Studies of Hypercholesterolemia in the Nonhuman Primate

II. Fatty Streak Conversion to Fibrous Plaque

Agostino Faggiotto and Russell Ross

This report presents the second portion of the morphologic studies on chronic, diet-induced hypercholesterolemia in nonhuman primates (Macaca nemestrina) examined sequentially between 5 and 13 months. A direct relationship was observed between the rate of cholesterol increase, the level and duration of hypercholesterolemia, and the changes in the artery wall that led to the formation of fatty streaks and their conversion to fibrous plaques. A loss of endothelial continuity was first observed in the iliac arteries between 3 and 4 months of atherogenic diet and appears to be a critical step in the conversion of many fatty streaks to fibrous plaques. With breaks in endothelial junctions and exposure of some of the macrophages in a fatty streak, many of the lipid-filled macrophages appeared to detach and enter the circulation. The number of circulating foam cells increased precipitously between 3 and 4 months, the time when increased sites of endothelial dysjunction and macrophage egress were observed. Exposure of subendothelial macrophages also permitted adherence of platelets to these macrophages and to exposed connective tissue. Fibrous plaques were found at similar anatomic sites where endothelial denudation had been observed at earlier time points but were more prevalent in the abdominal aorta and iliac arteries. These changes subsequently occurred at every level of the aortic tree and appeared to progress in a cephalad fashion with increasing rate, level, and duration of hypercholesterolemia.

The results of these studies stress the importance of following cholesterol levels of each animal throughout the entire period of the study and of sampling the entire arterial tree at every level with time. This helped us to understand the complicated interrelationships between the various cells in atherogenesis, provided further support for the "Response to Injury Hypothesis of Atherosclerosis," and helped to explain how hypercholesterolemia may be involved in the different stages of atherogenesis in nonhuman primates and possibly in humans. (Arteriosclerosis 4:341-356, July/August 1984)

In Part II of this report we describe the changes that occurred in a series of 15 pigtail monkeys fed a high-saturated-fat, high-cholesterol diet from 5 to 13 months. In Part I of this report we described the morphologic changes observed at each level of the arterial tree in each of 10 monkeys during the first 4 months of hypercholesterolemia induced by the same diet.

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This research was supported in part by Grant HL 18645 from the U.S. Public Health Service and by a grant from R. J. Reynolds, Incorporated.

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Received December 20, 1983; revision accepted March 2, 1984.

Dr. Alan Fogelman kindly acted as Guest Editor for the review of this paper.

Sporadic, apparently randomly localized, subendothelial macrophages occupied the intima of control monkeys, similar to observations reported in rats and swine. These macrophages were distributed focally directly beneath the endothelium and contained varying amounts of lipid. The presence of such sporadic macrophages distributed throughout the arteries of the control animals suggests that these cells probably act as physiological scavengers. Animals fed the high fat diet for 1 month and replaced on normal monkey chow for 9 months had fewer subendothelial foam cells than animals examined after 1 month on the diet, suggesting that some fatty streaks may be reversible. Fatty streaks began to form within 1 month concomitant with adherence of monocytes to the endothelium, followed by migration and subendothelial localization, where they become lipid-laden macrophages or foam cells. Clusters of these lipid-laden...
subendothelial macrophages represent the earliest form of the fatty streak.

With continued hypercholesterolemia, monocytes continued to attach to the endothelium and localize in the subendothelial space, causing the lesions to enlarge. Between 2 and 4 months, some of the intimal lesions accumulated smooth muscle cells beneath the macrophages and took on the more classical appearance of human fatty streaks. The number and size of the fatty streaks markedly increased between 12 days and 4 months. Due to their expansion, the endothelium was stretched exceedingly thin. Ultimately, breaks occurred in the endothelial continuity, resulting in macrophage emigration, and the appearance of foam cells in the circulation. Adherent platelet thrombi were observed on some exposed macrophages and at sites of exposed connective tissue where the endothelium had been denuded.

