Chapter 1

Vascular biology of atherosclerosis

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Introduction

The pathogenesis of atherosclerosis has been the subject of thousands of articles published over the past several decades. Currently over 5000 papers per year are being published related to atherosclerosis. To identify only eight articles from this vast literature that have had great impact on our understanding of the biology of atherosclerosis is a difficult task. It will be impossible to do justice to the hundreds of investigators that have shaped the field as it is currently viewed. Most of the fundamental concepts shaping the field of atherosclerosis were generated by pathologists through observational studies. The current view of atherosclerosis probably began with the work of Rudolph Carl Virchow, a professor of pathology, who published "*Cellular Pathology*" in 1858. Virchow put forth the novel notion that cells were affected by outside stimuli and that diseased cells arose from other diseased cells. Virchow suggested that the atherosclerotic lesion resulted from lipid accumulation and cellular proliferation in the arterial wall. During the same time period (1852), von Rokitansky suggested that atherosclerotic lesion development was preceded by fibrin deposition and that persistence of fibrin deposits might contribute to the formation of an atherosclerotic lesion. Many other pathologists preceding and during this time period had made similar observations and it is difficult to determine who should be credited with the original observations. Suffice it to say, many pathologists have described atheromatous changes in the vasculature but experimental data to support specific hypotheses were lacking during this time period. This review will therefore focus on papers from the more "modern era" of vascular biology.

The modern biology of atherosclerosis arguably began with a series of seminal primate studies described by Russell Ross. In 1973, Ross and Glomset described the cellular composition of atherosclerotic lesions and proposed a critical role for the vascular smooth muscle cell in atherogenesis. Ross and Harker went on to propose the "response to injury hypothesis" to explain the development of atherosclerotic plaques and establish the critical role of hyperlipidemia in the initiation and progression of atherosclerosis. In 1981, Ross Gerrity established the role of the monocyte in atherogenesis using a hypercholesterolaemic swine model. This work de-emphasized the contribution of the vascular smooth muscle cell in the growing atherosclerotic lesion and stressed the importance of foam cells derived from monocytes. In 1983, Erling Falk studied human autopsy specimens and demonstrated that coronary thrombosis developed when plaque rupture occurred at a site of pre-existing coronary stenosis. Further characterization of the "vulnerable plaque" composition was provided by Michael Davies from an autopsy series of patients who died suddenly of ischaemic coronary disease. Seymour Glagov and co-workers introduced the concept of vascular remodelling when he demonstrated that the vascular wall could actually enlarge to accommodate atherosclerotic lesion growth. Further elucidation of the complexity of atherosclerotic lesions was provided by Herbert Stary when he published results of an autopsy series that included infants through young adults. These studies demonstrated that growth of the atherosclerotic plaque begins very early in life and progresses through various stages of complexity. Following the establishment of the contribution of lipids to atherosclerosis by several investigators, Brown and Goldstein elucidated the major pathway responsible for cholesterol homoeostasis. This work would earn them the Nobel prize and lead to therapeutical breakthroughs towards the battle against atherosclerotic vascular disease.

Title 1

Atherosclerosis and the arterial smooth muscle cell

Author

Ross R, Glomset J

Reference

Science 1973; **180**: 1332–1339

Abstract

Proliferation of smooth muscle is a key event in the genesis of the lesions of atherosclerosis.

Summary

In this paper, Russell Ross reviews the current data regarding the vascular smooth muscle cell in atherosclerosis. “Proliferation of smooth muscle is a key event in the genesis of the lesions of atherosclerosis”. At the time this paper was written, little was known about the genesis of atherosclerosis. Previous studies had demonstrated that blood pressure, smoking and plasma lipid concentrations could influence the development of clinical symptoms of atherosclerotic vascular disease but the sequence of pathological events at the cellular level was largely unknown.

