Lipoproteins and the pathogenesis of atherosclerosis

DANIEL STEINBERG, M.D., PH.D.

ABSTRACT It is now clear that hypercholesterolemia can, in some instances, be a necessary and sufficient cause of premature atherosclerosis. This has been best established in patients with familial hypercholesterolemia, a deficiency of the low-density lipoprotein receptor. Although hypercholesterolemia is not the only cause of atherosclerosis, a large body of evidence has identified it as a determining cause in many cases. This article reviews current hypotheses regarding the mechanisms by which hypercholesterolemia accelerates atherogenesis. The role of the foam cell is discussed in detail because it is a characteristic feature of the earliest lesion, the so-called fatty streak. Once thought to derive exclusively from smooth muscle cells, the foam cell is now known to originate in large part from monocytes that enter the artery wall and alter their properties to become tissue macrophages. Recent studies of the biology of the macrophage-derived foam cell are providing new insights into the mechanisms by which it enters the arterial wall and interacts with various classes of native and modified lipoproteins. As our understanding of the biology of the foam cell and its precursors grows, it may become possible to intervene and slow the progress of atherosclerosis by new modalities that might act synergistically with measures to control plasma cholesterol levels.


IN THE BEST of all possible worlds, the atherogenic process would be a logical, linear series of events, triggered by a single, specific initiating factor and proceeding ineluctably and sequentially to the stenotic complicated lesion with its clinical consequences (figure 1). The pathologists have been trying to reconstruct such a chain of events from a series of “still photographs,” but any such sequence must remain speculative; there is no universal agreement on how the human atheroma evolves. Probably the closest researchers have come to identifying a simple schema is in the case of familial hypercholesterolemia. In this disorder, we know that A is a defect in low-density lipoprotein (LDL) receptor activity, B is a high level of plasma LDL, C is a high level of LDL in the artery, and so on. We are also aware of a certain number of consequences of hypercholesterolemia, but we do not really know which are most important or exactly how they fit together. In addition, we recognize that there are several other factors that will influence lesion formation in the face of any given level of hypercholesterolemia, such as damage to the endothelium, aggregation of platelets, and so on.

A more likely picture of what we are dealing with is the complex and interactive scheme shown in figure 2. A might again represent defective LDL receptor activity, and the pathway B-C-D the same sequence leading from that factor to atheroma. E might represent endothelial injury, followed by F, platelet aggregation, then stimulation of smooth muscle cell replication, and on to atheroma. Other initiating factors may also be operative, as in the case of cigarette smoking, about which we really know very little. The point is that several relatively independent pathways may be involved, all of which converge on a common end point, the atheroma. Also important is the concept that these several pathways are not likely to be totally independent, as previously discussed. If there is endothelial injury (E), for example, it will favor the penetration of lipoproteins into the artery wall (C) and promote the sequence of events that we associate with hypercholesterolemia even if cholesterol levels are not particularly high. We will not say much more about these aspects of atherogenesis because this symposium is limited to a discussion of the role of lipoproteins. One point is worth mentioning, however. It is striking that in countries such as Japan, where there is almost no hypercholesterolemia, even though cigarette smoking...
is rampant and even though hypertension is widely prevalent, there is very little coronary heart disease (CHD). The implication is that some minimum level of "hypercholesterolemia" is a prerequisite for atherosclerosis even in the face of other initiating factors.

Even if we accept that atherosclerosis can have multiple causes, it is possible that in any given case only one of these is the determining factor. In other words, several simultaneously operative causes may be present, yet correction of the dominant cause may be sufficient to arrest and even reverse the process. Patients with hypercholesterolemia, for instance, may be at a greater risk if an element of endothelial injury is present, but the marked elevation of LDL may be a determining cause. In that case, intervention to correct the hypercholesterolemia will be sufficient to make a significant impact on the progression of the disease and reduce the CHD risk. Optimal control, however, might require intervention on several fronts. If we can prevent endothelial damage or platelet aggregation, in addition to treating hypercholesterolemia, our intervention might be even more effective than it is when we control only hypercholesterolemia.

