Atherosclerosis and its complications constitute the most common causes of death in Western societies and Japan. Although several theories or hypotheses about atherogenesis have been proposed during the past decades, none can completely explain the whole process of the pathogenesis of atherosclerosis because this disease is associated with multiple risk factors. In spite of this, the concept that atherosclerosis is a specific form of chronic inflammatory process resulting from interactions between plasma lipoproteins, cellular components (monocyte/macrophages, T lymphocytes, endothelial cells and smooth muscle cells) and the extracellular matrix of the arterial wall, is now well accepted. Histologically, atherosclerotic lesions from the early-stage (fatty streak) to more complicated lesions possess all the features of chronic inflammation. It has been demonstrated that atherogenic lipoproteins such as oxidized low density lipoprotein (LDL), remnant lipoprotein (β-VLDL) and lipoprotein [Lp] (a) play a critical role in the pro-inflammatory reaction, whereas high density lipoprotein (HDL), anti-atherogenic lipoproteins, exert anti-inflammatory functions. In cholesterol-fed animals, the earliest events in the arterial wall during atherogenesis are the adhesion of monocytes and lymphocytes to endothelial cells followed by the migration of these cells into the intima. It has been shown that these early events in atherosclerosis are triggered by the presence of high levels of atherogenic lipoproteins in the plasma and are mediated by inflammatory factors such as adhesion molecules and cytokines in the arterial wall. The development of genetically modified laboratory animals (transgenic and knock-out mice and transgenic rabbits) has provided a powerful approach for dissecting individual candidate genes and studying their cause-and-effect relationships in lesion formation and progression. The purpose of this article is to review the recent progress regarding the inflammatory processes during the development of atherosclerosis based on both human and experimental studies. In particular, we will address the mechanisms of atherogenic lipoproteins in terms of inflammatory reactions associated with hypercholesterolemia. Understanding the molecular mechanisms responsible for inflammatory reactions during atherogenesis may help us to develop novel therapeutic strategies to control, treat and prevent atherosclerosis in the future. \textit{J Atheroscler Thromb, 2003; 10: 63–71.}

Keywords: Atherosclerosis, Inflammation, Hypercholesterolemia, Lipoprotein (a), Foam cells, Plaque rupture

Early stage of atherosclerosis

The accumulation of lipid-loaded cells underlying the endothelium of large arteries, namely, fatty streaks or dots, is a hallmark of early-stage atherosclerotic lesions (Fig. 1). Numerous studies of either human atherosclerotic lesions or cholesterol-fed animals have shown that these lipid-loaded cells mainly originate from blood-born monocytes subsequently differentiated into macrophages (1). These macrophages engulf a large amount of lipids deposited in the subintimal space and take on the appearance of foamy structures, designated as foam cells. It has become clear that a number of scavenger receptors on the cellular surface are involved in the lipid [mainly lipids derived from oxidized low density lipoprotein (oxLDL)] influx into macrophages (2, 3). Three major lipoproteins are often
observed in the lesions and are considered to be athero-
genic when elevated in the plasma: they include low den-
sity lipoprotein (LDL); especially small dense LDL, rem-
nant lipoproteins (also known as $\beta$-VLDL found in both
type III hyperlipidemia and cholesterol-fed animals) and
lipoprotein (a) [Lp (a)] (4). These atherogenic lipoproteins,
once deposited in the intima, are subjected to chemical
modifications, such as oxidation, thereby leading to a
series of biological reactions. Compared to oxLDL, which
is invariably associated with foam cells in the lesions, Lp
(a), although also present in the lesions, is seldom, if ever,
associated with foam cell formation. Lp (a) is inclined and
it tends to be associated with the extracellular matrix (Fig. 1).

The biological functions of Lp (a) and whether it plays a
role in lesion development remain unknown, but recent
studies in our laboratory using human apolipoprotein (a)
[apo (a)] transgenic rabbits have revealed that Lp (a) may
act as a pro-inflammatory mediator that augments the le-
sion formation (5, 6). In addition to the foam cells in the
lesions, T lymphocytes are scattered around macrophages
and foam cells (Fig. 2) (7, 8). Electron microscopic studies
of human atherosclerotic lesions have revealed that there
is an intimate interaction between T cells and macroph-
ages and that the T cells involved in this interaction are
usually in the activated state as they express major histo-
compatibility complex (MHC) class II antigen. We dem-
onstrated that in hypercholesterolemic rats, T cells actually
predominate in the incipient phase (cholesterol-rich diet

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**Fig. 1.** A typical fatty streak seen in human atherosclerosis. The lesion is composed of foam cells with clear cytoplasm. The lesion is stained with three monoclonal antibodies for monocytes (HAM), oxidized LDL (FOH1a/DLH3), and apo (a). These foam cells are derived from macrophages as they are stained by the HAM antibody. Oxidized LDLs, the most atherogenic lipoproteins, are closely associated with foam cells. On the other hand, Lp (a), another atherogenic lipoprotein, is basically deposited around the extracellular matrix.