In this report we present morphologic observations and monthly lipid values for animals examined between 5 and 13 months of hypercholesterolemia. Particular attention has been paid to a number of questions:

- When and where in the arterial vasculature is endothelial integrity lost?
- Is there evidence that fatty streaks evolve into fibrous plaques?
- At what time and where do fibrous plaques appear?
- What is the relationship among duration, level and pattern of hypercholesterolemia, number of circulating foam cells, and the severity of lesions?
- What is the relationship between the anatomic location of fatty streaks, endothelial denudation, platelet adherence, and the formation of fibrous plaques?
- How do the morphology and anatomic distribution of lesions resulting from diet-induced atherosclerosis in the pigtail monkey compare with those observed in humans?

Results

Response to the Experimental Diet

Although these animals were chosen from the population of animals screened for relative homogeneity of response to 1 month on the experimental diet (see reference 1), all animals did not respond in identical fashion in levels of lipoproteins, plasma cholesterol, and triglyceride. The changes in plasma cholesterol levels with time for each animal examined are presented in Figures 1 and 2. The morphologic changes observed at eight anatomic levels of the arterial tree were scored for the presence of the following: 1) normal morphology, 2) fatty streaks, 3) attached monocytes, 4) exposed macrophages, 5) exposed macrophages with adherent platelets, 6) endothelial denudation with adherent platelets, and 7) fibrous plaques.

Five Months of Atherogenic Diet

Cholesterol Levels

Two monkeys were hypercholesterolemic for 5 months. Each had similar plasma cholesterol levels at sacrifice; however, the plasma cholesterol of Animal 1 increased at a much faster rate so that by 3 months it had reached more than twice the level observed in Animal 2 (see Figure 1), but then it decreased after the 4th month to the level of Monkey 2. The morphologic changes observed in these two monkeys were quite different and may be related to the marked differences in their respective rates of increase in plasma cholesterol.

Lesion Type and Distribution

Both animals contained fatty streaks at every level of the arterial tree. The fatty streaks in the aortic arch and in the thoracic aorta were comprised of several layers of lipid-filled macrophages (Figure 3 A; see Figures 8 and 14 in Part I of this report). Macrophages were readily observed, possibly in the process of egress from the artery wall (Figure 3 B; see Figure 10 in Part I of this report). Fatty streaks in the abdominal aorta sometimes contained strands of elastic fibers and collagen surrounding small numbers of smooth muscle cells situated beneath the macrophages. These smooth muscle cells formed two to four layers and many of them contained lipid inclusions (Figure 3 B).

Animal 1 had extensive fibrous plaques in the internal and external iliac arteries, whereas Animal 2
Figure 1. Changes in cholesterol level with time for each animal examined. The anatomical distribution of the observed cellular changes and temporal pattern of hypercholesterolemia are shown for each animal.
Figure 2. Changes in cholesterol level with time for each animal examined. The anatomical distribution of the observed cellular changes and temporal pattern of hypercholesterolemia are shown for each animal.
had none at any level. The fibrous plaques that had formed in the iliac arteries of Monkey 1 occluded as much as 70% of the arterial lumen based upon the position of the internal elastic lamina, and had a characteristic fibrous cap consisting of numerous layers of smooth muscle cells (Figure 3 C). Accumulations of intracellular and extracellular lipid were present beneath the fibrous cap, and occasionally these fibrous plaques contained deep areas of necrosis. When this occurred, the underlying media appeared thinner. These proliferative smooth muscle lesions were located in the same anatomic regions that had shown endothelial rupture, macrophage egress, and platelet-macrophage interactions in monkeys that had been hypercholesterolemic for 4 months.1

Cellular Involvement

The fatty streaks located in the arch and thoracic aorta of both animals sometimes contained inflammatory cells in the luminal third of the lesions (Figure 4). These cells were principally lymphocytes together with varying numbers of eosinophils and plasma cells. Neutrophils, basophils, and mast cells were also occasionally observed in these regions. As observed previously,1 in both 5-month animals there were signs of endothelial cell injury, such as in-
creased cytoplasmic density; however, these cells remained attached to their neighbors. The luminal surface of the fatty streaks was highly irregular and covered by an extraordinarily thin endothelium (see Figure 9 in Part I), often showing adherent leukocytes. In transmission electron micrographs, these adherent cells appeared to be principally monocytes together with some lymphocytes. The leukocytes were attached to intact endothelium or, in denuded areas, to subendothelial connective tissue together with large numbers of spread, adherent platelets and lipid-laden macrophages, (Figure 5 B, C, and D). Platelet adherence occurred in denuded areas (Figures 5-7) or where cleavage planes appear to have occurred at the base of a fatty streak, resulting in partially detached endothelial cells some of which still overlay foam cells.

