Atherogenesis during Low Level Hypercholesterolemia in the Nonhuman Primate
II. Fatty Streak Conversion to Fibrous Plaque
Junichi Masuda and Russell Ross

This study focuses on the formation of lesions of atherosclerosis in the aortas and iliac arteries of nonhuman primates (Macaca nemestrina) maintained on a low level hypercholesterolemic diet (plasma cholesterol 200 to 400 mg/dl) for 2, 3, or 3.5 years. Advanced lesions, or fibrous plaques, were found in all of the animals. The extent and severity of the lesions were closely related to the level and duration of hypercholesterolemia. The presence of monocyte-macrophages, T-lymphocytes, and smooth muscle cells, and the interactions of those cells that precede fibrous plaque formation in these long-term, relatively low level hypercholesterolemic monkeys were similar to those observed in previously published studies of high level hypercholesterolemia in nonhuman primates, with one principal difference: the fibrous plaques in the longer-term, low level hypercholesterolemic animals contained increased amounts of fibrous connective tissue, more smooth muscle cells, and fewer macrophages. As in the studies with high levels of hypercholesterolemia, fibrous plaques were more frequently observed in the abdominal aorta and iliac arteries than in the thoracic aorta and aortic arch. Fibrous plaques were preferentially located at the branches and bifurcations of the arteries. These anatomic sites were consistent with those that contained fatty streaks and fibrofatty lesions in the animals fed the diet for shorter periods of time. These data are compatible with the proposal that many of the fatty streaks are converted to fibrofatty lesions, some of which ultimately become converted to fibrous plaques. (Arteriosclerosis 10:178-187, March/April 1990)

In the first article in this series, we described the cellular interactions that precede and are responsible for the formation of the fatty streak in nonhuman primates maintained on low level hypercholesterolemic diets. Deposition of lipid in the artery wall, adhesion of circulating leukocytes to endothelium, and subsequent entry of the leukocytes into the artery wall are the initial events observed during cholesterol feeding. These events parallel observations made in high level hypercholesterolemic nonhuman primates, swine, rabbits, rats, and pigeons. In our studies, many of the monocytes that enter and localize within the arterial intima became lipid-filled macrophages and formed the earliest fatty streaks. Small numbers of T-lymphocytes, principally CD8-positive cells with a few CD4-positive cells, were components of all of the fatty streaks. As the lesions progressed, the surfaces of the fatty streaks became increasingly irregular, and endothelial detachment and remodeling occurred, often resulting in exposure of the foam cells to the lumen. Platelet microthrombi were frequently observed attached to the surfaces of the exposed macrophages.

In this part of the report, we present the observations made during low level hypercholesterolemia in monkeys (Macaca nemestrina) maintained in this state for 2 to 3.5 years. We will focus on the cellular interactions and the changes that occurred during the progression of the fatty streaks to form fibrous plaques. The chronologic changes in the cellular components of the lesions were analyzed by light and electron microscopy together with immunohistochemistry to determine the distribution of monocyte-macrophages, smooth muscle cells, and T-lymphocytes. The very high levels of dietary cholesterol used in previous studies and the ensuing rapidity of lesion formation that results have raised the question of the validity of such an approach to the human disease, since most humans have much lower levels and human lesions presumably form slowly with time. By studying animals at lower levels of hypercholesterolemia, we have observed a similar sequence of events, and we demonstrate the validity of high level hypercholesterolemia as a model to study atherogenesis, its treatment, and its prevention.

Methods

Animals
The atherogenic diet used was the same as that presented in Table 1 of the accompanying report (page 166). Ten monkeys (Macaca nemestrina) were examined after 2, 3, or 3.5 years of cholesterol feeding. Plasma...
cholesterol (PC) levels were maintained between 200 and 400 mg/dl, as described in the accompanying report.

For examination by scanning and transmission electron microscopy, the animals were sacrificed after the following periods of cholesterol feeding: two animals after 2 years; one, after 3 years; and one, after 3.5 years. For immunohistochemistry, animals were sacrificed after the following periods on the atherogenic diet: two animals after 2 years; three, after 3 years; and one after 3.5 years.

