thematic review

Thematic review series: The Pathogenesis of Atherosclerosis

An interpretive history of the cholesterol controversy, part III: mechanistically defining the role of hyperlipidemia[1]

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Abstract In this third installment of the series, we point out that the absence of an explicit, detailed and plausible hypothesis linking hypercholesterolemia to the events in the artery wall was probably an important reason for continuing skepticism and for failure to treat elevated blood cholesterol levels. The rapid advances in understanding of lipoprotein metabolism in the 1950s and 1960s and the application of modern cellular biology in the 1970s provided the context for a modern consensus on pathogenetic mechanisms of atherogenesis.—Steinberg, D. **An interpretive history of the cholesterol controversy, part III: mechanistically defining the role of hyperlipidemia. *J. Lipid. Res.* 2005.** 46: **2037–2051.**

The thesis of this set of reviews is that substantial evidence for a causal relationship between hypercholesterolemia and atherosclerosis began to appear over 100 years ago and was already strong enough 40 years ago that it should have been persuasive. However, little or nothing was done about it, certainly not at the clinical level, until the 1990s. Even after the National Institutes of Health gave its blessings to treatment of hypercholesterolemia [after the 1984 Consensus Conference on Lowering Blood Cholesterol to Prevent Heart Disease (1)], practicing physicians paid little attention (2–4). In 1983, almost 50% of internists surveyed said they did not recommend *any* therapy, not even diet therapy, unless a cholesterol level was over 300 mg/dl! Over 40% of these internists recommended drug treatment only if the level was over 340; 27% said they *never* recommended drug treatment (3)! This history of the cholesterol controversy attempts to sort out the reasons it took so long to convince the profession and the public that correction of hypercholesterolemia should be a national public health goal.

Part I of this series (5) dealt with the landmark work of Anitschkow (6), which strongly indicted hypercholesterolemia as a sufficient cause of experimental atherosclerosis, and with that of Gofman et al. (7), which strongly suggested that the same was true for the human disease and first demonstrated the complexity of lipoproteins. Part II dealt with the several early lines of evidence that linked blood cholesterol to coronary heart disease (CHD) in humans, including the early clinical trials showing that cholesterol lowering by diet modification could indeed reduce risk (8). Here in Part III, we deal with one of the underlying reasons for the early skepticism about the lipid hypothesis, and that was the absence, until the 1980s, of an accepted, detailed hypothesis for lesion development that mechanistically linked lipoproteins to the pathogenesis of CHD.

THE IMPORTANCE OF UNDERSTANDING MECHANISM IN GAINING ACCEPTANCE OF A HYPOTHESIS

The lack of a well-delineated hypothesis is not necessarily a barrier to the acceptance of new directions in medical practice. The classic example is John Snow's demonstration that the 1854 cholera epidemic in London was attributable to contaminants in the water. When he removed the handle from the Broad Street pump, the number of cases in the area served by that pump promptly began to wane. Exactly what was in the water that caused the cholera would not be demonstrated for more than a quarter of a century. Still, the results of Snow's intervention were so dramatic that no one questioned the cause-and-effect relationship *even in the absence of an explicit hypothesis.* However, when the causal linkage is less obvious, the absence of a plausible hypothesis can be a significant deterrent to action.

To return to the case at hand, it was difficult for several reasons for physicians to accept that the concentration of blood cholesterol could be a major factor determining the

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chances of a myocardial infarction years or decades down the road. For example, as discussed previously (8), it was not appreciated that the *average* blood cholesterol level in the United States, the so-called *normal* level, was actually *abnormal*. It was accelerating atherogenesis and putting a large fraction of the so-called normal population at a higher risk for CHD. Also, very little was known about the structure and metabolism of these recently discovered and still mysterious cholesterol-protein complexes—the serum lipoproteins—and almost nothing about how they got into the vessel wall and contributed to the development of the lesions. A degree of skepticism was understandable.

The progressive enlightenment with regard to lipoprotein structure and metabolism in the post-Gofman decades, together with the development of a better understanding of the cell biology of the vessel wall, was critical in the fleshing out of the lipid hypothesis and is, therefore, an important part of this history.

EARLY ATTEMPTS TO DEFINE THE PATHOGENESIS OF ATHEROSCLEROSIS

Speculations about atherogenesis date back to the 18th century and earlier, but these speculations were not supported by much, if any, experimental evidence. Virchow's *insudation theory*, put forward in 1856, came closest to the mark but, like the others, it was a reconstruction based almost entirely on snapshots, i.e., on the gross and microscopic appearance of lesions at autopsy (9). He noted that lesion formation began with a thickening of the intima, which he attributed largely to "an increased imbibition of soluble components of the blood flowing past" the endothelium. He also noted the early occurrence of an increase in the numbers of subendothelial cells, an increase in their size, and the presence of lipoid substances, but had no way to deduce their origin or their significance. In the absence of a suitable animal model, the pathologist could not know the temporal relationships among the human lesions at autopsy.

Over 50 years later, Anitschkow beautifully described the foam cells in the lesions of the cholesterol-fed rabbit and concluded that they represented invading white blood cells (6). He reasoned this way: "One can see quite distinctly all conceivable transitional forms between the small lymphocytic and monocytic cells, on the one hand, and the large lipoid cells on the other . . . ." He also reported that "lymphoid or monocytic cells frequently seem to invade the aortic wall directly from the lumen." The development of the rabbit lesions and their severity depended on how high the blood cholesterol level was and on how long it was maintained at that level. Rabbits on normal chow showed no lesions at all, so lesion initiation could be taken to begin at the time cholesterol feeding began. Anitschkow could follow the evolution of lesions as a function of time by sacrificing animals after different periods on the cholesterol-rich diet (6). He reported that the very earliest change, a microscopic change anteceding the advent of grossly visible lesions, was the appearance of lipid in the space between the endothelium and the underlying inner elastic membrane. (In the rabbit, there are normally no cells between the endothelium and the inner elastic membrane.) In the next stage, he described the appearance in that space of "cells of a polyblastic or monocytic character" that contained lipid substances "in the form of little globules." These globules were anisotropic under polarized light, exhibiting "cruciform figures typical of cholesterin (cholesterol) esters." Anitschkow, for a number of reasons, favored the view that the foam cells represented mononuclear blood cells, as mentioned above, but he had no proof, and the origin of the foam cell remained an issue for some time. Readers interested in more details of the early history are referred to three chapters in the 1933 collection of essays edited by E. V. Cowdry (6, 10, 11).

Timothy Leary (father of the LSD guru of the same name), while a pathologist serving as Medical Examiner in Boston, made a detailed comparison of lesions in the cholesterol-fed rabbit and those in human coronary arteries and concluded, in 1934, that they were similar in most respects (12). His basic observations were in essential agreement with those of Anitschkow. However, he was struck by the large numbers of fat-filled cells in the liver of the cholesterol-fed rabbit, and he also observed a number of lipid-loaded macrophages in the general circulation. From these observations, he concluded that Kupffer cells loaded with lipids ("lipophages") exited the liver, entered the circulation, squeezed through the capillaries in the lungs, and penetrated the arterial wall, carrying their load of lipid in with them (13). In retrospect, the circulating "lipophages" probably represented foam cells escaping from fatty-streak lesions that had lost their endothelial cell cover (14). Although he was wrong about the cellular mechanisms involved, his work confirmed Anitschkow and, most important, suggested that the human and rabbit lesions were structurally similar and that lipids played a key role in both. His work was controversial at the time but helped revive interest in the possible etiologic role of blood cholesterol in the human disease.

