Aneurysm formation in experimental atherosclerosis: Relationship to plaque evolution

Christopher K. Zarins, MD, Seymour Glagov, MD, Dragoslava Vesselinovitch, DVM, MS, and Robert W. Wissler, MD, PhD, Chicago, Ill.

To determine whether aneurysms form in experimental diet-induced atherosclerosis, we reviewed our experience with cynomolgus monkeys (n = 268) and rhesus monkeys (n = 175) fed an atherogenic diet for various lengths of time. Many animals in long-term experiments were fed “regression” diets and cholestyramine to lower cholesterol levels after lesions were established. No aneurysms were found in animals on normal diet. There were no aneurysms in 252 animals fed an atherogenic diet with or without regression for 12 months or less. However, aneurysms formed in 13% of cynomolgus monkeys (4 of 31) and 1% (1 of 107) rhesus monkeys on an atherogenic regimen for 16 to 24 months. Four of the five animals with aneurysms were on a regression diet and cholestyramine for 4 to 12 months. The fifth was fed the atherogenic diet for 20 months without subsequent regression. Aneurysms were prominent and involved the thoracic and abdominal aorta, innominate artery, carotid arteries, iliac and femoral arteries, and formed in areas most involved with plaque formation in both species. Histologic evidence was found of thinning of the media and atrophy with loss of normal architecture. The higher incidence of aneurysms in cynomolgus monkeys was associated with greater media destruction than was noted in the rhesus. These data support the thesis that aneurysm formation is a manifestation of atherosclerosis. In primate atherosclerosis, aneurysms form only after prolonged exposure to the atherogenic regimen, even in the presence of declining serum cholesterol levels. Matrix fibers in plaques may provide structural support to the aortic wall where there is underlying atrophy of the media. With time or declining serum cholesterol levels or both, plaques may atrophy leaving an aortic wall too thin to support increasing mural tension, leading to aneurysmal enlargement. (J VASC SURG 1990;12:246-56.)

Although an association between atherosclerosis and abdominal aortic aneurysms has long been recognized, the mechanism by which atherosclerosis induces or predisposes to aneurysmal dilation is not clear. The rarity of aneurysm formation in experimental atherosclerosis and the absence of prominent atherosclerotic plaques in many patients with aortic aneurysms has led some investigators to question a role for atherosclerosis as a primary pathogenetic factor. A number of other causes have been proposed including increased proteolytic enzyme activity, abnormal trace metal metabolism and genetically determined deficiencies in connective tissue structure and enzyme function. Although aneurysms have been observed in animals with genetic disorders, and have been induced by copper deficiency, lathyris, or mechanical injury, aneurysms have seldom been reported in association with plaque formation in animal models of diet-induced atherosclerosis. The few reported instances are, however, noteworthy for they suggest that long periods of induction or induction and regression favor aneurysmal dilation. DePalma et al. reported an aneurysm in one of three dogs fed an atherogenic diet followed by a regression regimen for 60 months, whereas Strickland and Bond found four aneurysms in 750 squirrel monkeys (0.5%) fed an atherogenic diet. Aneurysms formed only in those animals fed an atherogenic diet for at least 12 months. In our own laboratories nonhuman primates have been subjected to atherogenic diets and regression regimens for both
short and extended periods. The major vessels have been examined and sampled systematically. In this investigation we reviewed the pathologic findings of 443 cynomolgus and rhesus monkeys studied over the past 15 years to determine to what extent and in what manner aneurysm formation is associated with atherogenesis.

