) complex on monocytes, and the migration in response to C5a was partially (75%) inhibited (92).

PAF enhances superoxide (O₂⁻) production, CD11b expression, and elastase/cathepsin G release by PMN, all essential components in the pathophysiology of severe inflammatory reactions. The oxygen radicals thus formed may in turn act synergistically with PAF to potentiate tissue injury (10).

In addition to upregulating cytokine/TF production, PAF may have further proatherogenic roles in enhancing the formation and action of minimally oxidized LDL (MMox-LDL) and promoting the induction of TNF-α production in concert with oxidized LDL, an important step in lesion formation (Fig. 1) (160).

G. Eicosanoid Products Involved in the Activation of Monocytes?

The failure of ASA in preventing the development of lesions in animals subject to a high-cholesterol diet has been taken as evidence for the notion that platelets do not have any important role in atherogenesis (444). This view has to be qualified in the light of more recent studies where ApoE-deficient mice were treated for 11 wk with either aspirin or the thromboxane antagonist S18886 (79). The administration of aspirin gave a pronounced decrease in TxB₂ levels, significantly more so than did S18886, indicating the greater efficacy of aspirin in preventing platelet synthesis of TxA₂. On the other hand, S18886 decreased the incidence of aortic root lesions and levels of ICAM-1 in serum, effects that failed to manifest when aspirin was administered. It was therefore suggested that such blocking of thromboxane receptors inhibited atherosclerosis by a mechanism that did not interfere with the binding of TxA₂ to the platelets, but rather with the binding of some other agonist. Certain nonenzymatic activation products of arachidonic acid capable of stimulating thromboxane receptors, such as F2-isoprostanes, were the suggested prime candidates for such atherogenic products. Alternative culprits suggested were HETEs, products that depend on leukocyte or endothelial lipoxygenases or nonenzymatic lipid peroxidation processes for their formation, and also have the capacity for promoting signal transmission via thromboxane receptors (520, 539). HETEs are increased in atherosclerosis (409, 539) and have been localized to atherosclerotic plaques (323).

Using an ex vivo model of whole blood, we recently found that the thromboxane receptor antagonist SQ 29548 significantly reduced LPS-induced TNF-α and TF, suggesting that very important mechanisms for the upregulation of activation products in monocytes are mediated via this receptor (135). This may in part be the explanation why ApoE deficiency mice appeared protected against lesion formation when the TxA₂ receptor was blocked by an antagonist (79). On the other hand, in the same model the TxA₂ synthesis inhibitor aspirin (ASA) enhanced LPS-induced TF and TNF-α (387), and in an in vivo human model of endotoxin effects TF was correspondingly enhanced (403). This is consistent with the failure of ASA to reduce or prevent the formation of lesions in animal models (54, 508).

H. PPAR-γ as Regulator of Monocyte/Macrophage Function

Based on a whole body of research over the last couple of years, there is an emerging understanding that the PPAR-γ has a role in relation to monocyte/macrophage functioning (263, 436). PPARs are ligand-dependent transcription factors belonging to the nuclear hormone receptor superfamily. Their function is to regulate genes involved in controlling growth, morphogenesis, cellular differentiation, and homeostasis (85, 262, 324). PPAR-γ only becomes functional once it is heterodimerized with the retinoid X receptor (RXR), the dimer functioning as a transcription factor regulating genes linked to lipid metabolism (33, 168, 234, 262).

Three subtypes of PPAR, indexed α, β, and γ, are known, all having distinct tissue distribution patterns and being associated with selective ligands (64, 261, 292, 460,
PPAR-γ is a nuclear receptor, reported to regulate glucose and lipid levels, and according to more recent studies it is involved in regulating the cell cycle and in the differentiation of monocytes and regulation of their function. The natural ligands for PPAR-γ are 15-deoxy-prostaglandin J2 (15d-PGJ2), polyunsaturated fatty acids such as linoleic acid and linolenic acid, oxidized LDL (527), and two oxygenated linoleic acid derivatives, 9- and 13-hydroxy-octadecadienoic acid (9-and 13-HODE) (360). Furthermore, PPAR-γ is activated by nonsteroidal drugs, as shown using indomethacin (287).