In their influential 1951 review (15), G. Lyman Duff, the Canadian doyen of pathology, and his young collaborator, Gardner C. McMillan, agreed that the accumulation of cholesterol and other lipids was "one of the most striking morphologic features of both human atherosclerosis and experimental cholesterol atherosclerosis." They also acknowledged the potential importance of the then very new findings from Gofman's laboratory (7) correlating elevations of certain classes of lipoproteins with premature CHD. However, they concluded, as did almost all investigators at the time, that "in the vast majority of cases with or without clinically demonstrable atherosclerosis the blood cholesterol level is normal." Here was another example of how the tendency to equate *average* with *normal* led people astray. The proposition that a significant fraction of apparently disease-free people could actually be heading to a myocardial infarction because of hypercholesterolemia seemed implausible.

In 1958, Poole and Florey (16), using light microscopy, noted the presence of macrophages laden with lipid in the vessels of cholesterol-fed rabbits, both on the lumenal surface and under the endothelium. They saw some macrophages apparently penetrating between endothelial cells but, as they pointed out, there were no arrows to indicate which way they were going. Their observations supported the identification of monocyte/macrophages as the progenitors of foam cells but left open the question of just how they became loaded with lipids.

By 1963, there was a better appreciation of the complexities of lesion structure and the beginnings of experimental studies on the sources of the lipid accumulating in the lesions. However, as is apparent from the presentations at a 1963 symposium on the Evolution of the Atherosclerotic Plaque (16), the role of lipoproteins remained unclear. For example, the question of whether the lipids accumulating in the lesion were the result of biosynthesis in the vascular wall or of deposition from plasma lipoproteins was still an issue to be settled. There was still disagreement about the relative contributions of smooth muscle cells and of fibroblasts to the bulk of the growing lesion, because there were no unambiguous markers for the several cell types. Using the electron microscope, Haust was able to show clearly that smooth muscle cells were the predominant cell type in larger, stenotic lesions, an important contribution. She also noted that some of these smooth muscle cells contained lipid droplets (17). Indeed, smooth muscle cells do take up lipoproteins, as we know now; remnant particles are taken up more avidly than are VLDLs, as shown by Bierman et al. (18, 19). Under the right conditions, smooth muscle cells can express scavenger receptors (20, 21) and take up modified forms of LDL. Still, only a relatively small fraction of the foam cells in the early fatty streak lesion are derived from smooth muscle cells. Haust, probably because she was focusing on the larger, space-occupying lesions, was less impressed by the lipid-loaded foam cells of macrophage origin. Another reason for the tendency to focus on smooth muscle cells and matrix deposition was that the degree of stenosis in the coronary arteries was at the time, and until quite recently, believed to be the best measure of the risk of myocardial infarction. Now it is recognized that thrombosis at the site of a ruptured plaque precipitates most infarctions. Much of the time, the site of the plaque rupture is a lesion with less than 50% stenosis, not the tightly stenosed lesions expanding into the lumen. But in the 1960s, the focus was on the degree of stenosis, and thus on the cellular growth and matrix deposition that caused the lesion to increase in size.

Important additional evidence favoring the lipid hypothesis was the demonstration in rabbits and in nonhuman primates that lesions could regress when the hypercholesterolemic diet was discontinued and that not only the lipid content but also, to some degree, the content of collagen and elastin could be reduced (22, 23). These findings indicated that the accumulated lipid was in some way contributing to the buildup of connective tissue matrix, possibly by stimulating smooth muscle cell growth.

Later studies by Gerrity (24), using electron microscopy, and by Fowler et al. (25), using lipid-laden cells isolated from the lesions of cholesterol-fed rabbits, left no doubt that many or most of the lipid-laden foam cells in early lesions were derived from circulating monocytes. However, some smooth cells also imbibe lipids and have at least some of the properties of foam cells. As so often happens in science, the answer to the smooth muscle cell versus monocyte/macrophage controversy was not either/or but both.

THE RESPONSE-TO-INJURY HYPOTHESIS OF ROSS AND GLOMSET

Because the bulk of the lesion is attributable to smooth muscle cells and the matrix they secrete, Russell Ross and John Glomset undertook studies of the growth of smooth muscle cells in culture (26, 27). In 1974, they made a pivotal discovery: serum from blood that had been allowed to clot ("blood serum") contained a growth factor for smooth muscle cells that was absent in serum separated from whole blood not allowed to clot ("plasma serum") (28). Similar findings had been reported for cultured fibroblasts (29, 30). The factor was evidently present in blood platelets and was released when the platelets aggregated. Ross and Glomset christened the growth-promoting material "platelet-derived growth factor" (PDGF). They put these observations together with the existing evidence that mechanical injury to the endothelium could lead to platelet aggregation and intimal thickening and proposed their response-to-injury hypothesis (31, 32) (see **Fig. 1**). The initiating event was presumed to be some still-unidentified form of "insult" to the arterial endothelium, followed by denudation with exposure of underlying matrix to which platelets adhered, releasing PDGF and possibly other growth factors. These now had access to the cells in the subendothelial space and could stimulate smooth muscle cell proliferation. Hypercholesterolemia was recognized as one possible source of injury but it was not considered to be an initiating factor in any other context. If blood lipid levels were high, there would be some accumulation of lipids in the developing lesion. However, the entry of lipids was not considered to play an obligatory role in initiation, although it might accelerate progression.

The impact of the response-to-injury hypothesis was great. Here was a way to account for smooth muscle proliferation, a prominent feature of the growing plaque, on the basis of a phenomenon observed in vivo—platelet adherence to the artery wall. The two-part review published by Ross and Glomset in the *New England Journal of Medicine* in 1976 (31, 32) quickly became the standard reference on the pathogenesis of atherosclerosis. Ross and Glomset's work proved to be importantly heuristic. It was the first to show how new approaches from cell biology could be brought to bear on the problem of atherogenesis.

As first proposed, the theory was that some "injury" caused endothelial desquamation, allowing platelets to adhere to the exposed intimal collagen (Fig. 1). Repetition