Focal accumulation of intimal smooth muscle cells was argued to be critical to the early stages of atherosclerosis. Ross argued that “the accumulation of smooth muscle cells necessarily precedes or accompanies both the deposition of lipid and the accumulation of extracellular connective matrix, because the lipid deposits occur either within smooth muscle cells or outside them in association with connective tissue matrix components which are secretory products of smooth muscle cells”. Ross stated that smooth muscle cell proliferation began when a breach of endothelial integrity occurred that would allow substances present in the plasma to stimulate cellular proliferation. Studies supporting these observations included the tendency of vascular smooth muscle cells to accumulate in the intima at arterial branch points, where endothelial permeability appeared to be increased. Stemerman and Ross had also demonstrated experimentally using macaques that vascular lesions could be induced by denuding the femoral artery vascular endothelium with balloon catheters. Three months after injury, the lesion contained as many as 15 layers of smooth muscle cells surrounded by collagen and immature elastic fibers. These lesions were described as identical in appearance to the “fibromusculoelastic” lesions seen in man. Ross also reviews evidence (*in vitro* and *in vivo*) that lipids appear to influence proliferation of vascular smooth muscle cells and that vascular smooth muscle cells are responsible for production of extracellular matrix.

Citation Count 986

Key message

Vascular smooth muscle cell proliferation plays a critical role in the development and growth of atherosclerotic lesions.

Strengths

The identification of vascular smooth muscle cells and the time course of proliferation and matrix production following vascular injury greatly enhanced the understanding of vascular lesion

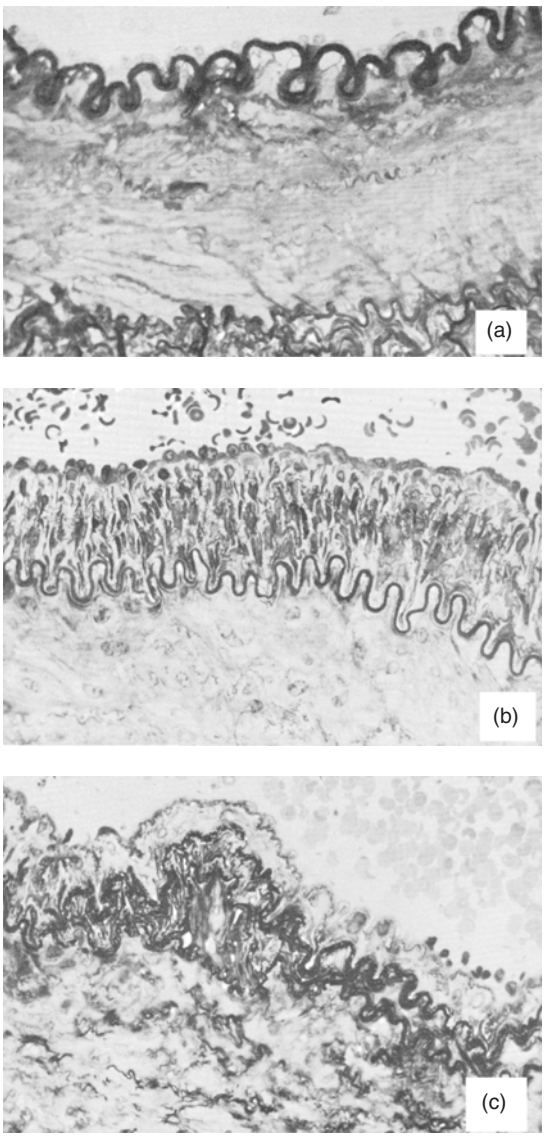

Figure 1 *Light micrographs demonstrating (a) a normal primate iliac artery (Mecaca nemestrina) (×550); (b) an iliac artery 3 months after de-endothelialization, showing a marked increase in intimal thickness due to accumulation of smooth muscle cells and extracellular matrix (×500); and (c) an iliac artery 6 months after de-endothelialization, by which time the intimal thickness had returned to one to two layers (×500). This sequence demonstrated the relative reversibility of an experimentally induced lesion in a monkey on a normal diet with normal concentration of plasma lipid.*

formation. This work also identified the smooth muscle cell as a therapeutical target towards the prevention of vascular disease.

Weaknesses

Conclusion regarding cell types and cellular proliferation in atherosclerosis were based on studies of femoral artery injury in primates. The lesions that develop following arterial injury with balloon catheters (neointima) are fibrous and are not the same as those that occur in naturally occurring atherosclerosis. In addition, accurate means of identifying cell types in atherosclerotic plaques were not available at the time of this paper.