Lipid infiltration hypothesis. In its simplest form, the lipid infiltration hypothesis proposes that hyperlipidemia (and, in particular, hypercholesterolemia) is a major contributing cause of atherosclerosis. The hypothesis does not limit itself to any one fraction of the lipoproteins, although, as we shall see, evidence for the linkage is strongest for LDL, the major carrier of cholesterol in plasma, and for \( \beta \)-very low density lipoprotein (\( \beta \)-VLDL), a fraction derived from chylomi-

Our certitude about the linkage between hypercholesterolemia and atherosclerosis is based on a wealth of experimental, clinical, genetic, epidemiologic, and intervention data that support the hypothesis:

1. Accumulation of cholesterol in the arterial wall is a hallmark of both experimental and human atherosclerosis.
2. Atherosclerosis can be produced in a wide variety of experimental animals by interventions that have the ability to raise plasma cholesterol levels.
3. Americans with high plasma concentrations of cholesterol are more likely to develop CHD at an early age and more likely to die from the disease than are individuals with low plasma concentrations of cholesterol.
4. Populations whose plasma cholesterol levels are, on the average, much lower than those of Americans (such as the Japanese) have a much lower incidence of CHD. When Japanese migrate (to Hawaii or San Francisco, for example), their plasma cholesterol levels and CHD incidence rise, probably because of changes in dietary habits. The low incidence of CHD in Japan is not primarily determined by genetics, therefore, but by environment.
5. Patients with familial hypercholesterolemia have a single, primary genetic defect — a deficiency of LDL receptors. Their high incidence of premature atherosclerosis must somehow stem from that defect, and the


FIGURE 2. Multiple, interactive-cause hypothesis for atherogenesis.
primary effect of the defect is to raise plasma cholesterol levels. Let us note parenthetically that children with the homozygous form of the disease have no other risk factors, yet die of myocardial infarction when they are as young as 10 to 15 years of age.

(6) Intervention studies have shown that the risk of CHD can be reduced when plasma cholesterol levels are lowered by dietary or drug treatment. In the recently completed Coronary Primary Prevention Trial, the reduction in risk was approximately 2% for each 1% decrease in cholesterol level.2 The results of clinical intervention trials are reviewed elsewhere in another article included in this symposium (see article by Tyroler).

In many epidemiologic studies, only the total plasma cholesterol level has been measured. More recently, such investigations have become progressively more sophisticated, and we are well aware of the quite different metabolic significance of cholesterol in various lipoprotein fractions. Indeed, the cholesterol level in the high-density lipoprotein (HDL) fraction actually varies inversely with risk of CHD.3 Most investigators agree that LDL and β-VLDL (which will be discussed in detail later) are both atherogenic, but whether to the same degree is not known. The question of whether the triglyceride-rich VLDLs are atherogenic remains controversial. According to some epidemiologic analyses, hypertriglyceridemia is not an independent risk factor, but this issue has not been completely settled. Most investigators agree, at least, that cholesterol in the LDL fraction and in the β-VLDL fraction is atherogenic. However, we are far from being able to spell out in detail exactly how they contribute to the deterioration of the arterial wall.

Two general categories of explanations have been offered. The first holds that the cholesterol-carrying lipoproteins induce or favor the progression of the atherosclerotic lesion as a direct or indirect result of the increased rate at which lipoproteins are taken up into the artery wall. This concept is much the same as the lipid infiltration hypothesis introduced by Virchow more than 100 years ago, but with modern trappings relating to lipoproteins and receptors. The second category of explanation suggests that the lipoproteins interact with other systems in such a way as to trigger lesion formation or lesion progression; for example, lipoproteins have been reported to favor platelet aggregation or to damage endothelial cells. In this case, the lipoproteins would contribute to atherogenesis and might concomitantly be taken up and degraded more rapidly. The uptake and degradation, however, might not be an obligatory component of their atherogenicity. Whichever of these hypotheses is correct, successful control of the hyperlipoproteinemia should help control the disease. If we had better insights into the cellular mechanisms associated with lipoprotein–arterial wall interaction during atherogenesis, it might be possible to intervene at the arterial wall level in addition to lowering lipoprotein levels and thereby obtain a synergistic or at least additive effect.