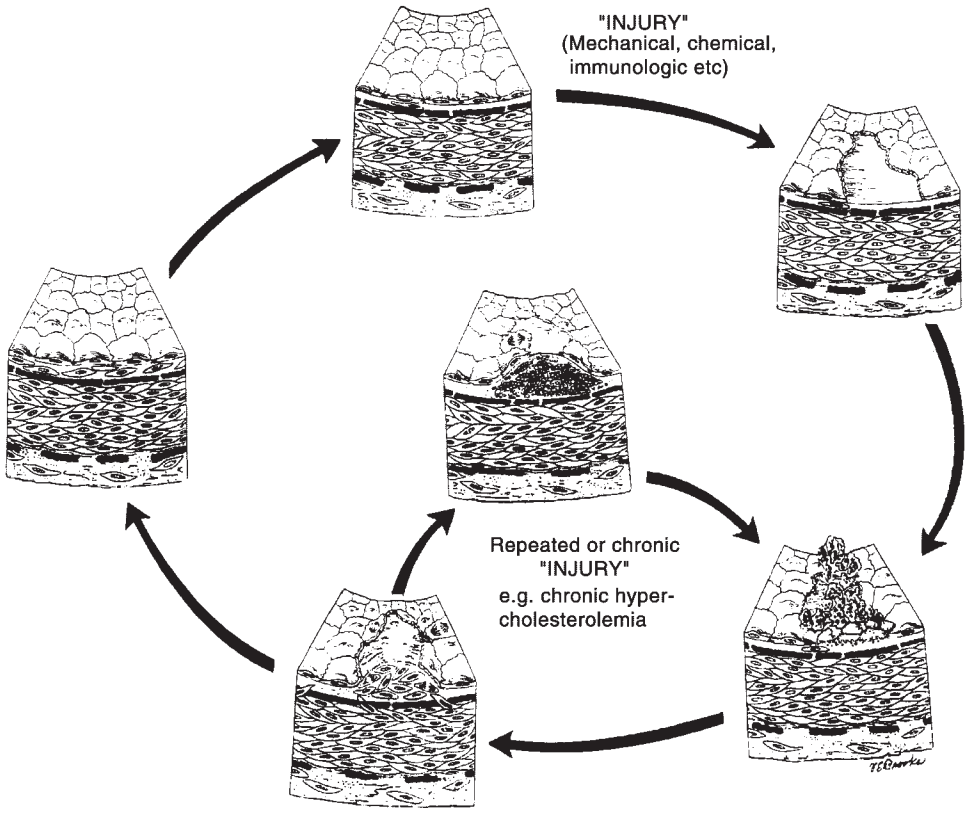

Fig. 1. Schema of the Ross/Glomset response-to-injury hypothesis. Reprinted with permission from Ref. (32). Copyright 1976, Massachusetts Medical Society. All rights reserved.

of such injuries over the years led to the complex later lesions. Later studies, however, clearly showed that the endothelial layer overlying the initial fatty streak lesion was actually unbroken (14, 16, 24, 33). These and other findings required later revisions of the theory, substituting functional injury for structural injury. However, the focus on the dialog between penetrating leukocytes and the cells of the artery wall—an "inflammatory" dialog—stood the test of time. In his last update of the hypothesis, published just a few months before his untimely death at age 69, Ross reviewed the factors that might be responsible for the "injury" to the endothelium (34). While acknowledging the possible role of hyperlipoproteinemia as an initiating factor that might promote inflammation in the artery, he gave it no greater weight than homocysteinemia, hypertension, infection, or other potential pro-inflammatory factors.

At about the same time that Ross and Glomset were developing their hypothesis, Benditt and Benditt, in the same department at the University of Washington, put forward their monoclonal hypothesis of atherogenesis (35). What they proposed was that the smooth muscle cells accumulating in any given localized atherosclerotic lesion had their origin in a single cell that had somehow been triggered to grow rapidly enough to become a benign tumor. In other words the atheroma was somewhat analogous to the leiomyoma, which had already been shown to exhibit monoclonality (36).

So we had the paradoxical situation that during the same years that the evidence for hypercholesterolemia as a primary causative factor was accumulating, the two most widely accepted hypotheses for the pathogenesis of atherosclerosis barely mentioned lipoproteins. Lipid accumulation was recognized to occur, of course, but it was almost regarded as an epiphenomenon. The response-to-injury hypothesis and the monoclonal hypothesis provided attractive schemes of pathogenesis that did not appear to involve hypercholesterolemia in any substantive way. Little wonder then that the skeptics could easily discount the lipid hypothesis as "case not proved."

UNRAVELING THE COMPLEX METABOLISM AND INTERACTIONS OF THE PLASMA LIPOPROTEINS

Generating a plausible cholesterol hypothesis would first require an understanding of the structure and metabolism of the macromolecules that carried it. The road to that understanding began with Gofman, as reviewed previously (5). Over the next several decades, there was a ferment of activity in many laboratories around the world that spelled out in detail the metabolic origins and metabolic fates of the lipoproteins, their transport functions, their complex interactions and transformations in the plasma, and the functional importance of the various apolipoproteins as cofactors and as the means of "addressing" lipoprotein particles.

It would require much more space than is available

here to do justice to this important chapter in atherosclerosis research. An informative and engaging historical review by Donald S. Fredrickson is warmly recommended (37), as are a number of additional reviews by some of the people who developed the field (38–42). Here we will limit ourselves to the question of how progress in the lipoprotein field contributed directly to understanding atherogenesis and gaining acceptance of the lipid hypothesis. It did so in several ways:

First, it made the blood cholesterol-CHD connection concrete at the biochemical and, ultimately, at the molecular level.

Second, it provided the basic science substratum on which atherosclerosis research was going to be built.

Finally, it provided the building blocks for the development of therapies that would one day make it possible to correct hypercholesterolemia and reduce CHD risk, thus establishing the validity of the "lipid hypothesis" once and for all.

In the '40s and '50s, atherosclerosis research was mostly the province of the pathologists, and it was largely descriptive. Very few basic scientists were attracted to the field, in part because they could see no obvious "hooks" on which to hang their biochemical hats. There were plenty of exciting questions in better-plowed fields. The opening up of the lipoprotein field provided the "hook." Here was a dynamic, complicated system for lipid transport that was being explored for the first time. More sophisticated tools for studying lipoproteins in experimental animals and also in humans were becoming available. If blood cholesterol played a role in atherosclerosis, then unlocking the mysteries of the complexes that carried it could be very important. Even if lipoproteins turned out to be unimportant in atherogenesis, an understanding of them would probably yield important general insights into other aspects of normal and abnormal lipid transport.

THE NATIONAL HEART INSTITUTE STORY

At almost exactly the same time that Gofman was beginning his lipoprotein studies in Berkeley, the National Institutes of Health (NIH), in 1948, established the National Heart Institute, with James A. Shannon as Associate Director for Research. Shannon, who had trained at New York University under the great guru of kidney physiology, Homer Smith, was widely known and respected for his personal scientific contributions and for his good judgment and wise leadership when he was Director of the Squibb Institute for Medical Research. It was now his job to recruit the best and brightest from their university ivory towers to this new federal laboratory outside Washington, D.C. This was not an easy job in 1949. At that time, the NIH was still not well known. It consisted of just three small buildings plus the Cancer Institute (the *only* categorical Institute at the time). Although there were ambitious plans for a huge new research building (which opened as the Clinical Center in 1954) and a great deal of enthusiasm in the Congress, the words "government laboratory" still had a musty connotation. Career-conscious young academicians instinctively felt that their futures were more assured in academia than in the U.S. Civil Service or in the U.S. Public Health Service. On the other hand Shannon had some powerful weapons going for him—more research money, better space (once the Clinical Center was finished), his own impeccable credentials as a scientific leader, and his own special brand of Irish charm. And, not unimportantly, he could offer draft deferments. With those weapons, he was successful in putting together a remarkable cadre of talented researchers to head up the divisions of the new Institute, including, among others, R. W. Berliner, R. Bowman, B. Brodie, S. Sarnoff, E. Horning, B. Witkop, and C. B. Anfinsen.

Anfinsen was a young assistant professor of biological chemistry at Harvard Medical School who came to Shannon with all-out recommendations from the department chair, A. Baird Hastings. Anfinsen's research interests were in basic enzymology and protein chemistry. Any connection between his research and heart disease was, to say the least, remote. However, Shannon assured Anfinsen that research with a direct linkage to heart disease was not a requirement in his new Institute. The Institute would support research that spanned from the most basic (Anfinsen's Laboratory of Cellular Physiology and Metabolism) to the most applied (the Cardiac Surgery Branch). Shannon promised that no one would pressure him to move away from the basic questions that interested him. Indeed, that pledge was honored. From the beginning, Anfinsen focused his energies on basic studies of the structure of RNase. He ultimately showed that its catalytic activity depended on its precise configuration, or folding, which, in turn was determined by the amino acid sequence and proper disulfide bonding. For that work, he shared a Nobel Prize in 1972 with William H. Stein and Stanford Moore of the Rockefeller University. So clearly, neither Shannon nor Shannon's successors interfered with Anfinsen's basic research. That having been made clear, I think I can now recount what may have represented a minor departure from this strict hands-off policy, a departure with a happy outcome.