Title 2

Hyperlipidemia and atherosclerosis

Author

Ross R, Harker L

Reference

Science 1976; **193**: 1094–1100

Abstract

Chronic hyperlipidemia initiates and maintains lesions by endothelial cell desquamation and lipid accumulation.

Summary

In this paper, Ross and Harker describe the effect of hyperlipidemia on the growth of vascular lesions, with and without mechanical injury, in primates. The article begins with an overview of the response to injury hypothesis to explain atherosclerosis development. According to this hypothesis (in 1976), everyone is susceptible to various forms of endothelial injury including mechanical, chemical, immunological and toxic sources. If the injury is a single event, the lesions would be reversible but continuous exposure to a toxic stimulus would lead to lesion progression which may be irreversible. Upon disruption of endothelial integrity, the vascular smooth muscle cells would be exposed to elements from the plasma that would stimulate proliferation. At the time of this paper, the principle mitogen present in blood serum responsible for the stimulation of smooth muscle cell growth was thought to be platelet-derived. In studies examining the effect of hyperlipidemia on vascular lesion formation, a group of monkeys were fed a hypercholesterolaemic diet and followed for up to 18 months after aorto-iliac balloon injury. At 6 weeks to 3 months following arterial injury, hypercholesterolaemic monkeys displayed thickened intima filled with lipid-laden cells which were presumptively identified as vascular smooth muscle cells. The number of cells comprising the intima in a control group of normolipidemic monkeys following injury was similar but the amorphous lipid inclusions were lacking. Long-term follow-up revealed that the lesions in the hyperlipidemic group had progressed while those in the normolipidemic group had regressed. Thus, hyperlipidemia promoted intimal growth after injury. Critical observations were then made in the non-injured iliac artery. By 10 months following initiation of hyperlipidemia, there were no differences between the injured and non-injured iliac arteries. Both arteries (injured and non-injured) contained 10–15 layers of lipid-laden smooth muscle cells surrounded by extracellular lipid and large quantities of newly formed connective tissue matrix. To search for evidence that hyperlipidemia was causing endothelial injury, Ross examined endothelial integrity in the hyperlipidemic animals using special staining techniques. In all of the hyperlipidemic animals, there was focal loss of endothelial cells accounting for approximately 5% of the aorto-iliac endothelial surface. In some areas, the longitudinal shape of the endothelial cells had changed to a polyhedral or round configuration, indicating abnormal endothelial regeneration.

Since platelet-derived factors were believed to be critical to the growth of vascular lesions following endothelial injury, measures of platelet activity were performed. Ross and Harker found that platelet survival was reduced from 8 days to 5.8 days in animals that were hyperlipidemic for more than 6 months. To determine whether the reduced platelet survival was due to increased platelet consumption at exposed endothelial surfaces or secondary to a direct effect of hyperlipidemia, labelled platelets were transfused from a normolipidemic animal into a hyperlipidemic