In areas where exposed foam cells had detached in sufficient numbers, the underlying matrix was exposed, and platelets adhered, forming several continuous layers. Platelet aggregates were often found in association with some of the remaining exposed macrophages (Figure 5 C, D). Figure 5 B shows an area of denudation containing a small number of exposed macrophages retained in situ by the edge of the endothelial cells that had previously covered the fatty streak. The remaining foam cells had presumably detached, exposing the connective tissue which became covered by a mural thrombus.

Some sites were covered by endothelium that no longer had a long axis in the direction of blood flow, suggesting that these areas may have been denuded and were re-covered by an irregular-appearing endothelium (Figure 5 E, F). Figure 5 A through E provide examples of endothelial denudation, presumably due to rupture of endothelial junctions, and suggest a sequence of events that starts with the exposure of intimal lipid-laden macrophages (Figure 5 A). We suggest that a series of changes may have occurred (pictured in Figure 5) in which some of the macrophages detached from the lesions and entered the circulation, exposing the floor of the fatty streak (Figures 5 B, E). In some instances, macrophages became covered by adherent degranulated platelets (Figures 5 B, C, D, 6, and 7), and some areas eventually became completely reendothelialized (Figure 5 F).

Seven and Eight Months of Atherogenic Diet

Cholesterol Levels

The changes in cholesterol levels in these four animals were more rapid and somewhat more homogeneous than in those monkeys observed at earlier or later times (Figure 1). The morphologic findings within this group were quite similar from animal to animal. These monkeys rapidly attained very high cholesterol levels, and the changes in the arteries in this group were more similar to those found in animals kept on the diet for 12 to 13 months, even though the latter had somewhat lower levels of cholesterol.

Lesion Type and Distribution

There is a clear difference in lesion distribution between the 7- and 8-month animals and those examined at earlier times (see Figure 1). Fatty streaks were present in the aortic arch and in the descending portions of the thoracic aorta. These lesions often contained some smooth muscle cells and fibrous connective tissue, and inflammatory cells were commonly observed. Fibrous plaques were observed for the first time in the arch and in the thoracic aorta. The abdominal aorta and iliac arteries contained numerous fibrous plaques, morphologically similar to those observed in the iliac arteries in the 5-month animals. Some of the fibrous plaques contained deep regions of necrosis and were vascularized with small vessels in their outer third. Cell type was often difficult to determine in the necrotic regions of the fibrous plaques, since many cells were disrupted and displayed signs of degeneration. These areas frequently contained cholesterol crystals, lipid and calcium deposits.

Cellular Involvement

Pale, edematous-appearing endothelial cells suggestive of cell damage were readily observed after 7 months, whereas they were rarer at earlier time intervals. Examination by scanning electron microscopy demonstrated that many of the fatty streaks had lost their endothelial cover, exposing clusters of 50 or more foam cells (similar to Figure 5 A). As before, individual macrophages were commonly observed, possibly in the process of emigrating into the circulation. Strands of endothelial cells often partially covered exposed foam cells, suggesting attempts at endothelial regeneration or, alternatively, incomplete separation of endothelial junctions (see Figure 12 in Part I). There were focal accumulations of attached leukocytes in a number of regions, some of which were observed between endothelial cells, possibly in the process of continuing entry into the artery wall. The areas of denudation containing adherent platelets were present at every level of the arterial tree (Figure 1).