METHODS

Since 1971 diet-induced atherosclerosis in primates has been studied extensively at the Specialized Center of Research in Atherosclerosis at the University of Chicago. Experiments have been performed to investigate the effects of diet composition and drug administration on plaque formation and plaque regression as well as to determine the role of hemodynamic and biomechanical factors in plaque and artery wall responses. Serum lipid levels were measured at regular intervals, and complete gross and microscopic postmortem examinations were performed. A variety of experimental diets and animal species have been used in a number of experimental protocols. The present review was limited to the findings in cynomolgus and rhesus monkeys fed induction diets containing 2% cholesterol and either 25% peanut oil or 12.5% coconut oil and 12.5% butterfat. Some animals were treated subsequently by dietary manipulations and drugs (cholestryamine) to produce regression of plaques. All were maintained according to the “Principles of Laboratory Animal Care” (formulated by the National Society for Medical Research) and the “Guide for the Care and Use of Laboratory Animals” (NIH Publication No. 80-23, revised 1985). All animals were males. Neither genetic background nor precise age of the animals was known. The present report is based on findings in 268 cynomolgus (Macaca fascicularis) and 175 rhesus (Macaca mulatta) monkeys studied over the past 15 years.

In many instances arteries were fixed in situ under conditions of controlled pressure perfusion at the time the monkeys were killed. Angiograms of these distended vessels were also available. Histologic preparations were stained with hematoxylin and eosin as well as by the Gomori-Wheatley trichrome and Weigert-van Gieson methods for selective staining of collagen and elastin matrix fibers. Although particular attention was paid to the presence or absence of aortic aneurysms, ectasias and aneurysms were found in other locations. Histologic sections were assessed for plaque thickness and composition and for features of artery wall morphology in regions of aneurysm formation and in corresponding locations where di-
Fig. 2. Total serum cholesterol levels in five monkeys that developed aneurysms after 16 to 24 months. Four of the five were on cholestyramine and had normal serum cholesterol levels at the time of death. The fifth had been on an atherogenic diet for 20 months and had a serum cholesterol of 935 mg/dl.

Table III. Serum cholesterol levels mg/dl

<table>
<thead>
<tr>
<th>Animal</th>
<th>Baseline</th>
<th>Mean</th>
<th>Peak</th>
<th>Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cynomolgus 29</td>
<td>124</td>
<td>791</td>
<td>1000</td>
<td>—</td>
</tr>
<tr>
<td>Cynomolgus 16</td>
<td>109</td>
<td>556</td>
<td>700</td>
<td>111</td>
</tr>
<tr>
<td>Cynomolgus 24</td>
<td>128</td>
<td>943</td>
<td>1150</td>
<td>105</td>
</tr>
<tr>
<td>Cynomolgus 25</td>
<td>164</td>
<td>1257</td>
<td>2130</td>
<td>124</td>
</tr>
<tr>
<td>Rhesus 18</td>
<td>162</td>
<td>601</td>
<td>864</td>
<td>122</td>
</tr>
<tr>
<td>Mean</td>
<td>137</td>
<td>830</td>
<td>1169</td>
<td>116</td>
</tr>
</tbody>
</table>

Dimensions appeared normal. As often as possible, samples of aneurysmal segments were compared with nearby aortic samples of near normal dimensions, or for paired vessels, with contralateral sections at similar locations.

The extent of multilayered foam cell lesion accumulation, the character and thickness of the fibrous cap, the relative proportions of lesion matrix fiber and foam cell accumulation, and the degree of media atrophy necrosis or degeneration was assessed in each of the sections studied.