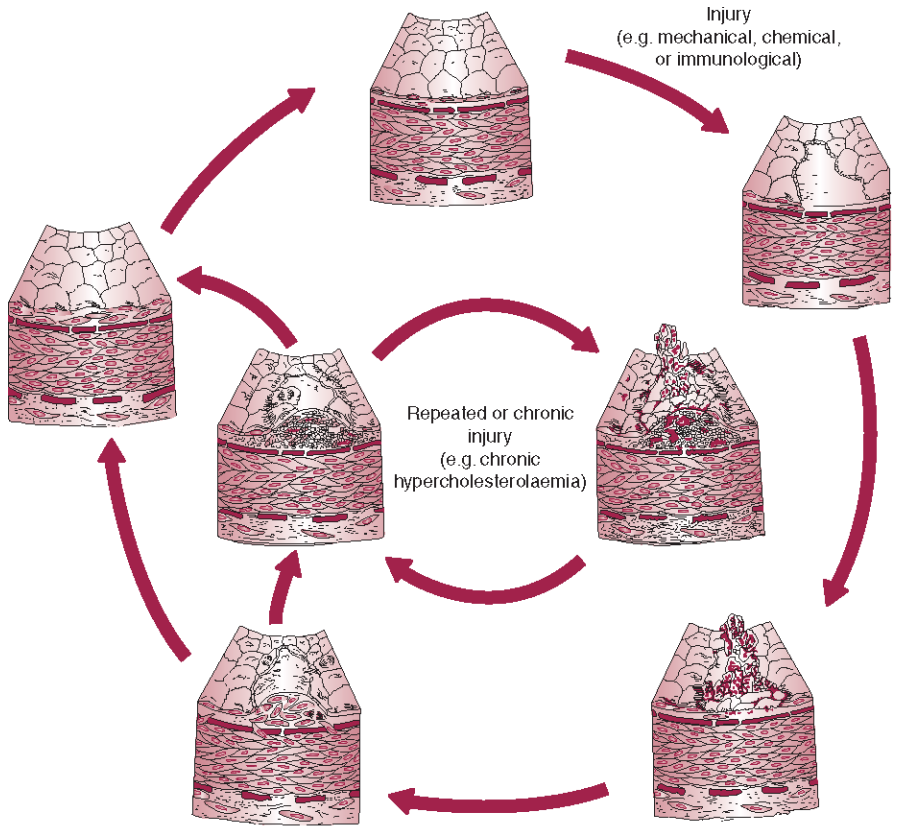

Figure 1 *In the response to injury hypothesis, two different cyclic events may occur. The outer or regression cycle may represent common single occurrences in all individuals in which endothelial injury leads to desquamation, platelet adherence, aggregation, and release, followed by intimal smooth muscle proliferation and connective tissue formation. If the injury is a single event, the lesions may go on to heal and regression occurs. The inner or progression cycle demonstrates the possible consequences of repeated or chronic endothelial injury as may occur in chronic hyperlipidemia. In this instance, lipid deposition as well as continued smooth muscle proliferation may occur after recurrent sequences of proliferation and regression, and these may lead to complicated lesions that calcify. Such lesions could go on to produce clinical sequelae, such as thrombosis and infarction.*

animal and from a hyperlipidemic animal to a normolipidemic animal. Platelets from the hyperlipidemic animals survived normally in the normolipidemic animals while survival of platelets from the normolipidemic animals was reduced in the hyperlipidemic animals. The authors concluded that reduced platelet survival was due to increased platelet consumption on exposed subendothelium and they went on to demonstrate a correlation between the amount of endothelium removed and platelet survival.

Citation Count

543

Key message

Hyperlipidemia is sufficient to cause endothelial injury, initiation, and growth of atherosclerotic plaques. This study also demonstrated the effect of the hyperlipidemic environment on the progression of vascular lesions following mechanical injury.

Strengths

In a primate model, the authors clearly demonstrated a critical direct role for a hypercholesterolaemic diet in the initiation and progression of atherosclerosis. The implications of these studies were that understanding and targeting factors involved in lipid metabolism could lead to therapeutic breakthroughs.

Weaknesses

The hypothesis that platelets or platelet products were essential to growth of the atherosclerotic plaque was not adequately tested. Thus the role of platelets in atherosclerotic lesion growth, based on *in vitro* vascular smooth muscle cell culture experiments and the transfusion experiments, was probably overemphasized.

Title 3

The role of the monocyte in atherogenesis: I. Transition of blood-borne monocytes into foam cells in fatty lesions II. Migration of foam cells from atherosclerotic lesions

Author

Gerrity RG

Reference

Am J Pathol 1981; **103**: 181–190, 191–199

Abstract

Paper I:
In a previous publication the author and his co-workers demonstrated that atherosclerotic lesion development in the aorta of hypercholesterolemic pigs was preceded by intimal penetration of blood-borne mononuclear cells, and that medial smooth muscle cells were not involved in the formation of early fatty lesions in this model. The current study shows that aortic arch lesions do not progress beyond the fatty cell lesion stage for up to 30 weeks of a moderate cholesterol/lard diet, although they become more extensive in area. Mononuclear cells were found adherent to the endothelium, in endothelial junctions, and in the intima during this period, and were ultrastructurally identified as monocytes by the presence of peroxidase-positive granules (peroxisomes) in their cytoplasm. In addition, lesion areas with nonspecific esterase activity correlated well with Sudan IV staining. Intimal monocytes and altered intimal monocytes with an enlarged cytoplasm and containing a few lipid droplets were both shown to be phagocytic by their uptake of ferritin, which had penetrated the intima after intravenous injection. Circulating monocytes and those adherent to the endothelial surface did not contain ferritin in these animals. The results indicate that blood mononuclear cells associated with lesion formation in this model are, in fact, monocytes, which subsequently undergo transformation into macrophage foam cells in fatty streak lesions. The absence of medial cell involvement indicates that monocytes are the major foam cell precursor in these lesions.