Tissue Preparation and Immunohistochemistry

The procedures of sacrifice, sampling of tissues, tissue preparation, and immunohistochemical staining were the same as those described in the accompanying report. The following monoclonal antibodies were used for immunohistochemical analysis: HAM56 for monocyte-macrophages, HHF35 for smooth muscle cells, 9.6 for CD2 of T-lymphocytes, OKTa for CD4 of T-lymphocytes, G10.1 for CD8 of T-lymphocytes, and 2H7 for CD20 of B-lymphocytes. The specimens were examined by light microscopy and scanning and transmission electron microscopy.

Results

Temporal Changes in Plasma Cholesterol and Triglycerides

Figures 1 and 2 show the temporal changes in PC levels, and Table 1 summarizes the analysis of the lipoprotein fractions and triglyceride levels. As reported in the accompanying article, fluctuation of PC levels was observed in each animal. Such fluctuation may be responsible for the variation observed in the different lesions. The increase in PC was due principally to an increase in low density lipoprotein (LDL) cholesterol and, to some extent, to an increase in very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and high density lipoprotein 3 (HDL3) cholesterol. On the other hand, HDL2 did not increase and showed a decreasing trend after 3 years. The temporal changes in triglycerides were not significant. The specific changes in differences in PC levels between animals are discussed for each group below.

Types of Atherosclerotic Lesions in Animals on the Atherogenic Diet

The atherosclerotic lesions were divided into three types (fatty streak, fibrofatty lesion, and fibrous plaque). This was determined by light microscopic observations made of paraffin-embedded sections and of 1-μm sections of plastic-embedded tissue, as described in the accompanying paper. Figures 3 and 4 summarize the distribution of various types of atherosclerotic lesions observed in the animals maintained on the low level hypercholesterolemic regimen for 2 years or longer.

Two Years on the Atherogenic Diet

Cholesterol Levels

Four monkeys (Animals 9 to 12) were sacrificed after 2 years on the low level hypercholesterolemic diet. The plasma cholesterol levels of these four monkeys fluctuated between 150 mg/dl and 500 mg/dl during the 2 years of cholesterol feeding. Animal 9 generally had higher PC levels than the others. Animals 10 and 12 maintained relatively low PC levels of approximately 200 mg/dl for the first year, and then developed relatively higher levels (between 300 and 400 mg/dl) for the second year. Animal 11 had cholesterol levels that were relatively higher for the first year and had lower levels during the second year. These differences appear to be at least partly responsible for the severity and extent of the lesions observed in these animals (Figures 1 and 3).

Types of Lesions and Distribution

Fatty streaks were still observed at most levels of the arterial tree, although fibrous plaque formation was evident at branch sites in the abdominal aorta and in the iliac arteries in all four animals. Fatty streaks were observed
which had a higher level of hypercholesterolemia during the 2 years, demonstrated an increase in the number of fibrous plaques throughout the aorta. All of these lesions contained numerous smooth muscle cells surrounded by extracellular matrix intermixed with numerous macrophages. Fibrous plaques were located principally at branch sites, including the iliac bifurcation of the abdominal aorta, and in their iliac arteries (Figure 3). These were the same sites where fibrofatty lesions and fibrous plaques were observed after 6 months and 1 year on the diet.1 Animal 9, which had a higher level of hypercholesterolemia during the 2 years, demonstrated an increase in the number of fibrous plaques throughout the aorta. All of these lesions contained large numbers of macrophages, forming a central atheromatous core. The other three monkeys had fibrous plaques only at the branches and bifurcations of the abdominal aorta and in their iliac arteries.

**Cellular Events**

The surface morphology of the fatty streaks observed by scanning electron microscopy was similar to that described after shorter times on the diet.1 Many of the fatty streaks demonstrated endothelial disruption, with exposure of lipid-laden macrophages and platelet thrombi on the irregular surface of the endothelium or on the exposed macrophages (Figure 5). In cross sections of the lesions, numerous macrophages were present not only on the surface of the lesions but also scattered throughout the thickened intima. The fatty streaks in these animals did not consist exclusively of macrophages but also contained many smooth muscle cells surrounded by extracellular matrix. The atherosclerotic lesions at the branch sites consisted of numerous smooth muscle cells, possibly because some of these had occurred on intimal cushions, which may have been converted to fibrofatty lesions. Immunohistochemical staining demonstrated the presence of T-lymphocytes in the macrophage-rich areas in virtually all of the fatty streaks and fibrofatty lesions. Most of the lymphocytes were CD8-positive, and a few were CD4-positive.