In 1950 or 1951, Shannon dropped by Anfinsen's lab for a chat. He asked Chris if he was aware of the work being done in California on lipoproteins and their possible relevance to heart disease, work of a young investigator named Gofman. No, Chris was not aware of it. Shannon said the work was getting a lot of attention and that some members of the Congress had asked him about it. Would Chris look into it and advise Shannon? After all, he said, we are the National Heart Institute and we should at least keep track of what's going on. Chris did look into it and, probably to his surprise, found something intriguing, something he thought would be fun to follow up, namely, the nature of what was then called the "clearing factor." The upshot was that Shannon assigned more positions and more lab space to Anfinsen and brought the NIH squarely into the lipoprotein era. The "clearing factor" story is a nice example of serendipity in science and it is worth taking a step back to recall it.

In 1943, P. F. Hahn had reported a chance observation

he had made while studying factors regulating the mass of red blood cells in dogs (43). The animals were supposed to be fasted overnight before they were studied, but one evening the technician forgot to remove the food from the cages. Hahn's protocol called for drawing a blood sample at the beginning of the experiment, then giving the dog an injection of heparin to prevent clotting, and finally drawing a second blood sample. On this particular day, the first samples were visibly cloudy, but the second samples, taken just 5 or 10 min later, were perfectly clear. Hahn had previously shown that adding heparin directly to the cloudy plasma did not have any clearing effect. The injected heparin must, in this case, be somehow eliciting the formation of a "clearing factor" in the dog's body. This was evident, because just adding some of the clear plasma drawn from a control dog after a heparin injection to a sample of cloudy plasma from a nonfasted dog caused it to "clear." Anderson and Fawcett confirmed these observations (44) and speculated that the clearing was due to some physico-chemical disruption induced by heparin-phospholipid complexes. D. M. Graham in Gofman's lab used the analytic ultracentrifuge to show that after heparin injection, there was a rapid decrease in the concentrations of large lipoproteins, accompanied by a concurrent increase in the concentrations of smaller, denser lipoproteins (45). Still the mechanism remained obscure. Anfinsen reviewed these data and "smelled" an enzyme. He and his newly recruited young colleagues, R. K. Brown and E. Boyle, quickly showed that heparin was actually releasing an enzyme into the blood stream. The clearing factor was a lipase (46, 47). A young postdoctoral fellow, E. D. Korn, joined the group shortly thereafter and succeeded in purifying the enzyme, and christened it "lipoprotein lipase" (48). Shannon was happy, the congressmen were happy, and Anfinsen had made his contribution to the disease-oriented mission of the National Heart Institute.

The lipoprotein group put together by Anfinsen expanded when the huge new research building, the Clinical Center, opened in 1954. The group in those early years included, among others, Richard J. Havel, Donald S. Fredrickson, Robert S. Gordon, Daniel Steinberg, Joseph H. Bragdon, James Baxter, Howard Goodman, and Howard A. Eder. Over the next two decades, the work of this group and their colleagues in other institutes, including, notably, Donald Frye and Robert W. Mahley, put the NIH on the map as one of the world's outstanding centers for lipoprotein and atherosclerosis research. It has continued to enjoy that reputation to this day. Anfinsen himself, having launched the enterprise, returned full time to RNase, but he had left his mark on the lipoprotein field—all because Shannon had gently suggested one day that "Gofman's stuff might be worth looking into."

BRINGING THE LIPOPROTEIN CONCEPT INTO CLINICAL PRACTICE

One of the young tigers recruited in 1953 by Anfinsen to join him was Donald S. Fredrickson, fresh out of a residency and fellowship at Massachusetts General Hospital. Fredrickson's talent was immediately evident, and he quickly rose to be the head of his own section within Anfinsen's Laboratory of Cellular Physiology and Metabolism. In 1966, he became Chief of the Molecular Diseases Branch of the National Heart Institute. Later, he would go on to become Director of the National Heart, Lung, and Blood Institute, then Director of the entire NIH, and finally, in 1984, President of the Howard Hughes Medical Institute, the single largest private philanthropy supporting biomedical research.

Fredrickson was persuaded of the correctness of Gofman's view that patterns of lipoproteins might contain valuable information beyond that given by measurement of the component lipids only (cholesterol and triglycerides). But he also realized that Gofman's analytical ultracentrifuge method was just too complicated and too expensive ever to be a practical clinical tool. Preparative ultracentrifugation, introduced by Havel, Eder, and Bragdon (49) in Anfinsen's laboratory, was a powerful research tool, but again not practical in clinical medicine. So when a young man named Robert S. Lees came into his laboratory as a postdoctoral fellow and showed him a wonderfully simple new method of separating plasma lipoproteins by paper electrophoresis (50), Fredrickson immediately saw its enormous clinical potential. Over the next few years Fredrickson and his collaborators, R. S. Lees and R. I. Levy, studied and classified the lipoprotein patterns in hundreds of patients referred to the Clinical Center at NIH (51–53). They found that most of them could be put into one of five types. These provided a context within which different lipoprotein disorders could be classified. Later studies would break down some of these patterns into subclasses with different underlying causes, genetic and environmental, but the availability of a relatively cheap and simple way of looking at lipoproteins sparked a wave of enthusiasm among clinicians around the world. The World Health Organization eventually adopted the Fredrickson system of classification as the international standard. It is fair to say that Fredrickson brought lipoproteins into the vocabulary of the practitioner, and this undoubtedly had a major impact on the clinical management of lipoprotein abnormalities.

MOVING FROM PHENOTYPE TO GENOTYPE

The Fredrickson classification was strictly phenotypic. Little or nothing was known about the origin and metabolism of the individual lipoproteins or their relationship to one another. The mechanisms underlying the five Fredrickson Types remained unknown. Nor was it clear which were genetically determined and to what extent.

In 1973, Arno G. Motulsky and his colleagues at the University of Washington published the results of a heroic study involving 2,500 relatives of 149 probands that had had a myocardial infarction and who had either hypercholesterolemia or hypertriglyceridemia or both. A major driving force and first author on two of these papers was a young postdoctoral fellow, Joseph L. Goldstein. Out of this work

came the first genetics-based classification of the hyperlipidemias (54–56). Three monogenic disorders were defined, and these were inherited in a Mendelian-dominant fashion. In many of the families, hypercholesterolemia was genetically determined but involved multiple genes. Finally, there were many cases of triglyceride elevation that appeared not to be genetic. The concordance between this classification and the phenotypic classification of Fredrickson was poor, sometimes two or more phenotype patterns appearing in a single genetic disorder. Over the next few years, more sophisticated studies of lipoprotein metabolism and improved methods of genetic analysis would confirm the basic correctness of this gene-based classification.

The high frequency of lipoprotein abnormalities in relatives of heart attack victims in this study again strongly suggested a causal relationship, but it by no means proved it. Skepticism about the lipid hypothesis continued to be the order of the day.