RESULTS

Cynomolgus monkeys

Aneurysms formed in 4 (1.5%) of the 268 cynomolgus monkeys involving one or more arteries. Focal or extended dilations of the aorta or of major
central arteries or both were evident on gross examination. All four of the affected animals were involved in long-term atherosclerosis experiments (Table I). No aneurysms were found in 44 monkeys serving as control animals and eating a normal diet with no cholesterol or fat supplementation. No aneurysms developed among 136 animals fed an atherogenic diet of 2% cholesterol plus 25% peanut oil or 12.5% coconut oil and 12.5% butterfat for 3 or 6 or 12 months. No aneurysms formed in 57 animals involved in experiments of 12 months’ duration. Among these, 21 were fed an atherogenic diet for the full 12 months, and 36 were fed the atherogenic diet for 6 months followed by a 6-month “regression” diet containing no cholesterol. Aneurysms developed in four of 31 animals involved in long-term dietary manipulation experiments for 16 to 24 months (13%). Three of the four had been fed an induction diet of 2% cholesterol (with 25% peanut oil (n = 1) or with 12.5% coconut oil and 12.5% butterfat (n = 2)) for 12 months followed by a period with no dietary cholesterol supplement and 2.5% cholestyramine for a period of 4 (n = 2) or 8 (n = 1) months. The fourth was fed a diet of 2% cholesterol and 25% peanut oil for 20 months of a planned 24-

Fig. 4. Postmortem cerebral angiogram of the cynomolgus monkey referred to in Fig. 3. Large aneurysms (arrows) were found at both carotid bifurcations (A, coronal view, B, lateral view). The carotid bifurcation is particularly prone to plaque formation in this experimental model.

Fig. 5. Postmortem angiogram of the aortic arch of the cynomolgus monkey referred to in Fig. 3. Note innominate aneurysm (A) and focal descending thoracic aortic aneurysm (B). Histologic sections of the descending thoracic aorta at level 1 (no aneurysm) and level 2 (aneurysm) are shown in Fig. 6.
Fig. 6. Histologic sections of descending thoracic aorta shown in Fig. 5. Section 1 is through an area without aneurysm formation. Note the presence of intimal plaque (1a) with preservation of a normal medial lamellar architecture throughout the circumference of the aorta. Section 2 is through the aneurysm. Note the larger size and the regions of thinning of the aortic wall. At (2a) the inner layers of media are replaced by a thick fibrous plaque with total wall thickness equal to (1b). At (2b) the plaque has atrophied and the medial lamellar architecture has disappeared. The wall is composed of fibrous tissue and total wall thickness is markedly reduced. Photographs of all sections taken at the same magnification (× 99).

Fig. 7. Aortic bifurcation of cynomolgus monkey that died of coronary artery occlusion after 20 months on an atherogenic diet. Aneurysms are present at the aortic bifurcation and common iliac arteries.

month diet induction period and died suddenly of a coronary artery occlusion. Twenty-eight of the 31 long-term animals had been studied in regression experiments with cholestyramine to lower serum cholesterol. Control animals in each experiment were killed at the end of the induction period to confirm the presence of lesions. All induction control animals had extensive plaque formation, including focal erosions of the inner media by contiguous extension of lesion components, including foam cells, from overlying lesions. Three of the 31 animals were long-term diet induction controls.

Rhesus monkeys

One aneurysm was found among 175 rhesus monkeys fed a diet containing 2% cholesterol plus 25% peanut oil or 12.5% coconut oil and 12.5% butterfat. No aneurysms formed among nine animals fed normal monkey chow (Table II). No aneurysms formed in 69 rhesus monkeys with diet and drug manipulation for 12 months or less. Among 107 monkeys in experiments of 12 to 24 months' duration, one (1%) aortic aneurysm was found.
The animal with an aneurysm was fed the atherogenic diet for 12 months followed by a 12-month regression period in which the same atherogenic diet included 2.5% cholestyramine. At autopsy this animal had no significant atherosclerotic plaques and a focal abdominal aortic aneurysm (Fig. 1). Microscopic examination revealed an atrophic aortic wall.

**Serum cholesterol changes and plaque formation**

Hypercholesterolemia developed in all monkeys while they were on the atherogenic diet. Total serum cholesterol elevation was variable and ranged from 350 to more than 2000 mg/dl. Animal response to the atherogenic diet was variable with extensive plaque formation in some and mild plaque formation in others. There was no direct relationship between the magnitude of total cholesterol elevation and extent of plaque formation. Animals with aneurysms reflected the variability of hypercholesterolemia (Table III). The time course of serum cholesterol in these animals is shown in Fig. 2. Regression regimens resulted in rapid return of serum cholesterol to normal values with regression of atherosclerotic lesions. More extensive lesions developed in cynomolgus monkeys than rhesus monkeys with more extensive aortic plaque deposition and greater medial thinning and destruction.