**Fig. 2.** Demonstration of T lymphocytes in human atheroscle-
rotic lesions. T lymphocytes (CD8 positive) are found around
foam cells, as shown by immunohistochemical staining with
OKT8 antibody (A). Electron microscopic observation shows
that a foam cell is surrounded by five lymphocytes in a rosette
pattern and an intimate cellular interaction between foam cells and lymphocytes and between lymphocytes (B).
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for 2 weeks) of the lesion formation and are gradually surpassed by monocyte infiltration in later stages (9, 10). Therefore, one can envision that these T cells are not simply bystanders in the lesions, but instead actively participate in the lesion progression, possibly through the release of diverse cytokines (9, 11, 12). Immunohistochemical studies also showed that a number of cytokines derived from different vascular cells are present in lesions at all stages (11). One of the most notable cytokines derived from T lymphocytes is interferon gamma (INF–γ), which has been shown to play diverse roles in mediating foam cell formation, the proliferation of smooth muscle cells and regulating the production of matrix metalloproteinases, thereby influencing plaque stability (see below). Taken together, these observations suggest that cell-mediated immunity mechanisms may be involved in the pathogenesis of atherosclerosis.

Mechanisms for the initiation and progression of atherosclerosis
Cholesterol-fed animals have been widely used for investigating the early events during atherogenesis, which cannot usually be studied in humans since the lesions develop over a period of several decades. A number of pioneer studies, including ours own, during the 1980s using cholesterol-fed animals, revealed that among the earliest events in the arterial wall of cholesterol-fed animals is the adherence of mononuclear cells to endothelial cells, as observed by electron microscopy and immunohistochemistry (13) (Fig. 3). Later, it was found that mononuclear cell adherence is triggered by a number of adhesion molecules on endothelial cells, such as vascular cell adhesion molecule–1 (VCAM–1), intracellular adhesion molecule–1 (ICAM–1), P–selecin and E–selecin (14–19). Increased expression of these molecules is considered to be responsible for the adherence to endothelial cells of monocytes and T lymphocytes and for their preferential binding to the arterial surface (20). These adhesion molecules are highly up-regulated by the elevation of the levels of atherogenic lipoproteins [oxLDL and Lp (a)] and cytokines in vitro (15). Blocking these molecules by injecting specific antibodies against them leads to a remarkable reduction of intimal cells in hypercholesterolemic animals (16). Direct evidence to support the notion that these molecules are critical in the initiation of the lesion formation has been derived from studies of several genetically modified mice in which these molecules are genetically deficient (19, 21). Cybulsky and coworkers generated VCAM–1–/– mice and showed by studying them in comparison to ICAM–1–/– mice that VCAM plays a more important role than ICAM–1 in initiating the monocyte adherence to endothelial cells during atherogenesis (21).