Although the occurrence of PPAR-γ was initially reported for whole tissue specimens, notably adrenal gland, spleen, adipose, and colon tissues, more recently expression of PPAR-γ in monocytes/macrophages has been confirmed (89, 239, 435). Like 12/15-LO, PPAR-γ is induced by IL-4 (104). IL-4-dependent expression of CD36 was impaired in 12/15-LO-deficient mice, and 12/15-LO has been shown to potentiate PPAR-γ activation by arachidonic acid. Evidence suggests that PPAR-γ may play some important dual role in atherogenesis, either promoting the process or protecting against it depending on the conditions. The promoting potential possibly stems from an upregulation of the scavenger receptor CD36 by PPAR-γ in combination with RXR ligands, leading to increased uptake of oxidized LDL and thereby enhanced foam cell formation (360, 527). On the other hand, PPAR-γ exerts negative transcriptional control of the genes for TNF-α, IL-6, IL-1β (239) MMP-9 (gelatinase B) (332, 436), and inducible nitric oxide synthase (95, 436) and inhibits gene expression and migration in human vascular SMC (331) (see Fig. 1).

Evidence for a direct role of PPAR-γ in atherosclerosis has been provided by the detection of PPAR-γ in atherosclerotic plaques of transgenic mice carrying the human Apo B100 and apolipoprotein (a) genes but lacking the LDL receptor (527). Furthermore, PPAR-γ has been detected in foam cells of human atherosclerotic lesions (332, 435), in a manner correlating with the presence of oxidation-specific epitopes (435).

Although PPAR-γ appears to serve both proatherosclerotic and anti-inflammatory and hence possibly antiatherosclerotic progresses, recent animal studies indicate that the overall effect of PPAR-γ activation tends to be predominantly antiatherosclerotic. This was documented using LDLR-deficient mice fed a Western-style diet in combination with either of two different synthetic PPAR-γ ligands, thiazolidinedione (TZD) and GW7845, both of which are capable of activating PPAR-γ (298). Either drug reduced the number as well as the size of lesions in male mice, an effect that for unknown reasons did not appear in females. An editorial commentary (442) suggested that CD36 expression, although it is required for foam cell formation, may not be rate limiting for the process under ordinary circumstances. Thus there is no evidence that increased expression of CD36 can further accelerate this process; rather, it was proposed that the levels of LDL cholesterol or the conversion of LDL to ox-LDL may be the rate-limiting factors.

PPAR-γ has been implicated in the regulation of the vascular inflammatory gene responses (112). Fibrates are synthetic ligands recognizing PPAR-γ (156), thereby mediating the lipid-lowering activity of these drugs (494). Significant evidence has emerged that the fibrates may also exert a more direct antiatherogenic activity independently of their lipid-lowering activity, as shown in rabbits fed a cholesterol-rich diet and treated with the PPAR-γ ligand fenofibrate. By this regime a decreased tendency of atherosclerotic plaque formation in the thoracic aorta of the rabbits was seen, even in the absence of discernably decreased plasma lipid levels (447). Furthermore, activation of PPAR-γ expression in mice was associated with a prolongation of the inflammatory response (116). Fibrates have a downregulating effect on the production of IL-6 and TNF-α also in humans (319, 494). The PPAR-γ-dependent mechanism by which fibrates inhibit the vascular inflammatory response appears to be via the interference of PPAR-γ with the NFκB and AP-1 transactivation capacity, involving direct protein-protein interaction with p65 and c-Jun (112).

VIII. INFECTION, MONOCYTES, AND ATHEROSCLEROSIS

Concurrent with the growing evidence that prevalence of CHD can only in part be explained by the classical risk factors, such as smoking, hypertension, and high cholesterol levels, there has been an emerging shift in focus that addresses the associations between atherosclerosis and certain persistent bacterial and viral infections.

The best evidence for a link between infection and CHD derives from seroepidemiological studies focusing mainly on three pathogens: Chlamydia pneumonia, Helicobacter pylori, and cytomegalovirus (CMV) (109). In a recent population-based study, strong evidence was found for a partial atherogenic role of persistent Chlamydia pneumonia infection, as judged by serological and clinical data (334). The seroepidemiological and histopathological findings are corroborated by the results of animal experiments and preliminary intervention studies. Recently it was shown that Chlamydia pneumonia infection may directly induce differentiation of monocytes to macrophages, and this may provoke the development of atherosclerosis (572).