**Aneurysm formation after a long induction period**

One of three cynomolgus monkeys in a 24-month diet induction experiment (2% cholesterol and 25% peanut oil) collapsed and died suddenly after 20 months. His baseline total serum cholesterol was 124 mg/dl. While on the diet, the mean serum cholesterol level was 791 mg/dl, with a peak of 1000 mg/dl (Table III). The final value at 20 months was 935 mg/dl. His weight was stable throughout the experimental period. Severe coronary atherosclerosis with total occlusion of the right coronary artery was found at autopsy along with multiple thoracic and abdominal aortic aneurysms as well as aneurysms of the iliac arteries and the innominate artery (Fig. 3). Aneurysms were also found at each carotid bifurcation (Fig. 4). No coronary artery aneurysms were found. The descending thoracic aorta had evidence of focal aneurysmal degeneration (Fig. 5) with thinning and atrophy of the media (Fig. 6). Gross aneurysmal enlargement was present just below the renal arteries and at the aortic bifurcation and common iliac arteries (Fig. 7). The aortic wall in the region of aneurysm formation was thinned, and the plaque was atrophic (Fig. 8). The medial lamellar architecture was extensively effaced (Fig. 9). It is noteworthy that the aneurysms formed in regions in which the greatest amount of plaque is usually found in these
animals. No aneurysms were found in the other two animals in this group.

Aneurysm development after induction and regression

Aneurysms developed in three of 28 cynomolgus monkeys while they were on a cholesterol lowering regression regimen after a 12-month period of hypercholesterolemia. Thoracic and abdominal aortic aneurysms as well as iliac and innominate aneurysms developed in two animals (Fig. 10). A very large femoral artery aneurysm formed in one. Regressing atherosclerotic lesions were most prominent in the infrarenal aortoiliac segment, and plaques were atrophic. As in other animals, aneurysms developed in the areas of most significant plaque deposition during induction.

STRUCTURAL CHANGES

Microscopic examination revealed alterations of the artery wall in all of the aneurysmal or ectatic regions. Atrophy or thinning of the artery wall was noted in the animals on the regression diets. In the animal subjected to 20 months of an atherogenic diet, changes ranged from erosion of the inner layers of the media to total disappearance of the media. In this animal, regions of destruction of the media were replaced by atherosclerotic plaque components in continuity with intimal plaque deposits (Figs. 6 and 8). Similar zones of media destruction underlying plaques were noted in nonaneurysmal regions, but these were both less extensive and less penetrating than in aneurysmal locations (Fig. 11). No evidence was found of focal or generalized media thinning, erosion, disorganization, or atrophy in the absence of directly associated plaque formation either in aneurysmal or nondilated regions.

Several features distinguished the changes associated with focal aneurysmal dilation or extensive ectasia in the five animals with aneurysms. In the animal on a prolonged diet (20 months) without a regression regimen, the media in affected locations was focally and extensively penetrated by plaques. Replacement of the entire thickness of the media was a prominent and frequent finding. The media defect was often replaced by collections of foam cells, but most of the plaques contained areas of collagen deposition, often in the form of dense fibrous caps. In general, segments showing aneurysmal dilation had relatively little collagen formation, or else the thickness of the collagen deposition was insufficient to restore the wall thickness to normal when compared to corresponding nearby or contralateral nonaneurysmal sections (Fig. 6). Although a similar sequence of changes was noted in nondilated regions, a lesser extent and degree of media penetration and greater augmentation of wall thickness by plaque collagen was evident.
The animals that were subjected to regression regimens after 12-month induction periods showed thinning and atrophy of the aortic wall with some residual minimal focal intimal thickening but few plaques. None showed focal total destruction of the artery wall. Rhesus monkeys had significantly less medial involvement or destruction by the atherosclerotic process.