After monocytes and T lymphocytes bind to the surface of the arterial wall, they migrate into the subendothelial space, where they then differentiate and are transformed into macrophages and foam cells. This subendothelial migration is induced by the presence of bioreactive mediators called chemoattractants in the intima. Several candidate chemoattractants have been documented as functional in this respect in vitro; all of these candidates must satisfy two requirements: they should be present in the intima and should act specifically as chemoattractants for monocytes or lymphocytes. During the past 20 years, a number of chemoattractants have been shown to be capable of inducing monocyte chemotaxis: they include oxLDL, Lp (a), cytokines [monocyte chemotactic protein (MCP–1), interleukin–1 (IL–1) and tumor necrosis factor–alpha (TNFα)] and degraded collagens and elastins (22). Among all the mediators reported thus far, MCP–1 and Lyso–PC (a component of oxLDL) may be the most important and are the best-characterized chemoattractants in the lesions (23). MCP–1 has been found in the lesions in the early stage and can be produced by endothelial cells and also macrophages themselves (24). The function of MCP–1 depends on the specific receptor CCR–2 present on the surface of monocytes. Deficiency in either MCP–1 protein or its receptor CCR–2 significantly reduces the le-
sion development in apoE KO mice (25, 26), suggesting that MCP–1 is a critical mediator of the recruitment of monocytes in the intima. This notion is further strengthened by the finding that CCR–2-deficient mice show increased susceptibility to tuberculosis, another type of chronic inflammation (27). More recently, using a specific CCR–2 receptor inhibitor, Yamashita et al. showed that administration of propagermanium to apoE-deficient mice reduces atherosclerosis by inhibiting macrophage infiltration (28). In addition to acting as a chemoattractant for monocytes, oxLDL can inhibit macrophage mobility (29). Although the mechanism of this phenomenon has not been fully clarified, such a dual function of oxLDL in terms of directing cell mobility may help to explain why macrophages or foam cells failed to return the circulation as they are supposed to do. These findings suggest that in future experiments we should test whether we can treat patients with coronary heart disease (CHD) or prevent atherosclerosis using drugs that can target either adhesion molecules or chemoattractants for monocyte/macrophages. Figure 4 summarizes the postulated mechanisms for the pathogenesis of atherosclerosis, with emphasis on inflammatory reactions.

**Plaque stability and inflammatory reactions**

Typical atheroclerotic plaque (also called atheroma or fibrous plaque) contains a lipid or necrotic core covered by a layer of fibrotic cap consisting of a mixture of smooth muscle cells and extracellular matrix (Fig. 5). The base of the lesion, called the “shoulder,” often contains a number of macrophage-derived foam cells and T lymphocytes. It is generally believed that these components can determine the fate of the plaque, so-called plaque stability and destabilization. Basically, two kinds of common plaque can be morphologically differentiated and are clinically relevant: stable plaque and unstable or vulnerable plaque (Fig. 5). Stable plaque is usually composed of a small lipid core and covered by a thick fibromuscular cap with more smooth muscle cells and extracellular matrix. Unstable plaque often contains a large pool of lipid core, and a thin cap and a large number of inflammatory cells, especially at the shoulder, may be present. Morphologically speaking, stable plaques are the major risks for
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Stenosis or occlusion when it causes a significant reduction of the vascular lumen. Vulnerable plaques, however, is fatal and the outcome of its formation is ominous, because regardless of its size, a vulnerable plaque usually leads to acute coronary syndrome if it ruptures or is associated with thrombosis. A representative unstable vulnerable plaque of coronary atherosclerosis is shown in Fig. 6. The mechanism(s) of plaque rupture is not fully understood, but the accumulation of macrophages and T lymphocytes may play a critical role in this regard because macrophages can produce matrix metalloproteinases (MMPs), which may destruct the thin cap and potentiate the rupture (30–32). Several cytokines in the lesions may also upregulate the secretion of MMPs such as TNF, IL–1 and MG–CSF (33). In addition, an intimate interaction between T lymphocytes and macrophages may also regulate the production of macrophage-derived MMPs. The CD–40 ligand on T cells can bind to the CD–40 receptor on macrophages, a process which has been shown to induce MMP synthesis (34). T lymphocytes can also influence plaque stability through the release of INF–γ, which inhibits smooth muscle cell proliferation and matrix production. In the future, it will be important to test whether the use of MMP inhibitors can reduce the risk of plaque rupture in CHD patients.

Lipoproteins and inflammation

Since atherosclerosis is a specific type of chronic inflammatory process, as discussed above, it is important to identify the inflammatory factors involved in atherosclerosis (35). The causal relationship between blood cholesterol and atherosclerosis is no longer in doubt, therefore, we should know which cholesterol or lipoprotein is bad or atherogenic (35). All atherogenic lipoproteins, once deposited in the intima, may exert direct or indirect proinflammatory effects. Table 1 summarizes the potential roles of oxLDL in atherogenesis, with special reference to its properties as an inflammatory mediator. As mentioned above, oxLDL can induce adhesion molecule expression on endothelial cells and trigger the migration of monocytes towards the intima. On the other hand, oxLDL can stimulate the production of many inflammatory mediators (e. g. endothelin–1) from other vascular cells, in turn resulting in diverse inflammatory responses in the arterial wall (36, 37). Using apoE KO mice, our laboratory demonstrated that the chronic administration of endothelin–1 A/B receptor antagonist SB209670 results in a significant reduction of atherosclerosis, independent of the plasma levels of cholesterol (37). Therefore, it is likely that, similar to lipid-lowering drugs (such as statins),

Fig. 5. Schematic illustration of stable and unstable plaques. Stable plaque usually has a well-preserved lumen and a thick fibromuscular cap in which there are numerous synthetic-type smooth muscle cells with abundant extracellular matrix. Lipid cores are almost always small. Vulnerable plaque usually has a large lipid or necrotic core and a thin fibrotic cap beneath which there are numerous macrophages together with T lymphocytes and smooth muscle cells.

