Mol. Nutr. Food Res. 2005, *49*, 995 – 998 DOI 10.1002/mnfr.200500081

Review

Hypercholesterolemia and inflammation in atherogenesis: Two sides of the same coin

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An abundance of experimental, clinical, and epidemiologic data capped by stunning interventional results with the statins has established hypercholesterolemia as a major causative factor in atherogenesis. In familial hypercholesterolemia and in animal models it is a sufficient cause. Some degree of hypercholesterolemia, perhaps 30 – 50 mg/dL, may even be a necessary cause. It is equally clear that from the very beginning atherogenesis has a strong inflammatory component, *i.e.*, it is characterized by penetration of monocytes and of T-cells into the developing lesion. These cells, through the secretion of cytokines and growth factors, through immune responses, and through complex cross-talk with elements of the artery wall modulate the growth of the lesion and affect its stability. But inflammation has to occur in response to something. What is that something? What is the "injury" in "response-to-injury"? The case will be made that oxidized lipids in oxidized LDL or generated in response to prooxidative changes in the cells of the artery wall should be considered a plausible candidate. There is no need to consider hypercholesterolemia and inflammation as alternative hypotheses. Both are very much involved. Optimal intervention and prevention will probably require attention to both.

Keywords: Atherosclerosis / Hypercholesterolemia / Inflammation / Oxidized LDL / Pathogenesis

Received: May 30, 2005; revised: July 21, 2005; accepted: July 25, 2005

1 Introduction

To say that atherosclerosis is an inflammatory disease is almost tautologic. Of course it is. The presence of leukocytes even in the very earliest fatty streak lesions was pointed out near the turn of the century by Anitschkow and confirmed in a number of laboratories [1 – 3]. In fact the earliest event observed after initiating cholesterol feeding in rabbits is an increase in the expression of leukocyte adhesion molecules at the arterial sites most susceptible to atherosclerosis [4]. Ross and his colleagues were among the first to try to systematize and integrate the multiple interactions among the cell types found in the progressing lesion: endothelial cells, smooth muscle cells, monocyte/macrophages, and T-lymphocytes [5]. The field has exploded in recent years and several excellent reviews document the extensive research in this area and the potential for finding interventions that may complement lowering of blood cholesterol [6 – 9]. There is no doubt that the rate of lesion *progression* can be importantly affected by inflammatory processes. Moreover, some of the variation in the rate of progression of disease in different individuals may relate to variations in individual sensitivities to inflammatory stimuli. Whether or not inflammation alone, *i.e.*, in the absence of some elevation of blood cholesterol, can initiate atherosclerosis is less clear. Arteritis can of course be generated but the lesions do not closely resemble those of human atherosclerosis.

To say that atherosclerosis is a disease of lipid and lipoprotein metabolism is equally self-evident. The central role of hypercholesterolemia in the pathogenesis is universally accepted. The case for the primacy of hypercholesterolemia has been made on the basis of extensive experimental studies, on clinical grounds, from epidemiologic data but most persuasively on the basis of the startlingly successful reduction of morbidity and mortality in the large-scale clinical intervention trials using the statins [10 – 12, 33].

The thesis of this paper is that atherosclerosis is both a disease driven by, and probably initiated by, hypercholesterolemia and, at the same time, also an inflammatory disease.

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Abbreviations: MCP-1, monocyte chemoattractive protein-1; **OxLDL**, oxidized LDL

2 Hypercholesterolemia can be in itself a sufficient cause for atherosclerosis. Can inflammation?

Beginning with the pioneering work of Anitschkow in 1913 [13] and over the ensuing years, it has been established that atherosclerosis can be induced in virtually any animal species if the blood cholesterol can be raised to a sufficiently high level. In some species, such as the rat or the dog, it is difficult to get the cholesterol level high enough but when, through dietary or hormonal manipulation, it gets up in the neighborhood of 300 – 800 mg/dL you do get atherosclerosis.

The most persuasive and clear-cut evidence comes from hypercholesterolemia induced by genetic modification, such as in the spontaneously LDL receptor-deficient rabbit [14] or the apoprotein E-deficient mouse [15]. In these models hypercholesterolemia is striking even on an ordinary chow diet (cholesterol-free). Note that no other manipulation is necessary. There are no other risk factors such as smoking or hypertension, or obesity or diabetes. There is no superimposed infection or other inflammatory process. In short, hypercholesterolemia is in these cases and in the LDL receptor-deficient patient, a sufficient cause for atherosclerosis.

Now this is by no means to say that if an inflammatory component is superimposed on the hypercholesterolemia in these models that it would not affect the disease, it might very well do so. However, it is not necessary.