Paper II:
A defined role in the atherogenic sequence is proposed for the circulating monocyte. The author has been able to demonstrate a “monocyte clearance system” in which large numbers of circulating monocytes invade the intima of lesion-prone areas in arteries, become phagocytic, and accumulate lipid. A fatty cell lesion results. Once lipid-laden, foam cells migrate back into the bloodstream by crossing the arterial endothelium. The ratio of penetrating monocytes to emerging foam cells decreases as fatty cell lesions develop until a one-to-one ratio is achieved in late fatty cell lesions, which do not progress further. Advanced fibroatherosclerotic plaques in the same animals do not show the same characteristics and have smooth muscle cell involvement. It would appear that advancement of the lesion is at least partially a result of failure of the monocyte clearance system to remove sufficient lipid. The invasion of monocytes and endothelial damage caused by foam cell clearance may, in late fatty lesions, contribute to plaque evolution by introducing growth factors from macrophages and platelets and allowing greater lipid influx. Elucidation of this system was facilitated by the examination of vessels from diet initiation onwards and by the observation of late nonprogressing fatty cell lesions. It is possible that this

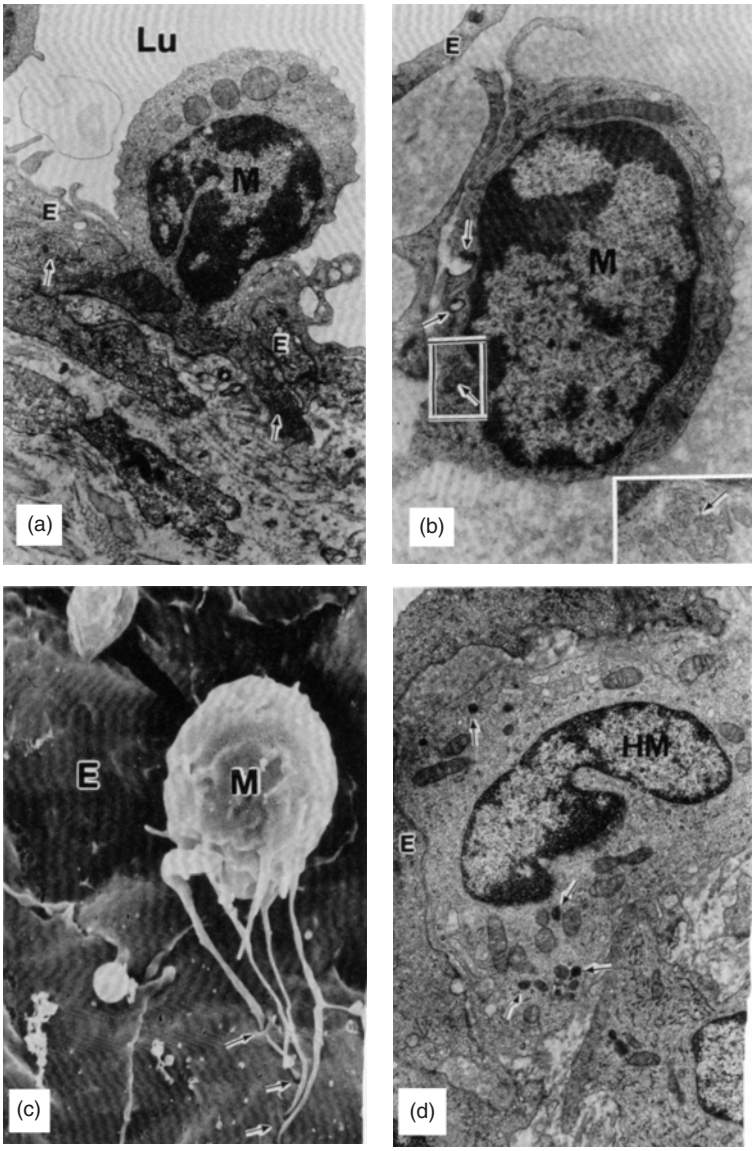

Figure 1 *(a) TEM of monocyte (M) trapped in junction of endothelium (E) from arch area of 12-week pig. Main body and nucleus of cell are in the lumen (Lu), with cellular extensions (arrows) spread below endothelium (uranyl acetate, lead citrate, ×12,400). (b) Monocyte (M) beneath endothelium (E) in a 15-week arch lesion from a pig injected with ferritin 15 min before death. Ferritin can be seen in phagocytotic vacuoles (arrows), one of which is open to the intimal space. Inset shows ferritin in vacuole in squared area (unstained, ×15,500; inset, ×61,000). (c) SEM of monocyte (M) adherent to endothelium (E). Cytoplasmic extensions from monocyte can be seen to indent endothelial plasma membrane (arrows); (d) TEM of "hypertrophied" monocyte (HM) beneath endothelium (E) in 30-week abdominal lesion. Peroxidase-positive granules (arrows) can be seen in the cytoplasm (uranly acetate, lead citrate; peroxidase-reacted, ×12,500).*