**Endothelial injury hypothesis.** The advanced atherosclerotic lesion impinges on the lumen of the artery and ultimately causes stenosis. The space-occupying lesion consists of lipid, in part, but also, very importantly, of smooth muscle cells and connective tissue matrix. To explain the cell proliferation, Ross and Glomset4 proposed that aggregation of platelets at sites of endothelial denudation released platelet-derived growth factor (PDGF), and that this was responsible for smooth muscle cell proliferation. Later studies have shown, however, that early lesions develop under areas in which the endothelial layer is morphologically intact.5-8 Involvement of “functional” injury remains a possibility. Also, we now know that many potential sources of growth factors other than PDGF might contribute to smooth muscle cell proliferation.9 Growth factors are released by smooth muscle cells, macrophages, and endothelial cells, and any or all of these may contribute to the overgrowth of cells that characterizes an atheroma (figure 3). More studies are needed on the generation of these various growth factors under conditions in vivo in the artery wall. Several questions remain: Which of these cell types actually release growth factors in vivo? At what rates are growth factors released? What are the factors that control release of growth factors? Until we know the answers to questions such as these, we can only speculate that one or another of

![FIGURE 3](http://circ.ahajournals.org/)

**FIGURE 3.** Some functions shared by endothelial cells, smooth muscle cells, and macrophages from the arterial wall that may play a role in atherogenesis.
the cells involved in lesion formation contributes factors that account for the overgrowth of cells. We still do not know, however, which are significant in vivo.

**Role of the monocyte/macrophage.** Over the past decade, a good deal of evidence has accumulated to show that many, if not most, of the foam cells in atherosclerotic lesions are derived from the circulating monocyte. For many years, the foam cell was thought to arise exclusively from smooth muscle cells that had migrated into the intima and begun to imbibe liquids. More recent studies have established that many foam cells are derived from circulating monocytes that adhere to the endothelium, penetrate into the subendothelial space, and there take up lipoproteins to become loaded with cholesterol esters. One of the earliest changes observed when experimental animals are placed on an atherosclerotic diet is adherence of circulating monocytes to the endothelial surface. Some of these cells have actually been "caught in the act" of penetrating between endothelial cells to gain entry into the intima. Because these cells are the hallmark of the earliest recognized atherosclerotic lesion — the fatty streak — a great deal of research interest has centered on them.

In experimental animals fed cholesterol to produce atherosclerosis, the increase in plasma cholesterol is to a large extent in the so-called \( \beta \)-VLDL fraction. This is a fraction of lipoproteins with the density of VLDL (less than the density of plasma — 1.006), but with the electrophoretic mobility of LDL — beta mobility. These lipoproteins bind tightly to receptors on the monocyte/macrophage and are taken up very rapidly, leading to foam cell formation in vitro.

Patients with the "garden variety" of hypercholesterolemia rarely have high concentrations of \( \beta \)-VLDL in their plasma, and their hypercholesterolemia is mostly due to an increase in LDL. Since LDL is atherogenic, one would expect that monocyte/macrophages would avidly take up LDL. Surprisingly, however, macrophages in culture take up native LDL only at a very low rate. Goldstein et al. were not able to convert macrophages to foam cells even on incubating them in the presence of high concentrations of LDL. These cells downregulate their LDL receptors and thereby protect themselves against storage of excess cholesterol esters. We should note parenthetically that the behavior of macrophages in situ in the artery wall may very well differ from their behavior in cell culture. Another point to keep in mind is that cell culture experiments are generally performed over relatively short time intervals (hours or days), whereas atherosclerotic lesions in man develop over much longer time intervals (months and years). Nevertheless, there appears to be a paradox, as indicated in table 1.