Helicobacter pylori has been associated with CHD in several studies (cited in Ref. 109). How this should be interpreted remains controversial, since these reports are seemingly contradicted by data not supporting the notion of Helicobacter pylori being a major independent risk factor.
factor for CHD (267). However, *Helicobacter* infections are associated with a prevalence of antibodies to the heat shock protein HSP60. Antibodies to bacterial heat shock proteins, notably HSP60, cross-react with the human antigen counterpart, and this has been suggested to evoke an autoimmunity response implicated in atherosclerosis (173, 571). In line with this scenario, bacterial HSP60 has been found colocalized with human HSP60 in atherosclerotic plaques (271).

HSPs are intracellular proteins generally perceived as serving protective functions under conditions of infection and cellular stress. There is a risk of autoimmunity associated with HSPs, due to their high interspecies sequence homology, throughout the range from prokaryotes to humans (586). Recently it was found that human as well as chlamydial HSP60, both detected in human atheroma, can activate vascular cells and macrophages (269) (Fig. 1). Furthermore, added *Chlamydia* HSP60 mediated activation of cultured peripheral blood monocytes and monocyte-derived macrophages in a CD14-dependent fashion, i.e., via the same receptor for which bacterial LPS is also a ligand (270).

Thus autoimmune stimulation via HSPs may be part of the mechanisms by which chronic infections may promote atherogenesis. Infectious organisms may also affect the atherosclerotic process by way of direct local interactions with the coronary endothelium, vascular SMC, and macrophages within the atherosclerotic lesion. Even more important and linked to the wide ranges of monocyte and platelet reactivities, under given conditions of hyperreactivity the infection may exert systemic effects via monocyte activation and ensuing cytokine production. Thus low-grade hypercoagulability states and inflammation may be promoted, eventually leading to lesion formation.

Whether a single microbial agent may be responsible for atherosclerosis has been questioned. It has been shown that nonspecific (endotoxin) stimulation of the immune system accelerates atherosclerosis in rabbits on a hypercholesterolemic diet (288). In line with this concept, it has been shown that seropositivity for one single pathogen did not have predictive power regarding risk for coronary artery disease (CAD), whereas on the other hand, the number of infection pathogens to which an individual has been exposed (infectious burden) did correlate with CAD (21).

**A. C-reactive Protein and Its Relation to Atherosclerosis**

An abundance of evidence based on acute phase protein markers in blood like CRP, fibrinogen, IL-6, albumin, and others suggests that inflammation is somehow linked to atherothrombotic disease in middle-aged populations (528). The fact that these markers may be of prognostic significance for events occurring even after considerable periods of time is in accordance with the notion that inflammation may be associated with all phases of atherothrombotic disease.

C-reactive protein (CRP) is an acute phase protein associated with inflammatory reactions and infections. Several recent studies have focused on its relation to cardiovascular diseases. Thus CRP has been found to have predictive power for future myocardial infarction and stroke in middle-aged men and women without clinical cardiovascular disease, independently of the incidence of other established risk factors (437).

The question has been raised, earlier for fibrinogen and more recently for CRP, whether these acute phase proteins are just markers of inflammation or somehow directly linked to the atherogenic process. Several mechanisms can be envisioned whereby CRP might contribute to the process, including induction of monocyte TF (83), complement activation (589), and cell-cell interaction (590).

In contrast to the tenet of the classical hypothesis regarding foam cell formation in atherogenesis, recently a mechanism for LDL uptake was suggested that did not hinge on any biochemical modification of LDL (591). In this study, LDL uptake turned out to be mediated by the plasma factor CRP. Thus uptake of CRP in complex with LDL was mediated by CD32 in a serum-dependent reaction, in line with former reports on CRP-mediated opsonization of biological particles (349).

**IX. THE PROINFLAMMATORY PLATELET AND ITS ROLE IN THE EARLY PHASE OF ATEROGENESIS**

Although platelets have been implicated in the pathogenesis of atherosclerotic lesions for a very long time (134), the issue whether they play an important role in the early phase of lesion formation has not been resolved. In our opinion, platelets are most likely part of the early mechanism of atherogenesis. This hypothesis is based on the proinflammatory properties of platelets.