WHICH LIPOPROTEINS ARE PROATHEROGENIC?

The question is more complicated than it may sound. For example, chylomicrons, because they are so large and get into the arterial wall much more slowly than even VLDLs, would appear not to pose much of a threat. And indeed, this appears to be the case, as long as the chylomicrons are not being broken down into smaller particles at any significant rate, as in the case of patients with familial lipoprotein lipase deficiency or in the case of cholesterol-fed diabetic rabbits. Compared with nondiabetic animals with equal elevations of total blood cholesterol, diabetic cholesterol-fed rabbits, paradoxically, have much *less* atherosclerosis (15). As shown by Zilversmit and colleagues (57, 58), this is nicely explained by the fact that chylomicrons and very large VLDLs are virtually excluded from the subendothelial space. On the other hand, if lipoprotein lipase is active and the chylomicrons are degraded to smaller, so-called remnant particles, they now can and do enter the artery wall and are decidedly proatherogenic. The clearance of lipoproteins into the artery wall in the rabbit decreases linearly with the logarithm of their molecular diameter (59).

When chylomicrons or large triglyceride-rich VLDLs are acted on by lipoprotein lipase with removal of most of the triglyceride, they retain their full complement of apolipoprotein B (apoB) and show a high content of apoE. These remnant particles are rich in both cholesterol and triglycerides and float at the density of VLDL but have β electrophoretic mobility (β-VLDL) (35). These remnant particles account for a major part of the hypercholesterolemia in patients with dyslipoproteinemia associated with the apoE-2 phenotype, because this isoform of apoE binds very poorly to hepatic receptors. These patients are at high risk for atherosclerotic complications, showing that these are decidedly proatherogenic lipoproteins. β-VLDL can be avidly taken up by macrophages, even without prior modification, probably by way of the LDL receptor rather than scavenger receptors (60).

LDL is the most important proatherogenic lipoprotein, because it is, by all odds, the lipoprotein fraction most commonly elevated in the garden-variety hypercholesterolemic patient at high risk for CHD.

Finally, HDL was suggested by the pioneering work of Russ, Barr, and Eder (61) and firmly established by Miller and Miller (62) to be a *negative* risk factor for atherosclerosis. The reasons for this were not at all clear but later studies, stimulated by the strength of the predictive power of HDL as a negative risk factor, led to the elucidation of the reverse cholesterol transport pathway. This was a striking example of the way in which epidemiologic correlations can point the experimentalist in the right direction.

DISCOVERY OF THE LDL RECEPTOR: THE REMARKABLE PARTNERSHIP OF BROWN AND GOLDSTEIN

Without question, the discovery of the nature of the defective gene in familial hypercholesterolemia (FH) by Goldstein and Brown was a major milestone in the lipoprotein field (63–65). In a remarkable series of elegant and insightful papers published in the ’70s and ’80s, they established that the cellular uptake of LDL absolutely requires the LDL receptor. In the complete absence of a functional receptor, the LDL cholesterol concentration can build up to 800 to 1,000 mg/dl. Because FH was clearly a monogenic disorder, it could now be said that the high LDL levels, secondary to the lack of LDL receptor function, must be the immediate and *sufficient* cause of atherosclerosis in these patients, including the devastating myocardial infarctions that sometimes occur as early as the first decade of life. The importance of the Goldstein/Brown work in supporting the lipid hypothesis cannot be overstated. They were awarded the Nobel Prize in Physiology or Medicine in 1985. How they became lifetime collaborators and how they made this seminal discovery makes a fascinating story.

They first met and learned to appreciate one another when they both served their internship and residency in medicine at Massachusetts General Hospital, from 1966 to 1968. Then they both spent the next two years at the National Institutes of Health, Goldstein working with Marshall W. Nirenberg, winner of a Nobel Prize in Physiology or Medicine in 1968, and Brown with Earl R. Stadtman, probably the most brilliant enzymologist at NIH (or anywhere else for that matter). Working as they were in different labs, there was no opportunity to do collaborative biochemical research at that time. However, they both wanted to do research on metabolic diseases and both were intrigued by the still-mysterious disorder of familial hypercholesterolemia. Goldstein, as a clinical associate responsible for the medical care of Dr. Donald S. Fredrickson’s research patients in the Clinical Center, saw these fascinating cases close up and discussed them with Brown. The two of them shared many common scientific interests, but the seeds of what would become a life-long partnership were sown not in the laboratory but over the bridge table. Both were duplicate bridge fiends!

Goldstein did his medical training at Southwestern Medical School at the University of Texas Health Science Center in Dallas. Dr. Donald W. Seldin, Chair of the Department of Internal Medicine, had a keen eye for talent and he saw that Goldstein was simply brilliant. Seldin was following a "grow your own" strategy to build the research strengths of his department, which was at the time already the most outstanding department in the medical school and one of the best in the country. So during Goldstein's senior year, Seldin "recruited" him. If Goldstein would agree to obtain graduate training in human genetics, Seldin would guide him through his residency and postdoctoral training and guarantee him a faculty position as head of a division of genetics on his return. Seldin had, and still has, the highest respect for excellence. Seldin also had, and still has, an intensity and charm that is irresistible. His was an offer that Goldstein could not refuse.

Brown got his M.D. at the University of Pennsylvania, where he was first in his class. As a medical student, he dove into research, spending three summers in the Smith-Kline laboratories. A rotation in Albert Winegrad's laboratory at Penn aroused his interest in lipid metabolism and in a research career. James Wyngaarden, Chairman of Medicine at Penn at the time, helped engineer the residency for Brown at Massachusetts General Hospital.

During their two years as clinical associates, Goldstein tried to "do a Don Seldin" on Brown, extolling the virtues of Southwestern Medical School in general and of Don Seldin in particular. "Come to Dallas, and together we'll solve the mysteries of familial hypercholesterolemia" may have been the bottom line. It worked. Brown, after working another year at NIH in Earl Stadtman's laboratory, accepted Don Seldin's offer of a faculty position in the Division of Gastroenterology. Brown, working with John M. Dietschy and Marvin D. Siperstein, partially purified and characterized the HMG-CoA reductase from liver (66), setting the stage for what was to follow when Goldstein returned to Southwestern.

Goldstein, after his two-year postdoctoral fellowship with Arno G. Motulsky, a world-class human geneticist at the University of Washington, returned to Dallas per agreement with Seldin. The Brown and Goldstein collaboration got under way—and it is still going. They have continued to work smoothly as a two-man team now for almost 35 years.

Goldstein and Brown start their search for the faulty gene in FH

When they started their collaboration, Brown and Goldstein knew that FH was due to a single gene mutation from the earlier studies of Wilkerson, Hand, and Fliegelman (67), Adlersberg, Parets, and Boas (68), and Khachadurian (69). But which gene? Marvin D. Siperstein, a senior member of the Department of Medicine in Dallas, was working at the time on the rate-limiting enzyme in the synthesis of cholesterol, HMG-CoA reductase. Goldstein and Brown picked this enzyme as a good starting place. They postulated that the cells in patients with FH might be producing cholesterol at an abnormally high rate, secondary to a genetic flaw in the reductase enzyme or in its regulation.