The relative ineffectiveness of LDL in generating foam cells led to the speculation that some modified form of LDL, generated after its formation from VLDL in the plasma, might account for the atherogenicity of LDL. Several chemical modifications of LDL — acetylation,\(^{17}\) acetoacetylation,\(^{18}\) and conjugation with malondialdehyde\(^{19}\) — convert it to a form taken up much more rapidly by the macrophage. These chemically modified forms are taken up by a specific receptor, originally designated the "scavenger" receptor,\(^{17}\) and the uptake is saturable, as is the case for any receptor-mediated uptake. Until the function of this receptor is better understood it may be best to designate it by the more neutral term "acetyl LDL receptor," recognizing the first ligand to be identified that reacts with it. So far, no firm evidence exists for the generation of chemically modified forms in vitro, and their pathophysiologic significance remains uncertain.

In 1981, Henriksen et al.\(^{20}\) showed that incubation of LDL with cultured endothelial cells leads to extensive modification of the LDL, both in its physical properties and in its biological behavior. The most important change was in the rate of uptake by resident mouse peritoneal macrophages, which increased four- to tenfold. Moreover, the increased uptake occurred mostly by way of the same receptor that recognized acetyl LDL and the other chemically modified forms of LDL mentioned above. Subsequent studies have shown that cultured arterial smooth muscle cells\(^{21}\) and even macrophages themselves are also capable of modifying LDL in a similar manner\(^{22}\) (figure 3). Recent studies have shown that these changes are probably all secondary to oxidative modification of the LDL.\(^{23,24}\) Antioxidants, such as \( \alpha \)-tocopheral or butylated hydroxytoluene, completely arrest the process. The modification does not take place if no metallic ions are present in the medium or if a metal-binding chelator is

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added. Accompanying the peroxidation is a marked breakdown of the LDL phosphatidylcholine to lyso-
phosphatidylcholine, with the release of the fatty acid from the 2 position, and extensive degradation of the
LDL apoprotein B.24 Inhibitors of phospholipase are
effective in blocking the modification — just as effective as antioxidants. Exactly how these modifications
interrelate remains to be determined, but the contribution of the cells appears to be primarily that of
catalyzing a peroxidative reaction by generating oxygen free radicals. Recent studies have shown that inhibi-
tors of lipoxygenases effectively inhibit the cell-in-
duced oxidative modification.*

There are two additional mechanisms by which oxidi-
vatively modified LDL might favor atherogenesis. Oxidized LDL is cytotoxic to cultured endothelial cells and
other cells in culture.25–28 In principle, then, it could
induce endothelial injury. However, damage in vitro is
prevented by the presence of serum, and it is uncertain
whether LDL in vivo can induce endothelial injury. A
second mechanism relates to the motility of macro-
phages and monocytes. There are many chemotactic
factors for the monocyte/macrophage, but it remains
unclear which of these play a role in vivo. All three of
the major cell types in the artery wall can release
chemotactic factors (figure 3). Recently, investigators
reported that oxidatively modified LDL potently inhib-
its the motility of the resident macrophage.29 It does not
inhibit the motility of the circulating human monocyte,
however. Recent studies have also shown that oxida-
tively modified LDL is actually chemotactic for the
circulating monocyte.30 The hypothesis that presents
itself is as follows (figure 4): (1) LDL entering the
arterial wall, at least at some sites, undergoes oxidative
modification, (2) the product acts as a chemoattractant
for monocytes, helping build up the mass of these foam
cell precursors in the subintimal space, (3) there the
monocyte assumes the properties of a tissue macro-
phage, and (4) now its movement is inhibited by the
same oxidized LDL that stimulated its entrance as a
circulating monocyte. According to this scenario, the
generation of oxidatively modified LDL would create
a sort of "lobster trap," luring monocytes into the intima
and then blocking their exit.