system exists in other models but has been overlooked by a predilection for the study of advanced lesions that prevails in the literature.

Summary

In this paper, parts I and II, Ross Gerrity described the progression of atherosclerotic lesions in a hypercholesterolaemic pig model. At the time of these studies the predominant cell type in growing

atherosclerotic plaques was controversial with much emphasis placed on the vascular smooth muscle cell. Although other authors had described monocytes in atherosclerotic lesions, this paper carefully studies the progression of atheroma at different time points and characterizes the lesion composition. Yorkshire pigs were fed a normal chow or high-fat chow and sacrificed at 6, 12, 15 and 30 weeks after the initiation of diet. At 15 weeks following high-fat chow, "lesions were always of a foam cell nature, confined to the intima, with no evidence of medial cell involvement in the intima or engorgement of smooth muscle cells with lipid". Monocytes were identified using various histological criteria. At 30 weeks following high-fat diet, fibrous lesions were described as fibrous caps overlying necrotic lipid cores. Gerrity hypothesized, based on this and earlier studies from his group, that blood-derived monocytes adhere to the endothelium, which is not necessarily associated with endothelial damage, and then penetrate into the intima. He also demonstrated that intimal monocytes are phagocytic at a time that lipid droplets appear in their cytoplasm. Thus, early lesions of atherosclerosis consist of monocyte foam cells that actively accumulate lipid and may be a source of a growth factor. The author also suggests a role of the monocyte in lesion lipid efflux.

Citation Count

Paper I: 1132, Paper II: 354

Key message

Blood-derived monocytes are the predominant cell type in early atherosclerotic lesions. This is a fundamental underpinning to our current understanding of atherosclerosis as an inflammatory disease.

Strengths

A detailed histological examination with elegant electron micrographs of lesions at various stages of development using a hypercholesterolaemic pig model.

Weaknesses

Primarily observational, descriptive data.

Title 4

A receptor-mediated pathway for cholesterol homoeostasis

Author

Brown MS, Goldstein JL

Reference

Science 1986; **232**: 34–47

Abstract

Not available.