What about the reciprocal question: Can inflammation in itself initiate the atherosclerotic process? Many investigators have tried over the years to produce atherosclerosis in animal models by inducing sepsis, by mechanical injury, by cauterization, or by damaging the endothelium (see [1] for a review). In the absence of hypercholesterolemia these attempts, while certainly producing some degree of arteritis and intimal thickening, have failed to mimic the human atherosclerotic lesion.

What about the many recent studies showing that cytokines and growth factors do indeed modify the severity of atherosclerosis in animal models, mostly in mice? It should be noted that almost all of these studies are carried out in mice that have atherosclerosis on the basis of a background of hypercholesterolemia induced by diet or, more commonly, by genetic modification. The point is that hypercholesterolemia of some degree is always present.

3 Can we propose a mechanism by which hypercholesterolemia itself initiates atherosclerosis?

The first visible lesion in atherogenesis is the fatty streak, consisting largely of lipid-loaded foam cells derived from monocytes that have penetrated into the subendothelial space and these lesions are the precursors of the later, more clinically relevant lesions [16, 17]. A major breakthrough in our understanding of atherogenesis came from the discovery in the Brown and Goldstein [18] laboratory that monocyte/macrophages could not be turned into foam cells by incubation with even very high concentrations of native LDL. The LDL receptor was down-regulated in the macrophage as it is in all cells when the cholesterol content of the cell starts to rise and the uptake was too limited to account for foam cell formation. Yet the ultimate source of the cholesterol building up in atherosclerotic lesions was known to be the circulating LDL. Consequently Goldstein and Brown inferred that some modified form of LDL must account for the generation of foam cells. They were able to show that acetylated LDL, made *in vitro* by incubation with acetic anhydride, could cause cholesterol accumulation in a specific, receptor-mediated manner. However, the uptake was not by way of the native LDL receptor but rather by way of a new receptor which they designated the acetyl-LDL receptor. This was later cloned by Kodama *et al.* [19] and renamed scavenger receptor A (SRA). However, there was no evidence that acetyl-LDL was ever produced *in vivo* (and there is still none). So the search continues for a modified form of LDL that might be generated *in vivo* and, like acetyl-LDL, be taken up specifically by macrophages and lead to the formation of foam cells.

Henriksen *et al.* [20] described a candidate form of LDL in 1981. They showed that simply incubating native LDL in the presence of endothelial cells overnight yielded a drastically modified form that was bound by the macrophage with high affinity and taken up rapidly. Most important, uptake was not down-regulated and the cholesterol content of the cells increased markedly. This modification of LDL, which turned out to be oxidation, could also be effected by incubation with smooth muscle cells or macrophages themselves. So oxidatively modified LDL (OxLDL) could perhaps account for foam cell formation and the initiation of atherogenesis.

In addition to inducing foam cell formation, OxLDL has a large number of other properties that make it proatherogenic. These have been discussed in detail elsewhere [21]. The most important evidence comes from studies in experimental animal models of atherosclerosis in which antioxidants, of several different kinds, have strikingly inhibited the atherosclerotic process [22].

We can visualize the following sequence of events that initiate fatty streaks:

(1) Hypercholesterolemia induces an increase in the expression of leukocyte adhesion molecules on the endothelium at susceptible arterial sites. Cybulsky and Gimbrone [4] showed that this antecedes the appearance of visible lesions.

(2) Well before any lesions are visible the concentration of LDL at susceptible sites in the artery wall of cholesterol fed rabbits is elevated, as shown by Schwenke and Carew in 1989 [23, 24]. They called special attention to the fact that the higher concentrations of LDL at these sites was not due to a higher rate of penetration but rather to what they called "selective retention". This facet of atherogenesis has been expanded on in more recent years by Williams and Tabas [25].

(3) Circulating monocytes and T-cells stick to selectins, roll and eventually penetrate into the subendothelial space under the influence of chemotactic factors, including OxLDL itself [26] and monocyte chemoattractive protein-1 (MCP-1) [27].

(4) LDL accumulating at susceptible sites undergoes oxidative modification to mildly oxidized (mmLDL) and then more heavily OxLDL, the latter being a ligand for macrophage scavenger receptors [28].

(5) OxLDL stimulates the release of MCP-1 from endothelial cells, speeding recruitment of monocytes, and also the release of macrophage colony stimulating factor (M-CSF) [29], favoring the growth and differentiation of macrophages accompanied by an increase in expression of scavenger receptors.

(6) A vicious cycle is now in place because macrophages can themselves oxidize LDL, and OxLDL can in turn, both directly and indirectly, help recruit more monocytes.

(7) OxLDL and/or its component oxidized lipids can participate in many of the immunologic and inflammatory processes in the complex progression of the lesion from a fatty streak to a fibrous plaque and on to the culprit or unstable plaque [30].