Nine and Ten Months of Atherogenic Diet

Cholesterol Levels

There was substantial variation in the patterns of plasma cholesterol among the four animals examined after 9 to 10 months. Two of the animals (8 and 10) reached relatively high cholesterol levels, one (8) much more rapidly than the other. In contrast, the other two animals had much lower cholesterol levels (Figure 2). This provided an opportunity to further determine whether there were correlations between degree and duration of hypercholesterolemia and arterial wall changes.
Figure 5. Examples of endothelial dysfunction leading to denudation and exposure of fatty streaks as seen by scanning electron microscopy. A. Large lipid-laden subendothelial macrophages that constitute the upper part of this fatty streak have become exposed due to the loss of endothelial continuity. Bar = 100 \mu. B. In this fatty streak there has apparently been endothelial dysjunction and contraction to expose the lesion. Many of the exposed macrophages have presumably been shed into the circulation exposing the connective tissue to which platelets have attached, adhered, and spread. They have formed a small thrombus below the upper endothelial edge. A few macrophages remain partially covered by the edge of the endothelium that covers the remainder of the lesion. Bar = 10 \mu. C. A platelet thrombus adheres to a few exposed macrophages of a typical oblong lesion. Bar = 100 \mu. D. A higher magnification of C. The platelets have aggregated on an exposed foam cell (note the several indentations of its surface due to the presence of intracytoplasmic lipid droplets) adjacent to the edge of the endothelium. Bar = 10 \mu. E. Platelets are absent from this denuded area, suggesting that if platelets had been present they may have detached and/or that a nonthrombogenic surface has been established. The remaining macrophages are exposed or partially covered by the edge of the endothelium. Bar = 100 \mu. F. This lesion shows no platelet participation and a few partially exposed macrophages. There appears to have been some attempt at endothelial regeneration at this site. Bar = 100 \mu.
Figure 6. Scanning electron micrographs of platelet adherence to exposed macrophages associated with fatty streaks.  **A.** Numerous platelet aggregates adhere to the exposed macrophages associated with a fatty streak. Attached monocytes, presumably in the process of entry into the lesion, are present on the surface of the fatty streak. Bar = 100 μ.  **B.** A higher magnification of one of the platelet thrombi attached to an exposed macrophage seen in A. A cross section of a similar thrombus is shown in Figure 7. Bar = 10 μ.
Lesion Type and Distribution

As seen in Figure 2, Monkey 9 maintained relatively low levels of plasma cholesterol, especially during the last 4 months on the diet. The arterial tree of this monkey was essentially normal with the exception of a few fatty streaks and occasional adherent monocytes. The cholesterol levels in Animal 7 also remained relatively low and gradually increased to just over 500 mg/dl starting at 6 months, and then dropped before sacrifice. Some endothelial denudation was observed in the abdominal aorta of this monkey with exposure of lipid-filled macrophages, which often had adherent platelets. Fibrous plaques were present in the iliac arteries, the aortic bifurcation, and in the lower abdominal aorta (Figure 2), again suggesting that proliferative lesions began in the same anatomic regions that had platelet interactions at earlier times.

In contrast to Monkeys 7 and 9, Animal 8 had fibrous plaques in the abdominal aorta and leg arteries. In this animal, plasma cholesterol values increased rapidly and were maintained at approximately 950 mg/dl for 4 months. Animal 10 exhibited a continuous increase in plasma cholesterol, reaching the highest level of the group, and showed an even greater incidence of endothelial injury, platelet interactions (67% of all segments at 9 to 10 months vs. 50% of all segments at 5 months), and fibrous plaque formation in 40% of the segments at 9 to 10 months (Figures 1 and 2).

Cellular Involvement

Adherent leukocytes continued to be observed on the endothelial surface in 33% of the segments of the arterial tree in all of the 9- and 10-month animals. Frequent breaks were observed in the endothelial cover of Animal 10, which contained exposed clusters of large lipid-laden macrophages, often covered by adherent platelets, similar to those observed at the earlier time periods. When the subendothelial connective tissue was exposed, larger numbers of adherent platelets were present. These platelet accumulations generally formed small mural thrombi in 44% of the arterial segments (for example, see Figure 5 B, C, D, and Figures 6 and 7).
The smooth muscle cells in the fibrous plaques were usually oriented parallel to the direction of blood flow, and were surrounded by a basement membrane as well as other connective tissue elements together with variable amounts of lipid (Figure 8 A, B).