Figure 6 shows a representative scanning electron micrograph of a fibrous plaque at a branch site in the abdominal aorta. Fibrous plaques were also located around the ostia of the branching arteries and had the same distribution as fatty streaks and/or fibrofatty lesions observed at earlier time points. The center of the elevated lesion shown in Figure 6 did not contain much irregularity of the endothelial surface; however, numerous adherent leukocytes were present. In contrast, at the shoulder of the fibrous plaque, the surface of the lesion was highly irregular and, as observed previously, there were numerous areas of disrupted endothelium with exposed mac-

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**Table 1. Lipoprotein Fractions of Plasma Cholesterol and Triglyceride in Low Level Hypercholesterolemic Monkeys**

<table>
<thead>
<tr>
<th>Time on diet</th>
<th>N</th>
<th>Total</th>
<th>VLDL</th>
<th>IDL</th>
<th>LDL</th>
<th>HDL&lt;sub&gt;2&lt;/sub&gt;</th>
<th>HDL&lt;sub&gt;3&lt;/sub&gt;</th>
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<tbody>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>10</td>
<td>91.6±19.7</td>
<td>2.9±1.8</td>
<td>2.4±1.5</td>
<td>33.1±10.6</td>
<td>39.6±10.3</td>
<td>6.9±1.2</td>
</tr>
<tr>
<td>6 mo</td>
<td>10</td>
<td>294.4±115.7</td>
<td>6.9±6.3</td>
<td>42.2±41.7</td>
<td>159.1±97.4</td>
<td>45.3±25.4</td>
<td>13.5±4.1</td>
</tr>
<tr>
<td>1 yr</td>
<td>10</td>
<td>312.0±91.9</td>
<td>6.0±5.1</td>
<td>33.8±16.4</td>
<td>187.6±82.0</td>
<td>34.0±12.5</td>
<td>19.0±3.5</td>
</tr>
<tr>
<td>2 yrs</td>
<td>10</td>
<td>215.5±98.9</td>
<td>2.8±3.3</td>
<td>19.1±17.3</td>
<td>114.0±83.4</td>
<td>51.7±20.6</td>
<td>13.1±4.1</td>
</tr>
<tr>
<td>3 yrs</td>
<td>6</td>
<td>408.0±168.1</td>
<td>17.5±11.1</td>
<td>87.8±38.0</td>
<td>246.7±123.4</td>
<td>22.7±6.3</td>
<td>19.0±3.4</td>
</tr>
<tr>
<td>3.5 yrs</td>
<td>2</td>
<td>425.0±125.0</td>
<td>42.5±29.5</td>
<td>157.0±87.0</td>
<td>168.5±0.5</td>
<td>11.0±3.0</td>
<td>18.5±1.5</td>
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<tr>
<td>Triglyceride</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>10</td>
<td>36.7±12.5</td>
<td>8.5±5.9</td>
<td>0.0±0.0</td>
<td>7.4±2.3</td>
<td>10.9±3.1</td>
<td>1.5±0.9</td>
</tr>
<tr>
<td>6 mo</td>
<td>10</td>
<td>26.2±12.2</td>
<td>5.5±4.5</td>
<td>4.7±3.3</td>
<td>7.5±3.2</td>
<td>7.2±5.5</td>
<td>1.0±1.2</td>
</tr>
<tr>
<td>1 yr</td>
<td>10</td>
<td>18.5±15.5</td>
<td>3.0±5.6</td>
<td>0.7±0.9</td>
<td>4.8±2.2</td>
<td>2.3±2.8</td>
<td>0.6±1.5</td>
</tr>
<tr>
<td>2 yrs</td>
<td>10</td>
<td>21.8±15.1</td>
<td>2.1±4.0</td>
<td>3.2±5.9</td>
<td>4.2±4.0</td>
<td>5.7±5.4</td>
<td>1.5±1.3</td>
</tr>
<tr>
<td>3 yrs</td>
<td>6</td>
<td>23.5±9.6</td>
<td>5.0±3.3</td>
<td>8.2±4.7</td>
<td>8.8±4.2</td>
<td>1.5±3.6</td>
<td>0.8±0.4</td>
</tr>
<tr>
<td>3.5 yrs</td>
<td>2</td>
<td>15.5±2.5</td>
<td>3.0±2.0</td>
<td>2.0±2.0</td>
<td>1.0±0.0</td>
<td>0.5±0.5</td>
<td>0.0±0.0</td>
</tr>
</tbody>
</table>

Values are given as mg/dl and are the means±SD. VLDL=very low density lipoprotein, IDL=intermediate density lipoprotein, LDL=low density lipoprotein, HDL=high density lipoprotein.