DISCUSSION

Our findings reveal that arterial aneurysms may develop in the presence of experimentally induced atherosclerosis in the nonhuman primate model. Aneurysms occurred only in animals maintained on atherogenic diets and only in those fed the diet for at least 12 months. None were found in animals maintained on the atherogenic diet for less than 12 months. Aneurysms were present in animals who were transferred to a regression regimen after a 12-month induction period and in an animal kept on the induction diet for 20 months without subsequent regression. No aneurysms were found in control monkeys kept for long periods in our animal facilities on standard chow.

These data indicate that spontaneous aneurysms do not form in the two species studied, and that a relatively long period of diet induction is necessary for aneurysm formation. Other reports that have identified aneurysms in the presence of experimental diet manipulation were also based on long-term studies. The incidence of aneurysm formation after 16 to 24 months of experimental atherosclerosis was 13% in cynomolgus monkeys but only 1% in rhesus monkeys, suggesting a difference in susceptibility for the two species studied. In general, diet-induced plaque formation in the cynomolgus is associated with greater media destruction than is usual in the rhesus. This difference tends to emphasize the important role of media involvement and atrophy, and supports earlier evidence that atherosclerotic lesions may evolve differently in the two species. Since duration of exposure to atherogenic stimuli appears to be a factor, observations of primates maintained for longer periods on atherogenic regimens may result in a higher incidence of aneurysm formation. The demonstration that a prolonged exposure to the atherosclerotic process in the experimental animal is necessary for aneurysm formation is consistent with clinical observations that patients operated on for abdominal aortic aneurysms are, on the average, 10 years older than those undergoing aortofemoral bypass procedures for occlusive disease.

Our histologic findings of destruction of the media in contiguity with overlying intimal plaques suggest that weakening of the aortic wall may predispose to dilation, ectasia, and aneurysm formation in relation to circumferential tensile stress. The elaboration of fibrous tissue is characteristic of plaque evolution and may provide structural support to the aortic wall where the media is eroded. In regions of aneurysm formation the reactive fibrotic component of the plaque may not be of sufficient thickness or density to provide the necessary reinforcement where the wall is markedly damaged. In those animals in which aneurysms developed after a period of regression, the artery wall was thinned and atrophic, and plaques were reduced in thickness or absent. These findings imply that some loss of media may have occurred during the induction period, and that during the period of regression the plaques receded removing the support that these lesions could have afforded to the underlying thinned media. Although plaque regression may have reduced tensile support of the media in the regression animals, the occurrence
of aneurysms in the animal with long exposure to an atherogenic diet but without regression suggests that long-standing progressive atherogenesis alone can result in aneurysmal dilation. Individual differences in reaction to the diet and to the regression regimen could help to account for differing susceptibilities to aneurysm formation.

Human atherosclerotic aneurysms, particularly those of the abdominal aorta, are also characterized by extensive atrophy of the media. The normal lamellar structure is almost totally effaced, and the wall is replaced by a narrow fibrous band. Atrophic changes are also evident in the overlying atherosclerotic lesions. These include relatively narrow plaques, little or no lipid, and the presence of dense fibrosis and calcification. Arteries usually enlarge as plaques form, both in nonhuman primates and in human vessels. The effect is to maintain a near normal lumen cross-sectional area until the disease is far advanced. Enlargement is due in large part to outward bulging of the atrophic media beneath the plaque. This apparent adaptive deformation may occur by mechanisms similar to those which eventually result in aneurysm formation in regions such as the abdominal aorta.