Twelve and Thirteen Months of Atherogenic Diet

Cholesterol Levels

With the exception of Monkey 12, the animals in this group reached similar cholesterol levels during the latter part of the study. In contrast, Animal 12 maintained consistently low levels of cholesterol (Figure 2). Animals 13 and 14 had early rapid rises in plasma cholesterol and then maintained these levels for the duration of the study.

Lesion Type and Distribution

Similar to Monkey 9, Monkey 12 maintained consistently lower levels of cholesterol and contained regions of normal morphology, as well as numerous fatty streaks (Figure 2). Furthermore, a few areas of endothelial denudation were present in the abdominal aorta and leg arteries of Animal 12 which were not observed in Monkey 9; however, no fibrous plaques were observed at any level. In contrast, the other four animals had numerous fibrous plaques as well as many regions that showed extensive loss of endothelial integrity. Animals 13 and 14 had the highest incidence of endothelial denudation and advanced atherosclerosis, as compared with Monkeys 11 and 15, whose cholesterol levels were consistently lower until the 9th month or later (Figure 2).

Cellular Involvement

After 1 year on the atherogenic diet, these monkeys had fatty streaks containing several layers of underlying smooth muscle cells. Endothelial denudation, exposed macrophages, adherent platelets, and leukocytes were common in the fatty streaks. Endothelial cells with a dense cytoplasmic matrix were present, as well as pale endothelial cells suggestive of endothelial injury (Figure 9). Platelet aggregates were most commonly found adherent to
exposed foam cells and often covered them, forming a thrombus that extended to the base of the fatty streak where the foam cells were attached (Figures 6 and 7). Serial sections of these platelet thrombi revealed that they were attached to the exposed connective tissue by a relatively small pedicle of degranulated platelets, suggesting that they might be subject to future embolization. Adherent leukocytes were observed throughout the arterial tree, often associated with mural thrombi (Figure 6). The size of the thrombi were variable, but many were as long as 150 μ and usually larger than 50 μ. The site of denudation was often difficult to visualize by scanning electron microscopy because it occurred deep in crevices where platelet aggregates apparently formed (Figures 6 and 7).

Circulating Foam Cells

The number of circulating foam cells was determined in peripheral blood smears. Comparable numbers of circulating foam cells were found in arterial and venous blood. There were no foam cells in the circulation of the control animals with the exception of one animal which had two foam cells per 1000 white blood cells (WBC). During the first month of hypercholesterolemia, the average number of foam cells increased but remained below 20 cells/1000 WBC. A sharp increase in the number of circulating foam cells was observed after the third month on the atherogenic diet (Figure 10). After this time, the number of circulating foam cells varied, but increased to remain between 25 and 70 cells/1000 WBC (mean 48 ± 16) (Figure 10). These observations of circulating foam cells must be interpreted as preliminary since studies were not conducted on all 15 monkeys in this investigation, but only upon nine of the animals. However, the observations were sufficiently reproducible (Figure 10) to warrant their inclusion as a preliminary report.

Discussion

Plasma Cholesterol Correlates With Lesion Formation

The role of the monocyte-macrophage in the establishment of the fatty streak and in the changes that precede the formation of fibrous plaques and complicated lesions is documented in the accompanying paper. The observations presented in this report indicate the particular importance of the rate of increase, level, and duration of a given level of hypercholesterolemia in relation to the type, extent, and distribution of lesion formation in each animal. For example, in Figure 1, the two animals that had been on the atherogenic diet for 5 months had comparable final levels of plasma cholesterol at the time of sacrifice. However, the cholesterol level increased in Monkey 1 much more rapidly than in Monkey 2, so that at 3 months its level was more than twice that of Monkey 2. Fibrous plaques were observed in the iliac arteries of Animal 1, suggesting that the early rapid rise in cholesterol may be related to these changes. Animal 2 had no proliferative lesions, but did demonstrate endothelial denudation with exposed macrophages and adherent platelets.

The correlation between the pattern of cholesterol levels and lesion formation is more striking after longer time intervals. For example, after 9 to 10 months there were clear disparities in the rate of increase, final levels of plasma cholesterol and the arterial changes among the four animals in this group. The plasma cholesterol level of Monkey 9 never rose above 500 mg/dl and was 200 mg/dl at sacrifice. As an apparent consequence of maintenance of this relatively mild level of hypercholesterolemia for 10 months, this animal contained only sporadic fatty streaks in the aortic arch and lower abdominal aorta, and had a modest number of surface sites containing adherent monocytes.