**Figure 3.** This diagram presents the anatomic distribution of the different types of atherosclerotic lesions observed in animals on low level hypercholesterolemic diets for 2 years.
FIBROUS PLACe IN LOW LEVEL HYPERCHOLESTEROLEMIA  Masuda and Ross 181

Three Years and 3.5 Years

Cholesterol Levels

Four animals were examined after 3 years (Animals 13, 14, 15, and 17) and two, after 3.5 years (Animals 16 and 18). The PC levels of all six animals remained principally in the range of 200 to 400 mg/dl, although some fluctuation was observed. Animals 13, 14, and 17 had cholesterol levels below 200 mg/dl for approximately 1 year during the middle of the period of the diet and showed a rapid rise in cholesterol at the time of sacrifice. The other three monkeys were relatively well controlled between 200 and 400 mg/dl over the 3 years and 3.5 years of study (Figure 2).

Types of Lesions and Distribution

All six animals in this group had fibrous plaques localized principally near branch sites and bifurcations. Animals 13, 14, 16, and 17 contained plaques only in the abdominal aorta or the iliac arteries, but not in the thoracic aorta or the aortic arch (Figure 4). The PC levels of these animals, except for Animal 16, were below 200 mg/dl for over 1 year during the experimental period. Thus the severity and extent of fibrous plaques appears to be closely related to the levels and duration of hypercholesterolemia (Figures 2 and 4).

Some sporadic fatty streaks were observed, and when they were present, they were located preferentially in the thoracic aorta and aortic arch. Many fibrofatty lesions were found throughout the aorta in all six animals. These observations suggest that the fatty streaks present in the same anatomic locations in animals that were hypercholesterolemic for shorter periods of time were converted to fibrofatty lesions. Some of these lesions ultimately became converted to fibrous plaques during the longer period of low level hypercholesterolemia.

Cellular Events

The surface morphology of the fatty streaks was similar to that observed after shorter periods of cholesterol feeding. They contained increased leukocyte adherence, irregularly shaped nodular elevations, and focal endothelial disruption, with exposure of macrophages to the circulation, frequently associated with adherent platelet microthrombi. Similar to the fatty streaks described in the animals fed the diet for 2 years, there was an increase in smooth muscle cells and associated extracellular matrix in the fatty streaks, suggesting transition from fatty streak to fibrofatty lesion.
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Advanced Lesions
Conversion of Fatty Streaks to high level hypercholesterolemic nonhuman primates
increased in numbers (Figures 12A, 12B, and 12C). Viewed advanced fibrous plaques were sometimes relatively de-calcification. In contrast, the macrophages in the ad-
tained an increase in their peripheral myofiiaments to-

T-lymphocytes (predominantly CD8-positive cells) were
was rich in collagen, elastic fibers, and extracellular

the fibrous plaques observed in the arterial tree con-

throughout the fibrous plaques. However, most of the

immunohistochemical staining. Associated with the

immunohistochemical staining. Associated with these observations suggest that fatty streaks are converted to fibrofatty
lesions and then to fibrous plaques. Such conversion
occurs earlier and more frequently in abdominal aorta and iliac arteries than in thoracic aorta and aortic arch, although
the precedent fatty streaks are observed throughout the aorta in animals on the diet for a shorter period of time. Similar
differences in distribution between fatty streaks and fibrous plaques have been described in humans. Mechanisms that explain such differences in lesion distribution are not clear; however, rheological factors may play a role in the localization of various types of lesions. Pre-existing smooth muscle cells in the intimal cushions at branch sites and in diffuse intimal thickening may also play a role in the process of initiation and formation of fatty streaks as well as conversion of fatty streaks to advanced lesions.