Summary

Animal models of hypercholesterolaemia, as described in the preceding papers, as well as humans with familial hypercholesterolaemia indicated an enormous potential for the role of cholesterol metabolism in atherosclerosis. Brown and Goldstein embarked on their studies of cholesterol homoeostasis in 1972, in an attempt to understand the human genetic disease, familial hypercholesterolaemia (FH), a disease characterized by marked hypercholesterolaemia with premature myocardial infarction. FH heterozygotes, carrying a single copy of a mutant low-density lipoprotein (LDL) receptor gene, are common accounting for about 1 in 500 persons. LDL levels are approximately doubled in these individuals and myocardial infarctions begin to occur in the 30 s and 40 s. The homozygous FH frequency is about 1 in a million persons and is characterized by a 6–10-fold elevation in LDL and myocardial infarctions beginning in childhood. The existence of the homozygous state facilitated the discoveries of Brown and Goldstein as they could study effects of the mutant gene without confounding effects of the normal gene. The authors demonstrated that 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase), the rate limiting step in cholesterol biosynthesis, was subject to negative regulation in cultured fibroblasts, i.e. activity was upregulated when lipoproteins were removed from the culture media and suppressed when they were added. This suppression only occurred with LDL, and at relatively low concentrations, suggesting the possibility of a high-affinity receptor. Homozygous FH cells had HMG CoA reductase activities that were extremely high and were not subject to regulation by LDL in the medium. This suggested that there may be a defect in the HMG CoA reductase gene that rendered it's expression resistant to LDL. However, when cholesterol was dissolved in ethanol so that it could enter cells passively, suppression of HMG CoA reductase activity was achieved. These studies supported the hypothesis of a cell surface lipid receptor. This was proven with radiolabelled LDL, which bound to normal fibroblasts and not FH-homozygous fibroblasts. The investigators and associates went on to demonstrate that cholesterol generated from LDL within the lysosome was the second messenger responsible for suppressing HMG CoA reductase activity. Cholesterol acts at several levels to influence lipid homoeostasis including suppression of transcription of the HMG CoA reductase gene and acceleration of the degradation of the enzyme protein. The LDL-derived cholesterol also activates a cholesterol-esterifying enzyme, acyl-CoA: cholesterol acyltransferase (ACAT), and suppresses the synthesis of LDL receptors by lowering the concentration of LDL receptor mRNA. Thus, the cells can adjust the number of LDL receptors to provide adequate cholesterol for metabolic needs without causing cholesterol overaccumulation.

At least 10 different mutations in the LDL receptor were identified by structural criteria and separated into 4 classes. These included mutations that lead to the absence of LDL receptors,

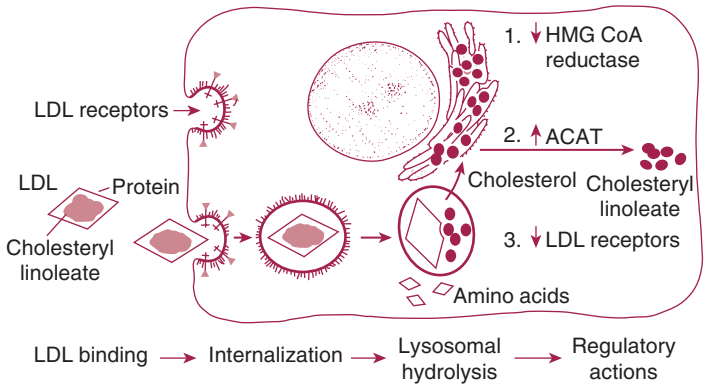

Figure 1 *Sequential steps in the LDL receptor pathway of mammalian cells. Vertical arrows indicate the direction of regulatory effects.*

mutations that alter receptor transport from the ER to the Golgi, mutations that lead to abnormal binding to LDL and mutations leading to failure of LDL receptors to cluster in coated pits.

Citation Count

2878

Key message

Cholesterol homoeostasis is under exquisite control by a complex cellular pathway initiated by a high-affinity LDL cell surface receptor.

Strengths

The pathways elucidated by Brown and Goldstein not only explained the cause of many cases of hyperlipidemia but have also served as the foundation for pharmaceutical discovery platforms aimed at prevention of coronary artery disease.

Weaknesses

None.

Title 5

Plaque rupture with severe pre-existing stenosis precipitating coronary thrombosis

Author

Falk E

Reference

Br Heart J 1983; **50**: 127–134

Abstract

Ruptured atheromatous plaques were identified by step-sectioning technique as responsible for 40 of 51 recent coronary artery thrombi and 63 larger intimal haemorrhages. The degree of pre-existing luminal narrowing at the site of rupture was decisive for whether plaque rupture caused occlusive thrombosis or just intimal haemorrhage. If the pre-existing stenosis was greater than 90% (histologically determined) then plaque rupture nearly always caused occlusive thrombosis. Clearly indicating the primary role of plaque rupture in thrombus formation were the frequent finding of plaque fragments deeply buried in the centre of the thrombus and the nature of the thrombus at the site of rupture where it consisted predominantly of platelets. Thus, a severe chronic stenosis seems to be a prerequisite for occlusive thrombus formation, but the thrombotic process itself is triggered by an acute intimal lesion.