Activated platelets secrete a number of chemokines of both the CC and CXC subgroups (414), most of which are known to be stored in the α-granules (247, 264, 452). The chemokine RANTES is characteristically targeting lymphocytes, monocytes, and eosinophils (247, 455), thus mediating release of histamine from basophils (279) and exocytosis of eosinophil cationic protein, and it also serves as a stimulant for leukotriene formation (445) and IL-8 production (559). Recently, it was shown that the deposition of RANTES by platelets triggers shear-resistant monocyte arrest on inflamed or atherosclerotic endothelium (543). Thus exposure of platelet-derived RANTES...
may support recruitment of monocytes from the circulation to the endothelium, epitomizing a proximal step in an emerging hierarchy (556). Platelet factor 4 (PF4) induces upregulation of monocyte activation (141), firm adherence of neutrophils on shear stressed endothelium, and release of neutrophil granule components (407).

In addition to the proinflammatory products mentioned above, two interleukin substances have been identified in platelets. A cell-associated form of IL-1β was demonstrated on ADP- and epinephrine-activated platelets, active in inducing the expression of ICAM-1 and the production of IL-6, IL-8, and GM-CSF by endothelial cells (208, 251). Furthermore, epinephrine was found to upregulate IL-8 production in a platelet-dependent reaction in LPS-stimulated blood (142).

A link between platelets and atherogenesis has been suggested in studies demonstrating that platelets are activated by very low, physiologically relevant concentrations of oxygen free radicals generated chemically by leukocytes (for a review, see Ref. 236). This could also be part of the explanation why the white blood cell count, which is a measure primarily of the numbers of granulocytes, proficient oxygen radical generating cells, is an independent risk factor of CHD (108, 557).

One of the principal proinflammatory attributes of platelets is their propensity for P-selectin exposure and subsequent induction of various proinflammatory products. P-selectin is stored in the α-granules and is translocated to the platelet surface in an exocytotic process comprising fusion of the α-granule membrane with the platelet outer membrane. As mentioned earlier in this review, exposed P-selectin will then interact with its ligand PSGL-1 on monocytes and neutrophils (for a review, see Refs. 82, 111). Such binding has been shown to prime PAF synthesis and phagocytosis in monocytes (140). An ensuing activation of leukocytes has also been suggested, since induction of TF in monocytes upon P-selectin-PSGL-1 interaction has been reported (80). However, other groups were not able to affirm this (383, 560). Furthermore, P-selectin exposed on activated platelets has been found to induce monocyte superoxide anion production (530), NFkB translocation, and secretion of MCP-1 and monocyte IL-8 (560). In a whole blood system it was shown that epinephrine upregulates IL-8 production in a platelet- and P-selectin-dependent reaction (142).

Recently it was reported that platelets contain the CD40 ligand (CD40L) and that it is expressed on the surface of activated platelets (212). Upon ligation, the cognate receptor CD40, which is present on B cells, monocytes, macrophages, and endothelial cells, may trigger inflammatory reactions (540). The ligation of endothelial CD40 to CD40L on activated platelets was shown to induce adhesion molecule expression, chemokine secretion, and TF expression in endothelial cells (212, 487) and monocytes (307).

A recent study suggested a more direct link between platelets and development of atherosclerosis based on the evidence for a role of vWF in atherogenesis (343). It has been established that vWF, present in plasma, platelet α-granules, Weibel-Palade bodies of endothelial cells, and the subendothelium (546) is involved in thrombus formation at the site of atherosclerotic plaque rupture (26). On the other hand, the notion that vWF should have any role in atherosclerotic lesion formation has been less clearly underpinned. In a study using vWF-deficient mice interbred with an atherosclerosis-susceptible LDLR −/− strain, and fed a diet rich in saturated fat and cholesterol, it was found that in the absence of vWF lesion formation was reduced relative to the LDLR −/− control, primarily at flow-perturbed regions of the aorta typically prone to atherosclerosis. vWF expression is particularly prominent near branch points and bifurcations (470). Further evidence that platelets may play a role in the early phase of atherogenesis has emerged from a study where hypercholesterolemia primed platelets for recruitment via vWF, glycoprotein IB (GPIB) alpha, and P-selectin to lesion-prone sites, before lesions are detectable (521). It can be concluded that vWF may recruit platelets and with them leukocytes to the lesion in a flow-dependent manner. Platelets thus interacting with an activated endothelium in a vWF-dependent manner may contribute to the atherosclerotic process by releasing growth factor and other proinflammatory products. In addition, many years ago it was shown that platelets adhere and aggregate to the endothelium of aortas of mice not exposed to any experimental injury (244). It was suggested that the adherence of platelets to the vessel wall induced injury of the intimal tissue through the release of vasoconstrictive agents from the platelets.