Figure 10. Circulating foam cells as a function of time on the atherogenic diet. A sharp increase occurs between the second and fourth months. This correlates with the first easily observable breaks in the endothelium and with the appearance of macrophage egress.
thelial rupture, suggesting that such injury may have occurred during the earlier, more acute rise in plasma cholesterol and that sufficient time had elapsed for endothelial repair to occur. It is also possible that lesion formation in this animal may have occurred by some other means than endothelial denudation.

Similar correlations can be observed by comparing monkeys that were hypercholesterolemic for 9 to 10 and 12 to 13 months. Animals 9 and 12 maintained relatively low plasma cholesterol values (200–400 mg/dl) for 10 and 12 months, respectively, and only Animal 12 contained evidence of endothelial injury, including sites of platelet adherence in the iliac arteries and abdominal aorta, however there were no proliferative lesions. In contrast, Animals 11, 13, 14 and 15 had either early, rapid rises or a continuous increase in plasma cholesterol to reach 600–900 mg/dl. All these monkeys contained both platelet-macrophage interactions and fibrous plaques (Figure 2).

These data suggest that, dependent on individual genetic susceptibility, a given rate of increase and/or maintenance of a sufficient level of hypercholesterolemia may lead to cellular alterations similar to those observed in the present investigation in monkeys, and possibly in humans. Five months on an atherogenic diet with a plasma cholesterol of 800 mg/dl or more was associated with advanced proliferative lesions in the iliac arteries. Much lower levels of cholesterol, prolonged for a longer time period, also lead to fibrous plaque formation beginning in the lower arterial segments. In contrast, animals, such as Monkeys 4, 6, and 10, which rapidly became hypercholesterolemic and remained so for 7, 8, and 10 months, respectively, had fibrous plaques throughout their arterial tree. The changes observed in the other animals (Tables 1 and 2) fall between these two extremes. These observations confirm that the type and extent of lesions formed, as well as the integrity of the endothelium, as demonstrated by exposed macrophages with or without adherent platelets, appear to depend more on the pattern and consistency of elevation of plasma cholesterol rather than the specific level at the time of sacrifice.

The reasons for the apparent cephalad progression of the proliferative lesions are not clear. However, in each case, platelet interactions with denuded areas often associated with exposed macrophages appear to precede proliferative lesions in a given arterial segment by 1 to 2 months. These observations indicate an important point that may help to resolve some of the conflicting data in the literature. If one were to limit examination to specific anatomic sites at specific time points, one might find a variety of lesions that would be difficult to interrelate. It is not known if similar or different patterns occur in other species including humans. However, although little is known about the pathogenesis of human atherosclerosis, severe plaques are generally found in the abdominal aorta and lower extremities in humans, as was the case in this study.

The plasma cholesterol levels reached in most of these monkeys are similar to those that occur in several of the human genetic lipid disorders associated with accelerated atherosclerosis. Most hypercholesterolemic patients, however, do not suffer from familial lipid disorders with their associated absence of LDL receptors. It has been suggested that the high levels of cholesterol induced by diet in most individuals may, in combination with subtle genetic abnormalities, somehow lead to changes such as those observed in the present study. Thus, the changes observed in this study may provide a basis for understanding the cellular events that lead to the onset and progression of atherosclerotic lesions. Similar changes might occur in diet-associated hypercholesterolemia in humans if they are present for a sufficiently long period of time in an appropriately genetically susceptible individual.

Endothelial Injury

Changes such as pale or dense endothelial cells were suggestive of cell damage. The reasons for these changes are not clear; however, it is possible that endothelial cells may become more susceptible to insult due to alterations in amounts of cholesterol and type of phospholipids in their plasma membranes that result from chronic exposure to elevated levels of lipoprotein.