A distinct feature that helps to characterize the fibrous plaque as different from the fatty streak is the formation of a discrete fibrous cap composed largely of smooth muscle cells and surrounded by large amounts of extracellular matrix, including collagen, elastic fibers, and extracellular liposomes (Figure 11). The smooth muscle cells contained an increase in their peripheral myofilaments together with dense bodies and a poorly developed rough endoplasmic reticulum, as compared with those observed in the earlier lesions (Figure 11 in the accompanying paper, see page 174). There was often evidence of calcification. In contrast, the macrophages in the advanced fibrous plaques were sometimes relatively decreased in numbers (Figures 12A, 12B, and 12C). Viewed by scanning electron microscopy, at this stage of fibrous plaque formation, there was a decrease in the surface irregularity observed in the fatty streaks and in the shoulder regions of the early fibrous plaques.

Discussion
Conversion of Fatty Streaks to Advanced Lesions
Our previous studies of diet-induced atherosclerosis in high level hypercholesterolemic nonhuman primates (600 to 1000 mg/dl) showed that hypercholesterolemia increases the extent and severity of fibrous plaque formation and that such advanced lesions appear to form first in the iliac arteries and subsequently in a cephalad direction in the abdominal aorta, thoracic aorta, and aortic arch. The present long-term, lower level hypercholesterolemic study shows a similar correlation in the level and duration of hypercholesterolemia with the extent, severity, and distribution of fibrous plaques.

Figures 3 and 4 demonstrate that fibrous plaques occur more frequently in the iliac arteries and abdominal aorta than in the thoracic aorta and aortic arch. In addition, the lesions are principally located at or near branch sites and bifurcations. Such anatomic sites are consistent with those previously affected by fatty streaks and fibrofatty lesions in the animals fed the diet for shorter periods of time, and suggest that blood flow and its rheological properties may be an important factor in lesion formation and progression. These observations suggest that fatty streaks are converted to fibrofatty lesions and then to fibrous plaques. Such conversion occurs earlier and more frequently in abdominal aorta and iliac arteries than in thoracic aorta and aortic arch, although the precedent fatty streaks are observed throughout the aorta in animals on the diet for a shorter period of time. Similar differences in distribution between fatty streaks and fibrous plaques have been described in humans. Mechanisms that explain such differences in lesion distribution are not clear; however, rheological factors may play a role in the localization of various types of lesions. Pre-existing smooth muscle cells in the intimal cushions at branch sites and in diffuse intimal thickening may also play a role in the process of initiation and formation of fatty streaks as well as conversion of fatty streaks to advanced lesions.

A distinct feature that helps to characterize the fibrous plaque as different from the fatty streak is the formation of a discrete fibrous cap composed largely of smooth muscle cells and surrounded by large amounts of extracellular matrix, including collagen, elastic fibers, and extracellular liposomes. Key events that lead to fibrous plaque formation include smooth muscle migration from the media and proliferation of these as well as of pre-existing intimal smooth muscle cells, which produce the extracellular matrix in the lesions.

The surface of the mature fibrous plaque does not contain the irregularities observed during the advanced stages of fatty streak formation. However, the shoulder region of the early stage of the fibrous plaque does contain numerous surface irregularities and disrupted endothelial junctions, retraction of endothelium to expose the underlying macrophages, and adherence of platelet microthrombi to some of the exposed macrophages. This suggests that a series of dynamic cellular interactions may participate in fibrous plaque formation and perhaps in fibrous plaque expansion in the shoulder regions.

Role of Endothelial Injury and Growth Regulatory Molecules
Several growth regulatory molecules may be chemo-
tactic and/or mitogenic for smooth muscle cells and may

Figure 7. A scanning electron micrograph of the shoulder region of a fibrous plaque. There are numerous surface irregularities due to subendothelial macrophages, loss of covering endothelium, and exposure of the underlying macrophages. Platelets can be seen adherent to some of the exposed macrophages surrounded by retracted and perhaps regenerating endothelial cells. x150