The similarity in morphologic changes in aneurysms associated with experimental diet manipulation and in atherosclerosis-prone human vessels suggests a mechanism of aneurysm formation that involves the relationship between plaque evolution and artery wall responses over time. Intimal plaque deposition may result in metabolic and functional alterations in the media directly beneath the plaque, which may lead to atrophy of the media. Early plaque formation results in adaptive enlargement of the artery wall to maintain a normal lumen caliber. As the vessel enlarges the increased radius would be associated with higher than normal tangential tensile stress on the media, which would be expected to stimulate fibrogenesis. Increased fibrous matrix deposition would strengthen the wall and prevent progressive dilation. The atherogenic process, in addition to lipid deposition, involves cellular proliferation and the elaboration of fibrous tissue in both the intima and media. The formation of a fibrous cap on the surface of plaques is a prominent feature of lesions. These atherosclerotic processes may reflect an ongoing healing response that tends to sequester the degenerative plaque components from the lumen and to provide tensile reinforcement for the faltering media. Thus the balance between the adaptive processes tending to enlarge the artery to compensate for developing lumen encroachment, and the restructuring repair and support processes to maintain structural integ-

Fig. 11. Cross section of aorta in a cynomolgus monkey demonstrating prominent fibrous cap (FC) overlying large necrotic core (N) with marked atrophy of underlying media (M). The fibrous cap acts to isolate the necrotic core from the lumen and may also provide structural support to replace that lost because of erosion and atrophy of the media. Material on the lumen surface is gelatin used to distend the vessel after pressure-perfusion fixation. (Magnification ×99).
rity, may determine whether enlargement progresses or wall stability is achieved.\textsuperscript{24} The formation of aneurysms may be a consequence of excessive vessel enlargement before stability is reached or may result from gradual or episodic progression of the atherosclerotic process such that plaque sclerosis does not keep pace with media destruction and atrophy.

Determinants of individual differences in aneurysm formation in both nonhuman primates and humans may include the rapidity with which atherogenesis proceeds, the duration of periods of plaque stability, periods of progression and regression related to control or absence of risk factors, and the intensity and time of imposition of hemodynamic or pressure-related changes favoring lesion progression or regression or both. Differences in tissue reactivity in relation to lesion healing and fibrosis would also be expected to play a role. Genetic differences may account for individual variations in relation to any of the factors listed above. Heredity and specific genetic predisposing conditions are well documented risk factors for susceptibility to atherosclerosis. Genetically based differences in susceptibility to aneurysm formation in the presence of atherogenesis may also occur. Genetic predisposition alone, however, cannot account for aneurysm formation in our experiments, for no aneurysms occurred in animals not subjected to atherogenic dietary regimens. The animals were of various ages and were obtained at different times from the wild. An atherogenic diet was always necessary for the production of aneurysms. This is equally true for the reported cases by others of aneurysms in nonhuman primates.\textsuperscript{16-19}

The mechanisms suggested above emphasize the role of atherosclerosis in the pathogenesis of aneurysmal dilation in disease-prone regions. This explanation does not, however, exclude the possible influence of genetic or acquired predisposing conditions other than predisposition to atherosclerosis, which may modify matrix fiber composition and organization in the artery wall. As a consequence of such conditions plaques may be forming on an abnormally extensible media, and plaque matrix fibers formed during atherogenesis may be abnormal in composition and lack sufficient resiliency and tensile strength to contribute significantly to wall stability. The wall may also be exposed to abnormal quantities of specific proteases or specific tissue inhibitors of degradation may be absent. In either case reduction in effective supporting matrix fibers may weaken the wall. If metabolic and enzymatic deviations contribute to the pathogenesis of aneurysms in susceptible regions, evidence from both experimental models and human disease indicates that long-standing atherosclerosis is, nevertheless, a necessary precipitating or inducing condition. Further detailed studies of the factors affecting plaque evolution in both animal models and in human disease are needed to reveal precisely how specific conditions operating during the atherosclerotic process may act to potentiate aneurysm formation in some individuals and obstructive disease in others.

REFERENCES