The extraordinary thinness of the endothelium covering the macrophages in the fatty streak, coupled with the close proximity of these two cell types, raises the possibility that endothelial injury could result from mechanical stretching of cells expanded to their limit, or possibly to the release of substances from the underlying macrophages. These macrophages could phagocytose altered lipoproteins or other substances that may have been processed by the endothelial cells and possibly be stimulated to release potentially toxic substances. They also may play other, as yet undefined, roles in inducing endothelial damage.

The Monocyte-Macrophage

It is well known that the blood monocyte can, in response to a chemotactic signal, enter tissues and become a macrophage. During the first 4 months of hypercholesterolemia, the most striking changes in the artery are those principally associated with the monocyte-macrophage-endothelial interactions, and the evolution of the fatty streak. Adherent monocytes were observed at every arterial level throughout the entire period of the present study, and monocytes continued to migrate between endothelial cells at all time periods. It will be important to determine what triggers monocyte adhesion to the endothelium. Their presence in focal regions throughout the arterial tree suggests that these leukocytes may be responding to local chemotactic stimuli. Smooth muscle cells and other constituents
Fatty Streak Progression to Fibrous Plaque

Although it has long been suggested that fatty streaks progress to fibrous plaques, we know of no studies that provide the chronology of events described in this report. The dynamics of the events that may lead to the formation of the proliferative lesions are shown diagrammatically in Figure 11. With increasing duration of hypercholesterolemia, monocytes adhere to the surface of the endothelium, migrate into the intima, take up lipid, and become foam cells, which may lead to the formation of the proliferative fibrous plaque. Fatty streaks may progress to fibrous plaques in cases where endothelial integrity is preserved (Figure 11).

The response to injury hypothesis of atherosclerosis suggests that “injury” to the endothelium leads to a series of interactions between blood components and the arterial wall that lead to the formation of the fibrous plaque, a proliferative smooth muscle lesion. The concept of “injury” includes a wide spectrum of endothelial alterations, from subtle functional changes to clear morphologic evidence of cell damage such as endothelial denudation.

Circulating Foam Cells

Circulating foam cells were observed in increasing numbers with increasing time on the diet, but their numbers were variable. Rare occasional foam cells were observed in blood smears of some of the controls, suggesting a physiological mechanism for clearance of lipids from the vessel wall. The numbers of these cells were low and slowly increased during the first 3 months on the atherogenic diet. However, at 4 months, when the first breaks in the endothelium were observed, lipid-laden macrophages were readily observed, presumably in the process of egress from the vessel wall. After the fourth month, concomitant with the presence of numerous large breaks in the endothelial surface and the exposure of large clusters of lipid-laden macrophages, circulating foam cells were found in increasing numbers in peripheral blood smears. These preceded the appearance of more advanced proliferative lesions, suggesting that the presence of such circulating cells may be indicative of the state of endothelial integrity in experimental animals and possibly in humans. It will be interesting to determine whether the presence of such cells could be used to determine potential activity for lesion progression.

Platelet Interactions

Different patterns of endothelial injury and platelet adherence were observed in this study. The most frequent form appeared as breaks in the endothelium over fatty streaks where platelets adhered to macrophages and exposed matrix and formed a mural thrombus. Next in frequency were adherent platelets at sites of cleavage planes at the base of fatty streaks. Small numbers of foam cells remained attached to the exposed matrix and some were covered by a flap of remaining endothelium. The least frequent type of platelet interaction occurred at sites of endothelial separation in areas uninvolved with fatty streaks. In these areas, remnant strands of endothelial cells bridged the exposed areas which were covered by several layers of adherent, degranulated platelets (not shown).

Many mural thrombi attached to exposed macrophages were present in monkeys fed the atherogenic diet for 1 year (see Figure 2). Platelets derived from hypercholesterolemic blood may be in a hypersensitive state, as suggested by in vitro experiments and might be susceptible to embolization and adherence downstream. This might help to explain why the lesions of the abdominal aorta and iliac arteries appeared to progress more rapidly than those in the upper part of the arterial tree.

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Subsequent exposure of connective tissue could initiate adhesion and aggregation of platelets together with macrophages, both of which could release factors capable of inducing metabolic, chemotactic, and mitogenic signals for smooth muscle cells leading to the generation of the fibrous plaque. Cultured endothelial cells are also capable of synthesizing growth factor(s) in vitro. Should this occur in vivo, they may also play a role in angiogenesis and in atherogenesis.