Yet another indirect clue for a role of platelets in early atherogenesis has been provided by the demonstration that activated platelets induce significantly enhanced secretion of MCP-1 (also shown before in Ref. 559) and surface expression of ICAM-1 and αvβ3 by cultured endothelium (125). Furthermore, it was shown that activation of NFkB, which also regulates transcription of the MCP-1 gene, was significantly increased in EC treated with activated platelets by way of an IL-1-mediated mechanism.

Another aspect of the contribution of platelets to lesion formation may be their essential role in thrombin generation (for review, see Ref. 326). Furthermore, platelets upregulate tissue factor expression in monocytes in a P-selectin-dependent reaction (195). Although the role of thrombin in lesion formation is still debated, it has been shown that patients with impaired thrombin generation have reduced atherosclerosis (50). However, the very recent report by Sramek (492) found no link between decreased coagulability and atherogenesis based on observations in individuals with a hereditary bleeding tendency.
(hemophilia and/or von Willebrand disease). It was suggested that the protection against myocardial infarctions that has been reported in hemophilia patients probably is primarily caused by a decreased tendency to form occluding arterial thrombi.

Based on our own observation of an association between myocardial infarction and large platelets (large mean platelet volume, MPV) in men aged 45–65 years belonging to a number of families with apparently inherited hyperactive platelets with large MPV, in the absence of any other risk factors of CHD (68), and recent new data linking platelets to lesion formation, we postulate that platelets may play an important role not only in thrombus formation at plaque rupture, but even in the early phase of atherosclerosis.

We infer from the available data that the trait of inheritable large platelets predisposes for early development of atherosclerosis and that this in turn is a reflection of a pivotal role of platelets in atherogenesis. This hypothesis is corroborated by the recent documentation that platelet-derived microparticles, which are enhanced in large platelets (490), are capable of activating platelets as well as monocytes and endothelial cells (37).

X. CONCLUSIONS

The important role of monocytes in the early phase of atherogenesis has been established through studies on the regulation of their recruitment to the intima by adhesion molecules and chemokines that are essential to the process. Because this process is initiated by LDL, the monocyte activation and function is greatly influenced by trapping of LDL in the vessel wall. New data emphasize the role of the cellular inflammatory system associated with the potential for oxidative lipoprotein modulation. This process unfolds as an inflammatory response involving monocytes and macrophages in concert with lymphocytes and endothelial cells, crucial for the excessive macrophage consumption of oxidized LDL leading to foam cell generation.

The importance of monocyte/macrophage activities in atherogenesis has been explored through studies on cellular signaling, notably pertaining to the roles of phospholipases, i.e., sPLA2 and cPLA2, and lipooxygenases, which convert arachidonic acid into active oxidative metabolites, HETEs, and leukotrienes. Corroborative as well as new evidence for many of the signal mechanisms that are currently seen as playing a mandatory role in atherogenesis has been obtained from studies using transgenic animals, particularly mice, systematically exploring the effects of lesion formation of abolishing or overexpressing certain pathways. The emerging realization that PPARs are involved in regulating the production and activities of activation products of monocytes/macrophages such as oxidative metabolites, cytokines, and growth factors, gives further credence to the notion that these cells have an important role in the development of fatty lesions.

The recent observation of polymorphism of the gene of the CD14 receptor on monocytes/macrophages and how it relates to myocardial infarction in individuals with no other risk factor may provide a link for substantiating the indications that chronic infections, particularly Chlamydia pneumoniae, may enhance the development of atherosclerosis. Although not established, we hypothesize that platelets may be playing an important role in the early phase of atherogenesis through their proinflammatory properties.

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