Light microscopic observations of the fibrous plaques in these animals revealed a localized intimal thickening, usually near branch sites (Figures 10A, 10B, and 10C). Immunohistochemical staining with HHF35 demonstrated the predominance of smooth muscle cells and the distinct formation of a fibrous cap, which was continuous with smooth muscle cells in the intimal cushion at the branch point. HAM56 demonstrated that the core of the fibrous plaques as well as macrophages scattered among the smooth muscle cells in the fibrous cap and elsewhere. A small number of T-lymphocytes were scattered throughout the fibrous plaques. However, most of the T-lymphocytes (predominantly CD8-positive cells) were associated with macrophages and could be identified by immunohistochemical staining. Associated with the longer duration of the lower level hypercholesterolemia, the fibrous plaques observed in the arterial tree contained more fibrous connective tissue matrix between the smooth muscle cells. Ultrastructurally such fibrous tissue was rich in collagen, elastic fibers, and extracellular liposomes (Figure 11). The smooth muscle cells contained an increase in their peripheral myofilaments together with dense bodies and a poorly developed rough endoplasmic reticulum, as compared with those observed in the earlier lesions (Figure 11 in the accompanying paper, see page 174). There was often evidence of calcification. In contrast, the macrophages in the advanced fibrous plaques were sometimes relatively decreased in numbers (Figures 12A, 12B, and 12C). Viewed by scanning electron microscopy, at this stage of fibrous plaque formation, there was a decrease in the surface irregularity observed in the fatty streaks and in the shoulder regions of the early fibrous plaques.

Discussion
Conversion of Fatty Streaks to Advanced Lesions
Our previous studies of diet-induced atherosclerosis in high level hypercholesterolemic nonhuman primates
Figure 8. A fibrous plaque from the abdominal aorta of a monkey on the diet for 2 years. A. Hematoxylin and eosin stain. B. Immunoperoxidase with anti-macrophage antibody, HAM56. C. Immunoperoxidase with anti-muscle antibody, HHF35. ABC method with nickel chloride modification of paraffin-embedded tissue sections counterstained with methyl green. A discrete fibrous cap is present, composed largely of smooth muscle cells that cover the central core of lipid-filled macrophages. ×24

Figure 9. Immunoperoxidase staining of a fibrous plaque in the abdominal aorta of an animal on the diet for 2 years. Serial frozen sections stained by ABC method with OsO₄ intensification, counterstained with nuclear fast red. A. Anti-muscle antibody, HHF35. B. Anti-macrophage antibody, HAM56. C. Anti-CD8 antibody, G10.1. D. Anti-CD4 antibody, OKT4a. ×90

be produced by the cells involved in the process of atherogenesis. For example, macrophages can produce platelet-derived growth factor (PDGF), transforming growth factor-α (TGF-α), transforming growth factor-β (TGF-β), tumor necrosis factor (TNF), and interleukin-1 (IL-1). Endothelial cells, smooth muscle cells, and platelets are also capable of forming many of these same factors. PDGF, for example, is capable of inducing migration and proliferation of smooth muscle cells and of enhancing lipid accumulation. We have preliminary data from Northern analysis of the monkey fibrous plaques that suggests that messenger RNA is increased for the B-chain of PDGF and for IL-1 (unpublished data). Barrett et al. reported that messenger RNA
Figure 10. Fibrous plaque at a branch site from the abdominal aorta of a monkey on the diet for 3 years. A. Hematoxylin and eosin stain. B. Immunoperoxidase staining with anti-macrophage antibody, HAM56. C. Immunoperoxidase staining with anti-muscle antibody, HHF35. ABC method with nickel chloride modification of paraffin-embedded tissue sections counterstained with methyl green. The accumulated macrophages are covered by a thin fibrous cap containing smooth muscle cells, which are continuous with smooth muscle cells in the intimal cushion at the branch point. ×48

Figure 11. A transmission electron micrograph of the fibrous cap region of a fibrous plaque from the abdominal aorta of a monkey on the diet for 3 years. The lesion is largely composed of smooth muscle cells containing some lipid vacuoles and abundant extracellular spaces occupied by numerous collagen fibrils, elastin, and extracellular liposomes. ×16,000

for PDGF A- and B-chain are induced in carotid atherosclerotic plaques and are correlated with increased message for α-actin and c-fms, respectively. Wilcox et al. also reported that PDGF A- and B-chain mRNA are present in mesenchymal-appearing intimal cells and capillary endothelial cells in carotid atherosclerotic plaques. TGF-β, which can be formed by most cells including platelets, endothelial cells, smooth muscle cells, and macrophages, is also capable of inducing chemotactic migration of smooth muscle cells and macrophages. It is a potent inducer of extracellular matrix synthesis, and in some situations may induce smooth muscle differentiation and induce or inhibit the proliferation of smooth muscle cells. These observations can be interpreted to support the response to injury hypothesis of atherosclerosis; however, the actual roles of these cells and the factors they form in vivo and their regulatory control mechanisms remain unknown.