Note that this hypothetical pathogenetic pathway, while initiated by LDL and/or other atherogenic lipoproteins, almost from the beginning involves monocytes and, as the immune system comes into play, lymphocytes also. The critical issue here is that OxLDL and/or oxidized lipids interact with many of the same systems that are considered proinflammatory and proatherogenic. The extent to which the rate of lesion progression requires the continuing presence of OxLDL and oxidized lipids is not known with certainty.

4 Findings that support a centrally important role for hypercholesterolemia in atherosclerosis

(1) Animals, with some rare exceptions, do not spontaneously develop atherosclerosis. Yet they all will if their blood cholesterol is elevated by some means or other and maintained for a long enough time. The LDL level in most animals is 50 mg/dL or less [31]. Human LDL levels are in the range of 130 – 160 mg/dL.

(2) The striking results of the large-scale statin trials over the past decade show that decreasing LDL, whether initially very high or not, decreases coronary heart disease risk. Current recommendations are that physicians should strive to bring the LDL level down at least to 100 and very recent results indicate that there is additional benefit by lowering it to 70. Note that none of these trials have included any antiinflammatory drugs. The statin drugs themselves have some antiinflammatory effects as well, but most of the beneficial effect probably relates to cholesterol lowering. This conclusion is based on the fact that the benefit from statin therapy is roughly equivalent to that obtained with cholesterol lowering interventions of other kinds (*e.g.*, cholestyramine, clofibrate, diet) when the degree of cholesterol lowering is comparable.

(3) In Japan in the 1960s, when total cholesterol levels were only about 160 mg/dL (and LDL therefore about 100 mg/dL) the coronary heart disease death rate was only about 10% of that in the United States and the rest of the developed countries in the world. This was true despite the fact that the Japanese held the world record for average numbers of cigarettes smoked *per* day a higher than average incidence of hypertension and a prevalence of diabetes similar to that in the Western world. These observations suggest that hypertension, cigarette smoking and diabetes – some of the most prevalent risk factors for coronary heart disease – only become major contributors to risk when the LDL level is above some threshold value. What that level is we do not know but in view of the statin results discussed in Section 5, the values in other animals and these observations in Japan a good guess would be somewhere between 50 and 75 mg/dL.

(4) Low HDL cholesterol predisposes to atherogenesis but even zero HDL fails to induce atherosclerosis in apoA-deficient mice. If their LDL, which is vanishingly low in the wild-type, is elevated by introducing the human apoB gene, the apoA-deficient animals show protection against the atherogenesis caused by the elevated LDL. It is also worth noting that patients with Tangier disease, having almost zero circulating HDL, while they do have more coronary heart disease than the normal population, do not have the kind of striking premature coronary heart disease seen in patients lacking the LDL receptor [32]. Schaefer has suggested that this is because their LDL levels are very low, averaging a little less than 50 mg/dL (for reasons not known). Here is another example of the theme: Some minimum degree of LDL elevation is necessary for premature expression of atherosclerosis and its symptoms whatever other factors are operating to speed the process.

5 The statins and the "goal" for LDL in treatment

The LDL goal has dropped progressively since the introductin of the statins. Most recently it has been shown in the Treatment to New Targets study that lowering it to about 77 mg/dL confers significantly more benefit than lowering it to only 101 mg/dL [33]. Looking at all of the statin data, it is shown that the decrease in risk falls in a linear fashion with the level of LDL achieved during treatment. The curve does not appear to plateau but continues dropping linearly. The present author has taken the liberty of extrapolating the line to zero coronary event rate and the intercept lies at about 40–50 mg/dL, which is the sort of LDL level that is found in atherosclerosis-resistant animal species! It is thought that we should be wary of extrapolations but there may be a message here.

6 Concluding remarks

Atherosclerosis is a disease involving BOTH hypercholesterolemia AND inflammation. However, it is probably initiated by elevated levels of LDL (and other proatherogenic lipoproteins). The best current hypothesis for initiation is that OxLDL is the "injury" that accounts for dysfunctional endothelium, expression of leukocyte adhesion molecules and monocyte recruitment. There follows a broad array of complex inflammatory and immune responses. OxLDL and its component oxidized lipids interact at many points with these pathways.

Hypercholesterolemia is not the only factor contributing to atherosclerosis and its complications. It is, however, the best established and possibly a necessary element in the pathogenesis.

Optimal treatment and prevention will probably require BOTH reduction of blood lipids AND interventions at one or more critical sites in the inflammatory pathways.

Rather than look on inflammation and hypercholesterolemia as alternative choices it may be more positive to regard them as "partners in crime", as discussed in detail elsewhere [30], or as "two sides of the same coin".

7 References

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