Cholesterol synthesis in skin fibroblasts in cell culture

The technique of growing cells in culture was still relatively new in 1972. The notion that cultured cells might allow pinpointing of metabolic errors was still newer. At that time, the liver was believed to be both the source of the blood lipoproteins and the site at which they were removed from the blood. Brown and Goldstein would therefore have liked to study liver cells in culture but it was difficult to justify the risks associated with liver biopsies purely for research purposes. On the other hand, human skin fibroblast cells were readily grown in culture and if the gene defect were global it might be apparent in skin fibroblasts. Several gene abnormalities underlying metabolic disorders had already been discovered and characterized using skin fibroblasts, so Goldstein and Brown decided to give it a try. Their first hypothesis was that the rate of cholesterol synthesis would be abnormally high. They would assay the activity of the reductase enzyme and take that as a measure of the rate of cholesterol synthesis.

In their very first set of experiments, they found that in normal cells grown in the presence of serum, the rate of cholesterol synthesis was low. However, when the serum was removed and the cells were incubated overnight in a simple, protein-free medium, the rate of cholesterol synthesis rose sharply, as much as 10-fold. They showed that the suppressive activity of the serum on the normal cells resided in the LDL fraction.

In contrast, cells from patients with FH always showed a high rate of cholesterol synthesis, even in the presence of serum. Moreover, the addition of LDL to the medium, which reduced synthesis 10-fold in normal cells, had absolutely no inhibitory effect in FH cells. At that point, Brown and Goldstein postulated that the gene defect must be in some "hitherto unidentified gene whose product is necessary for mediation of feedback control by lipoproteins." It was known that cholesterol in the diet suppressed the rate of cholesterol synthesis and that cholesterol synthesis in tissues was suppressed by cholesterol in the incubation medium. So at this point, they were presumably visualizing a system for regulating the synthesis of cholesterol that was faulty in FH patients because one of the genes involved in that "feedback" regulation was defective.

The problem is getting LDL inside the cells!

Then they discovered that the FH cells, while not responsive to LDL in the medium, responded nicely if pure cholesterol (dissolved in alcohol) was added to the culture medium instead of LDL. The response of the cells to free cholesterol in the medium was no different from that of normal cells. In other words the FH cells *could* respond just as well as normal cells, provided the cholesterol got into the cells. The FH cells could not take up cholesterol when it was offered as a component of LDL, but could respond normally to cholesterol once it got inside the cell. That is how the concept of the LDL receptor was born. It provided a mechanism for the transfer of LDL, with its cholesterol, from the surrounding medium to the inside of the cell. Goldstein and Brown went on to characterize

the receptor, delineate the "LDL receptor pathway" and, ultimately, to clone the receptor.

These were elegantly simple experiments, carefully conducted and correctly interpreted. This was the first transport receptor to be characterized. Hormone receptors also bind their respective ligands with high affinity, but they regulate the cell's metabolism by molecular signaling ("second messenger" systems). The concept of receptor-mediated endocytosis represented a major contribution to biology in general. Large numbers of other receptors that function in this way have, of course, since been identified and characterized. The LDL receptor of Brown and Goldstein was the prototype (70). The schema shown in **Fig. 2** became arguably the most reproduced "logo" in cell biology in the 20th century.

These results considerably strengthened the lipid hypothesis. The key point was that a monogenic defect was sufficient to raise plasma LDL markedly, and that, apparently, was in turn *sufficient* to cause atherosclerosis. The atherosclerosis was not the result of some other pathway affected by the LDL receptor gene mutation. There was, however, one caveat. Patients with FH have elevated levels of intermediate density lipoproteins (IDLs) as well as LDL. This is because, normally, a large fraction of the IDL (and some VLDL) is taken up, like LDL, by way of the LDL receptor. But that uptake is linked not to binding of apoB but to that of apoE on the lipoprotein particles (39). Also, the apoE-rich IDLs (β-VLDL) are avidly taken up by macrophages and could account for foam cell formation, as mentioned above (60, 71). So at one point, the notion was seriously entertained that it might be the IDL rather than the LDL that was mainly responsible for the atherosclerosis in FH. The later discovery of patients with defective apoB-100, caused by a mutation at residue 3500, resolved the question (72, 73). The LDL in these patients binds very poorly to the LDL receptor, and so LDL levels can build up to values almost as high as those seen when the LDL receptor itself is defective. But these patients do not have any elevation of IDL levels because their LDL receptors are normal and the affinity of their remnant lipoproteins for the LDL receptor is normal. As pointed out by Myant (74), their cholesterol levels, while variable, can be comparable to those in FH, and the severity of their atherosclerosis is similar. In this way, the 3500 apoB mutation, raising LDL levels in an entirely different way and without causing a build-up of IDL, further supported the lipid hypothesis and pinpointed LDL as the key atherogenic lipoprotein. This is not to say that IDL never plays a role; when present at high levels, as in patients with dyslipoproteinemia, IDL can certainly play an atherogenic role.

Brown and Goldstein: an appreciation

The Goldstein-Brown partnership published its first joint paper in 1973. Seldom has there been such a fruitful blending of two talents. Over the next 12 years, they published an average of ten to twelve papers a year, and every one of them was highly significant. Many scientists publish (and, sad to say, even re-publish) minor findings to pad their bibliographies. Brown and Goldstein never did that. They were quickly recognized by everyone in the field as a major new force. Some spoke of them with amazement (and probably a touch of jealousy) as the "Dallas Paper-of-the-Month Club." It was said that they sustained the breathtaking pace of their research by dividing responsibilities on a rotating basis: one would run the lab while the other wrote the papers! Every original paper they wrote was jointly coauthored—and they alternated the order of authorship religiously between Brown-Goldstein and Goldstein-Brown. This was a partnership in a class with Gilbert and Sullivan, Rodgers and Hammerstein or, more aptly, Stein and Moore (who shared the Chemistry Nobel Prize in 1972 with C. B. Anfinsen). Goldstein and Brown were made full professors by age 36, were elected to the National Academy of Sciences at age 40, and won the Nobel Prize in 1985 at age 45. It was my honor to introduce them at a conference in San Diego in 2005, and I pointed out that the Nobel Prize had not damaged their productivity,

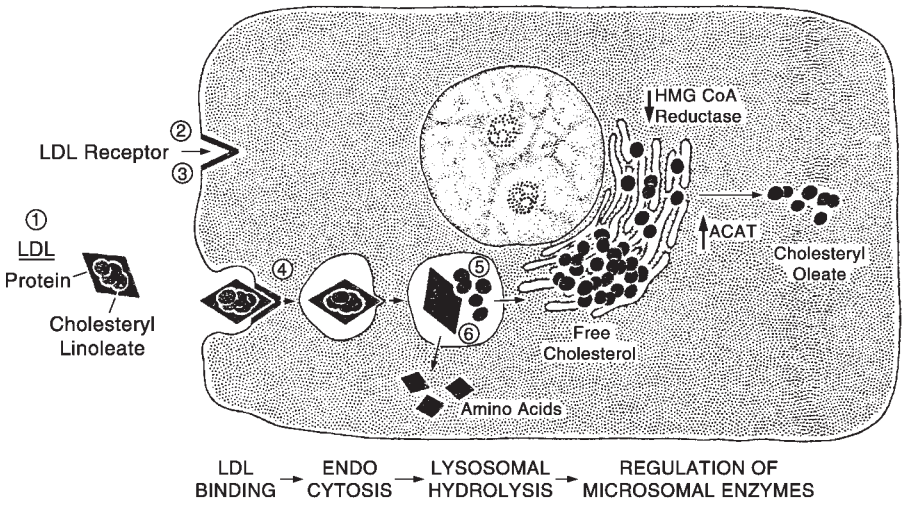

Fig. 2. The LDL receptor pathway for endocytosis as originally proposed by Brown and Goldstein in 1976. Reprinted with permission from Ref. (70). Copyright 1976 AAAS.

as it often has for other winners. In 2003, they won the prestigious Albany Prize and the citation specifically declared that it was being awarded for work done *after* 1985 (**Fig. 3**).