Fig. 6. A typical unstable plaque of human coronary atherosclerosis. The lesion is stained with antibodies HAM for monocytes, HF35 for smooth muscle α–actin and apo (a). The lesion contains a thin cap beneath which a number of macrophages (B and C) are present. In the cap of the lesion, there are almost no smooth muscle cells (D). Presumably, macrophage-derived MMPs may reduce plaque stability and cause a rupture. It is notable that Lp (a) is also present in the lesion but is apparently not associated with macrophages (E).
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anti-inflammatory agents may be useful for the treatment of atherosclerosis in the future. In addition to oxLDL, Lp (a), another atherogenic lipoprotein, may also lead to an inflammatory process by inducing the expression of adhesion molecules on endothelial cells, the chemotaxis of monocytes, and the proliferation of smooth muscle cells (38). In one of our recent studies using transgenic rabbits expressing human apo (a), we demonstrated that Lp (a) in transgenic rabbits which do not have endogenous apo (a) showed increased aortic atherosclerosis in comparison with control rabbits. Atherosclerotic lesions in transgenic rabbits are characterized by increased cellular proliferation, and Lp (a) is often deposited in the lesions. We initially hypothesized that Lp (a) may stimulate smooth muscle cell dedifferentiation in addition to proliferation and found that a large number of intimal cells are positively stained with SMemb, a monoclonal antibody against the smooth muscle myosin heavy chain isoform (38). In addition, Lp (a) has the ability to inhibit fibrinolytic activity by increasing plasma plasminogen activator–1 production (38). More recently, we demonstrated that Lp (a) enhances advanced atherosclerosis and vascular calcification in WHHL transgenic rabbits (39). These results again suggest that atherogenic lipoproteins exert diverse effects by inducing chronic inflammatory reactions during lesion formation. Importantly, these atherogenic lipoproteins can augment the production of cytokines by vascular cells, and through the autocrine and paracrine mechanisms, the inflammatory reaction may lead to a vicious cycle resulting in lesion progression. In contrast, HDL, an anti-atherogenic lipoprotein that protects against atherogenesis via reverse cholesterol transport, plays an important role as an anti-inflammatory factor. Several beneficial functions of HDL have been documented (Table 2) and it is expected that the therapeutic use of HDL elevation may open avenues for the treatment of atherosclerosis in the future (40).

**Perspectives**

Vascular biology research has progressed remarkably in the last decade, resulting in a better understanding of vascular cell responses to inflammatory stimuli. The role of vascular cells and dyslipidemias is critical during inflammation and is of particular importance in atherosclerosis. It is clear that most vascular inflammatory responses are mediated through the nuclear factorκB system. In addition, PPARs (steroid hormone nuclear receptors) have been shown to act as ligand-activated transcription factors controlling the expression of a number of genes in the vascular wall (41–44). Understanding the molecular mechanisms of these phenomena may not only provide insights into the pathogenesis of atherosclerosis but may also help us to develop new therapeutic strategies to treat atherosclerosis in the future. In this respect, it will be important to determine how to control inflammatory pro-

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<td>Adhesion of monocytes to endothelial cells ↑</td>
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<td>Monocyte and T lymphocyte chemotaxis ↑</td>
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<td>Scavenger receptor A and CD36 ↑</td>
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cesses during the lesion formation and progression and whether anti-inflammatory agents are clinically beneficial. Macrophage foam cells in the lesions may be a target for therapeutic intervention in the future (45). At present, treating patients with hypercholesterolemia is still the primary clinical task, but inhibiting the inflammatory process using various anti-inflammatory antagonists may help to prevent plaque rupture and reduce myocardial infarction. It can be anticipated that in the next few years, new anti-inflammatory agents may be developed as therapeutic tools as the molecular mechanisms of atherosclerosis and dyslipidemias are clarified.

Acknowledgements: This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan, the Japan Society for the Promotion of Sciences (JSPS-RFTF96I00202) and TARA project of the University of Tsukuba.

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