Examination of the data in Figures 1 and 2 also provides an opportunity to determine the relationship between the anatomic location of cellular changes and fibrous plaque formation. Table 1 represents a compilation of the data from Figures 1 and 2 in relation to the number of times exposed macrophages (M), macrophages with adherent platelets (MP), denudation with adherent platelets (DP), and fibrous proliferative lesions (FP) were observed in the thoracic versus the abdominal aorta and leg arteries.

Figure 11. Schematic diagram of the different phases of atherogenesis as we have observed them in the chronic markedly hypercholesterolemic pigtail monkey. The first alteration consists of newly attached monocytes that probe between endothelial cells (A) and which localize subendothelially to become foam cells and form early fatty streaks (B). Smooth muscle cells gradually accumulate under these foam cells (B,C,D). At some point, endothelial junctions appear to separate, resulting in foam cell exposure, and possibly permit some foam cells to enter the circulation (C). At other times these exposed foam cells appear to become foci of platelet thrombi (D). Ultimately, many of these sites become foci of active smooth muscle proliferation and fibrous plaque formation. It is possible that fibrous plaques form from sites of platelet interaction, but they may also form directly from fatty streaks with or without endothelial separation, suggesting that in each of these sites macrophages may play a key role in this event.
+ MP + DP represents all areas of endothelial retraction, whereas MP + DP represents denuded areas with adherent platelets. FP/MP + DP provides an estimate of fibrous plaque in regions according to their anatomic location. Examination of the data obtained by this approach suggests a prevalence of fibrous plaques in the abdominal versus the thoracic aorta. Platelet adherence may not be a necessary event, but if it occurs it may be a more important event at sites in the abdominal aorta and iliac arteries in these animals, and possibly in humans.

The results of this study suggest that monocyte-macrophage-endothelial interactions lead to fatty streak formation. These are followed by endothelial dysjunction that may be accompanied by platelet interactions and release of platelet constituents, possibly platelet-derived growth factor. Either or both of these phenomena may be key events in the formation of proliferative lesions of atherosclerosis in chronic hypercholesterolemia (Figure 11). New questions have been raised concerning the causes of endothelial injury, the nature of endothelial-macrophage interactions, and of smooth muscle proliferation.

The recent Lipid Research Clinic Study demonstrates that lowering the cholesterol level decreases the mortality and morbidity due to coronary atherosclerosis. The changes observed in our study may provide a basis for explaining the ways that hypercholesterolemia induces atherosclerotic lesions.

Acknowledgments

We acknowledge the important role played by Kenneth Porte in these experiments, who took the majority of the thousands of scanning electron micrographs that became the data base for the observations in both manuscripts. We are indebted to Stephanie Larra for expert technical assistance and to Mary Hillman for skillful typing. We particularly thank the many individuals in the laboratory for hours of helpful discussion and Elaine Raines and Michael Rosenfeld for their critical appraisal of the manuscript.

References

15. Henriksen T, Mahoney EM, Stelnberg D. Enhanced macro-

Table 1. Data Reduction from Figures 1 and 2

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Data are compiled from Figures 1 and 2 in relation to the number of times exposed macrophages (M), macrophages with adherent platelets (MP), denudation with adherent platelets (DP), and fibrous proliferative lesions (FP) were observed in thoracic versus abdominal aortas and leg arteries.
phage degradation of low density lipoprotein previously incubated with cultured endothelial cells: Recognition by receptors for acetylated low density lipoproteins. Proc Natl Acad Sci USA 1981;78:6499–6503


41. Grotendorst GR, Seppa HE, Kleinnan HK, Martin GR. Attachment of smooth muscle cells to collagen and their migration toward platelet-derived growth factor. Proc Natl Acad Sci USA 1981;78:3669–3672


Index Terms: hypercholesterolemia • fatty streaks • fibrous plaque • artery wall • atherogenic diet • macrophages • endothelial dysfunction • nonhuman primates • foam cells
Studies of hypercholesterolemia in the nonhuman primate. II. Fatty streak conversion to fibrous plaque.
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doi: 10.1161/01.ATV.4.4.341

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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