Before leaving the "B and G" story, it should be said that they have been unfailingly generous to colleagues and trainees. Almost every one of their trainees has gone on to hold a major chair here or abroad. I never had the pleasure of collaborating directly with them, but I have my own direct experience of their magnanimity. In 1975, Nicholas B. Myant at Hammersmith Hospital arranged a Ciba Symposium in London at which Brown and Goldstein and our group from La Jolla were invited to present. Olga and Yechezkiel Stein from Jerusalem, pioneers in the study of lipoproteins and atherosclerosis, were at the time visiting scientists in my lab in La Jolla. Together with my postdoctoral fellow, David B. Weinstein, we had studied LDL metabolism in cultured cells that Myant had sent us from one of his patients with homozygous FH. Our data nicely confirmed the basic Brown-Goldstein finding: these cells could not degrade LDL. However, we failed to see any defect in LDL binding and proposed that in this particular patient (and possibly in other FH patients), the defect was not in the binding to the cell surface but in the mechanisms by which the LDL, once bound to the surface, was internalized. At the London conference, when I got up to present our results, I put the names of the four authors on the blackboard—Stein, Weinstein, Stein, and Steinberg—and referred to it as the "Vierstein" paper (75). When Brown got up to make his presentation, he said that in Dallas, they, unfortunately, had only one Stein—and, quick on the draw as always, scrawled "Einstein" on the blackboard.

It turned out that they were absolutely right with only one Stein, while we were wrong despite our four Steins. We had been misled by the general "stickiness" of LDL and were measuring a lot of nonspecific (irrelevant) binding that masked the binding defect that was actually there. Goldstein and Brown's subsequent work with the same cell line from Myant's patient showed that there was a clear deficiency in binding, in addition to the defect in degradation. Ironically, Brown and Goldstein later discovered an FH patient in whom the receptor did bind normally but failed to internalize (76). Throughout this contretemps, Goldstein and Brown were generously nonjudgmental, sharing data with us and offering advice. Nor did it stop them from inviting me to Stockholm in 1985 as one of the six colleagues the winners are allowed to invite as special guests of the Nobel Committee. They are good at forgiving.

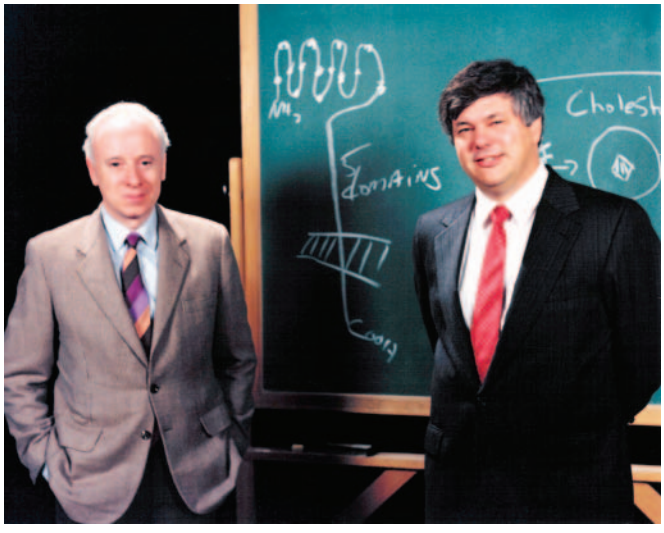

Fig. 3. Joseph L. Goldstein and Michael S. Brown at the time they were awarded the Nobel Prize in Physiology or Medicine in 1985. (Photo courtesy of Drs. Brown and Goldstein.)

DISCOVERY OF THE SCAVENGER RECEPTOR ON MACROPHAGES

The discovery of the LDL receptor by Brown and Goldstein represented a turning point in the history of lipoprotein research (65), but equally important was their discovery of the scavenger receptor on the macrophage (77, 78) and the nature of the ligands for it (79). They were struck by the fact that most of the cells from patients with homozygous FH take up LDL at very low rates. Indeed, the slow uptake by the liver accounts for the very high steady-state concentrations of LDL in their blood. However, the cells in xanthomas and in atherosclerotic lesions are heavily loaded with cholesterol, suggesting that they might be taking up LDL rapidly. Yet many of these patients have no functional LDL receptors. Therefore, the uptake has to be by some alternative mechanism(s).

Knowing that foam cells in lesions are largely derived from circulating monocytes, they tried to generate foam cells in vitro by incubating mouse peritoneal macrophages or circulating monocytes with high concentrations of LDL. Even at very high concentrations, uptake was slow and no foam cells developed. Because the ultimate source of the cholesterol stored in xanthomas and in arterial lesions had to be plasma LDL, they reasoned that the circulating LDL must undergo some modification and that it was the modified form that entered the macrophages. They explored a number of chemical and enzymatic modifications of LDL but the only one that worked was chemical acetylation. Acetyl-LDL bound with high affinity to macrophages and was taken up rapidly enough to strikingly increase the cell content of cholesterol. Moreover, this uptake had all the earmarks of a receptor-mediated process, and they christened the receptor the acetyl-LDL receptor. However, there was no evidence that acetyl-LDL was generated in vivo, and they considered it unlikely that it would be. Fogelman et al. (80) showed that malondialdehyde-treated LDL bound with high affinity to macrophages and suggested that such a modification might occur in vivo when platelets aggregated. However, there was no evidence that such a modification ever occurred in vivo.

Other chemical modifications involving conjugation of lysine residues with malondialdehyde (80) or by acetoacetylation (81) were shown to mimic acetylation, but

none of these modifications of LDL were shown to occur in vivo. The search for the modified LDL postulated by Goldstein and Brown went on, and several candidates emerged.

OXIDIZED LDL

In 1979, Henriksen, Evensen, and Carlander, in Oslo (82), and Hessler, Robertson, and Chisolm, in Cleveland (83), independently observed that cells cultured in the presence of native LDL and in the absence of serum underwent severe damage, beginning to die within 24 h. This cytotoxicity was inhibited by serum or by HDL. Henriksen, interested in the mechanisms underlying this cytotoxicity, came as a visiting scientist to the Specialized Center of Research on Arteriosclerosis in La Jolla. He found in 1981 that during the incubation of LDL with cultured endothelial cells, the LDL was drastically altered, becoming more dense, more electro-negative and, most important, becoming a ligand for receptor(s) on the macrophage. This so-called "endothelial cell-modified LDL" could increase the cholesterol content of the macrophage, and it was added to the list of LDL modifications that might solve the paradox of how macrophages become foam cells (84). Then Hessler et al., in Cleveland, demonstrated that the changes induced in LDL by incubation with endothelial cells were due to free radical modification (85). Steinbrecher et al., in La Jolla, independently came to the same conclusion with regard to the mechanism by which endothelial cells converted LDL into a ligand for macrophage receptors (86). Addition of vitamin E or a low concentration of normal serum blocked both the oxidation and the conversion of LDL to its more atherogenic form. Together with later findings showing that oxidized LDL was chemotactic for circulating monocytes but inhibited the motility of macrophages (87), the hypothetical scheme for lesion initiation shown in **Fig. 4** was proposed.