Involvement of the Immune Response

In our earlier studies, we did not look for the presence of T-lymphocytes because at that time it was not appreciated that they might be a component of the lesions. It is clear from our observations that T-lymphocytes, principally CD8-positive as well as some CD4-positive cells, are present throughout the different stages of lesion forma-
tion. In most cases, T-cells are found in regions where macrophages are present. Several investigators have reported the presence of T-lymphocytes in atherosclerotic lesions in humans, not only in the fibrous plaques, but in fatty streaks. However, there is variation among the different reports concerning the subsets of T-lymphocytes in the atherosclerotic lesions. It is difficult to compare the results of these different studies because the monoclonal antibodies used were different. It will be necessary to expand our data concerning the distribution of subsets of T-lymphocytes in the various types of atherosclerotic lesions, not only in humans, but in experimental animals as well. The presence of T-cells suggests that an immune response may be important in lesion formation and progression; however, this needs further study. Whether this response is associated with the etiology and progression of the lesion, or whether it represents a secondary manifestation of immunity during lesion development, remains to be ascertained.

**Increased Connective Tissue In Advanced Lesions**

The advanced lesions, or fibrous plaques, observed in the animals on diets for 2 years or longer are similar in most respects to those observed in our previously published reports of high level hypercholesterolemia in nonhuman primates, with one important difference. Two types of fibrous plaques were found in the present study: the first was similar to that observed in the acute, high level hypercholesterolemic monkeys. In contrast, the second contained much more fibrous connective tissue around the proliferated smooth muscle cells. In the latter, macrophages were fewer in number, sometimes scattered throughout the fibrous tissue, and an atheromatous core was less evident in some cases. The more fibrotic lesions were readily observed in animals on the diet for 3 years or longer. Smooth muscle cells in such lesions, containing large amounts of connective tissue, were ultrastructurally closer to what has been described as contractile-type smooth muscle cells, whereas those in the less fibrotic tissue contained more protein synthetic apparatus. This may be the case if the fibrotic lesion had formed during an earlier period by synthetic-state cells that subsequently reverted to a contractile state, as discussed by Manderson et al.

Haust divided advanced lesions (atherosclerotic plaques) in humans into two categories: one consists of atheromatous plaques containing a basocentral atheroma and a fibrous cap, and the other consists of a white or pearly-white fibrous plaque without a basocentral atheroma. The two types of fibrous plaques that we observed in the nonhuman primates on the low level hypercholesterolemic regimen are consistent with those described by Haust in humans. This may be the result of the increased period of time available for lesion formation, connective tissue matrix turnover, and lesion remodeling.

**Relevancy of Nonhuman Primate Atherosclerosis to That In Humans**

The demonstration that it is possible to develop early, intermediate, and advanced lesions of atherosclerosis in nonhuman primates on a relatively low level hypercholesterolemic diet for prolonged periods of time suggests that the lesions that form in these animals are similar to those observed in most humans who are hypercholesterolemic. As demonstrated by Cornhill et al., lesions are often found at the entrance regions of vessels and at the lateral edges of the flow divider. In the case of these monkeys, lesions were found on the flow divider itself (Figure 6), which suggests that this animal model will also be useful in studying various therapeutic agents that may potentially be helpful in the prevention of lesion formation and in the induction of lesion regression. The fact that the advanced lesions that form in these animals are very similar to those that occur in animals on high level hypercholesterolemic regimens suggests that such studies can probably be performed for shorter periods of time by using higher levels of hypercholesterolemia, unless one is particularly interested in studying the aspects of connective tissue formation. A longer time interval is required to obtain fibrous plaques rich in connective tissue similar to that found in many advanced human lesions.

These studies have further defined the nature of the cells involved during lesion initiation, formation, and progression and suggest the importance of studying the roles of growth regulatory molecules in the process of...
atherogenesis. They demonstrate the usefulness of the nonhuman primate and the use of short-term, high level hypercholesterolemia as a reflection of similar changes that occur during long-term, low level hypercholesterolemia. These approaches should enable studies to develop antagonists to interfere with or prevent lesion formation. It should now be possible to utilize investigations such as these to study both interruption and prevention of lesion formation, with possible application to this disease as it occurs in humans.

Acknowledgments

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