In closing this section, it must be stressed that there
is only very limited evidence for oxidative modification
in vivo. Studies by Goldstein and Hoff and their
colleagues31, 32 have provided evidence that some
kinds of modified forms of LDL are found in the aortic
wall, and Raymond et al.33 have shown that modified


![Figure 4](image_url)  
**FIGURE 4.** A hypothetical scheme suggesting that oxidatively mod-
ified LDL may enhance the accumulation of macrophages by attracting
circulating monocytes while inhibiting tissue macrophage motility.

LDL can be isolated from inflammatory fluids. Addi-
tional work is needed, however, to characterize and
quantify these modified forms of LDL further and to
confirm their possible role in atherogenesis.

**Unifying hypothesis.** For several years, the lipid infil-
tration hypothesis and the endothelial injury hypothesis
seemed not only distinct, but contradictory. Closer
examination, however, reveals numerous ways in which
they are very closely related and may even be
thought of as two faces of a single, Janus hypothesis.1
As already mentioned, two or more different initiating
factors may well lead to the same or similar end point.
An atheroma is extremely complex in structure and
could, quite conceivably, be generated by different re-
action sequences. Some such possibilities will be men-
tioned here. If the endothelium were damaged, there
would be an increased rate of penetration and the reac-
tions proposed for both pathways would be initiated.
Of course, if high concentrations of LDL were able to
damage the endothelium, as they can in vitro, then high
concentrations of LDL would be a sufficient cause for
initiating both chains of events. Another possibility is
that the foam cells formed in response to high levels of
LDL and residing just beneath the endothelium may
release toxic substances that induce endothelial damage
in that way. In either case, one can see how these two
hypotheses are not really distinct and independent.
Elsewhere, we have pointed out several other potential
interactions between the two hypotheses.1 One more
example may be cited: When smooth muscle cells
proliferate, perhaps in response to PDGF, they syn-
thetize and secrete connective tissue matrix. The latter
serves as a trap for lipoproteins because of their high
affinity for glycosaminoglycans, elastin, and other
matrix elements.

**"Protective" effect of HDL.** A wealth of epidemiologic
evidence has firmly established the very strong negative correlation between levels of HDL cholesterol in the plasma and risk of CHD. The strength of this negative correlation is at least as good as (and probably better than) the strength of the positive correlation with plasma levels of LDL cholesterol. Although there is no question about the strength of the predictive value of HDL cholesterol levels, we are still uncertain as to the reasons for it. The best current hypothesis is that suggested by GLomset34 some years ago. He proposed that HDL serves as a carrier for the removal of cholesterol from peripheral tissues, thereby transporting it, directly or indirectly, back to the liver, where it can be converted to bile acids and excreted. Although this “reverse cholesterol transport” hypothesis is compatible with everything we know, it should be stressed that there is very little direct proof in vivo that the mechanism operates in the manner proposed.

If the effectiveness of this reverse cholesterol transport system is directly proportional to the concentration of HDL in the plasma, the negative correlation between HDL levels and risk might indicate that reverse cholesterol transport is also important in moving cholesterol out of the arterial wall. However, we still lack any direct evidence that changing the HDL levels in an individual will in fact change his risk. In the case of LDL, we do have such evidence — intervention studies showing that lowering the LDL cholesterol level lowers risk; no comparable evidence is available with respect to HDL. We know that in experimental animals we can induce atherosclerosis by raising LDL levels; there is no comparable evidence that we can induce it by lowering HDL levels. Therefore, our confidence that lowering LDL levels is therapeutic is much greater than our confidence that raising HDL will be beneficial. Until more information about HDL becomes available, the emphasis must continue to be on lowering plasma cholesterol levels, and LDL levels in particular.

References


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