The findings in Cleveland and in La Jolla were strongly heuristic, stimulating a burst of activity in many laboratories. Over the next 10 years, over 300 papers were published relating to oxidized LDL and its possible role in atherogenesis, and the number now stands at over 3,000.

The "oxidative modification hypothesis" is now strongly supported by a number of lines of in vitro and in vivo evidence, and the interested reader is referred to several comprehensive reviews (88–92). The epidemiologic evidence and the evidence in experimental animal models of atherosclerosis were sufficiently persuasive to lead to recommendations that clinical intervention trials be undertaken (93). However, the trials to date, most of them using vitamin E as the antioxidant, have been negative (94, 95). The possibility that other antioxidants, possibly started earlier in life or acting by other mechanisms, may prove effective has not been ruled out (92, 94). Here we want only to record how the availability of an explicit hypothesis potentially linking LDL directly to atherogenesis helped enlist support for the recognition of hypercholesterolemia as a centrally important causative factor in CHD.

OTHER LIGANDS AND OTHER MACROPHAGE RECEPTORS THAT MAY CONTRIBUTE TO FOAM CELL FORMATION

Lipoprotein remnant particles rich in apoE can effectively load macrophages with cholesterol and could play a role in foam cell generation (60). These lipoproteins are apparently recognized by the LDL receptor rather than the scavenger receptor, so they would not account for foam cell formation in patients with no functional LDL receptors but could play a role in other cases of hypercholesterolemia. Further research is needed.

Aggregated LDL is much more rapidly taken up by macrophages than is native LDL, but again the uptake appears to occur via the LDL receptor (96). Aggregation of LDL

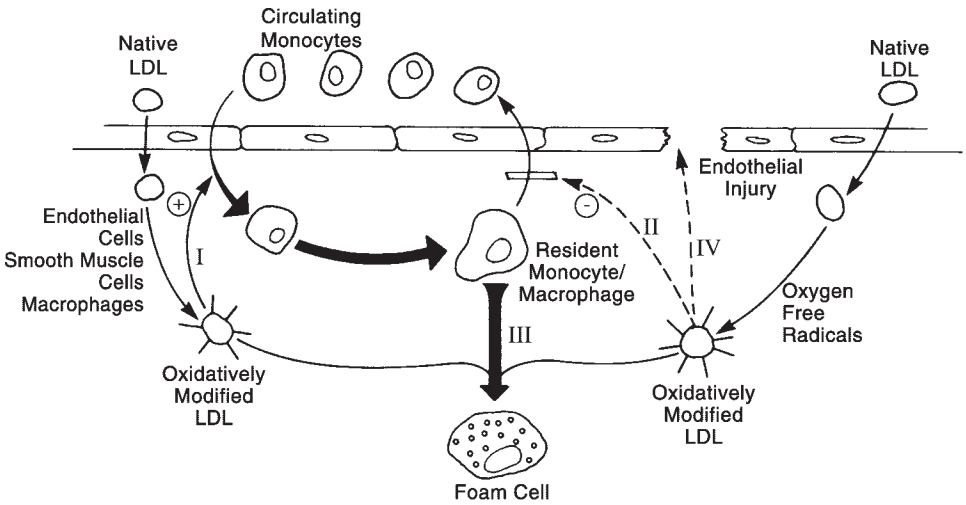

Fig. 4. Schema showing how oxidized LDL might be involved as an initiator of atherosclerosis, based on the work of Henriksen et al., (82, 84), Hessler et al., (85), and Quinn et al. (87). Reprinted with permission from (87).

in the subendothelial space has been demonstrated (97) and this may be encouraged by the proteoglycans in the artery wall to which LDL binds avidly (98).

Immune complexes of LDL with antibodies can enhance macrophage uptake via the F_c receptor (99), another possible mechanism to account for foam cell formation, in this case even in the absence of LDL receptors.

Finally, it should be noted that macrophages express more than one "scavenger receptor." The first scavenger receptor, SRA, was characterized by Kodama et al. in Krieger's laboratory (100) Early studies by Sparrow, Parthasarathy, and Steinberg (101) provided evidence that mouse macrophages must contain one or more additional receptors recognizing oxidized LDL, and in 1993, Endemann et al. (102) cloned CD36 and characterized it as a second oxidized LDL receptor. Subsequent studies showed that CD36 is the dominant receptor for uptake of oxidized LDL (103, 104). The amelioration of atherosclerosis by knocking out of either SRA or CD36, receptors for modified forms of LDL, added strength to the case against LDL and supported the oxidative modification hypothesis (104, 105). These scavenger receptors recognize and take up oxidized LDL but do not recognize native LDL, β-VLDL, or aggregated LDL, the other modified forms of LDL that have been reported to lead to foam cell formation in vitro. The results imply that foam cell generation reflects uptake of oxidized LDL (and possibly other similarly modified forms of LDL) and that foam cell generation is essential for atherogenesis, at least in these mouse models.

INFLAMMATION IN THE PATHOGENESIS OF ATHEROSCLEROSIS

If the term "inflammation" embraces any disease process in which circulating leukocytes are recruited to the disease site and participate in the progression and/or clearing of the disease process, then certainly atherosclerosis is an inflammatory disease. We have referred above to the early recognition that mononuclear cells are already present in the very earliest lesions (6, 16, 24). Indeed, the earliest observed event after inducing hypercholesterolemia in rabbits is an increase in the expression of vascular cell adhesion molecule-1 (VCAM-1) on the arterial endothelium overlying atherosclerosis-susceptible sites (106).

As first stressed by the work of Ross (107), the progression of atherosclerotic lesions involves the interactions among the several cell types that characterize the lesion, including, notably, the monocytes and T-lymphocytes, and the immune system is clearly involved. Several excellent reviews document the extensive research in this area and the potential for finding interventions that could complement lowering of blood cholesterol (34, 108–110). Whether inflammation alone, i.e., in the absence of some elevation of blood cholesterol, can initiate atherosclerosis is not clear. Various forms of arteritis can, of course, be generated, but the lesions do not closely resemble those of human atherosclerosis. Nevertheless, the rate of progression of lesions, once established, is almost certainly influenced by the multiple growth factors and cytokines produced within the lesion (or reaching it from elsewhere in the body) and by the immune system. It is worth noting that in nonhuman primates, simply removing the cholesterol from the diet is enough to induce impressive regression of lesions, with loss of lipid, decrease in cellularity, and decrease in the volume of connective tissue matrix (22, 111). These results suggest that the continuing presence of hypercholesterolemia is needed for lesion maintenance and progression, at least in the case of relatively early lesions. Rather than looking on inflammation and hypercholesterolemia as alternative choices in the pathogenesis of atherosclerosis, it might be more profitable to regard them as "partners in crime," as discussed in detail elsewhere (112).

STATUS OF THE LIPID HYPOTHESIS IN 1980

The development of a pathogenetic scheme showing how LDL and other proatherogenic lipoproteins penetrate into the artery wall and give rise to foam cells, the hallmark of the initial lesion, together with specific details about the macrophage receptors involved, made it easier to accept the lipid hypothesis. At the same time, the new insights into the inflammatory facets of atherogenesis for a time diverted attention from the role of hypercholesterolemia and may have delayed acceptance of the central importance of treating it aggressively. However, even with these advances in understanding, there was little enthusiasm for lowering blood cholesterol levels as a preventive measure. It would take a definitive large-scale clinical intervention trial to make an airtight case and justify a national preventive program.

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