Summary

At the time of this paper, there was general agreement that clinical complications of atherosclerotic vascular disease, such as myocardial infarction and stroke were due to thrombotic vascular occlusion. However, the underlying substrate or triggering mechanism for these thrombotic events was not well understood. In this paper, Erling Falk studied 47 patients with suspected fatal ischaemic heart disease. Postmortem angiography was performed along with detailed histological examination of the coronary arteries. Forty of 103 sites of plaque rupture identified were associated with significant recent luminal thrombosis, 95% of which were occlusive. In general, the greater the degree of underlying stenosis, the higher the risk of occlusive thrombosis. An underlying ruptured atheromatous plaque was identified in 82% of recently thrombosed coronary segments. No obstructions due to intimal haematoma (haemorrhage) were identified in this study.

Citation Count 548

Key message

Thrombotic complications of coronary atherosclerosis are due to plaque rupture.

Strengths

A combined postmortem angiographical and coronary histological study with excellent histological examples of plaque rupture and occlusive thrombosis.

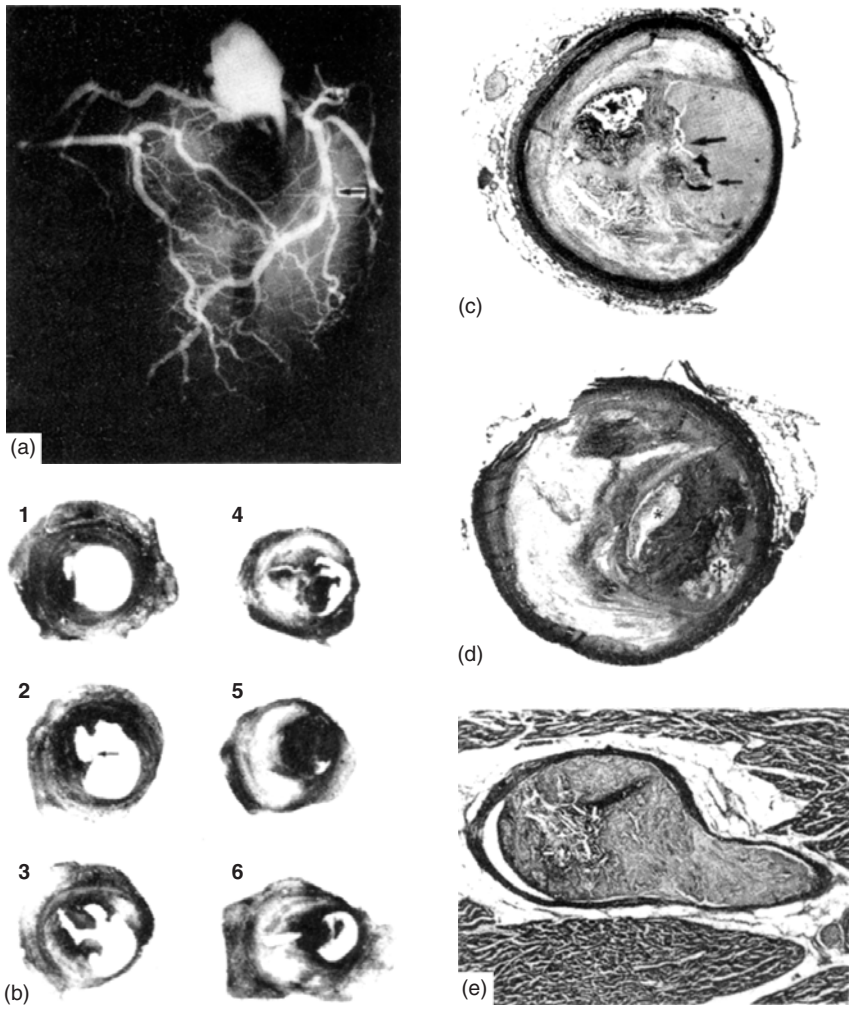

Figure 1 *A case clearly illustrating the primary role of plaque rupture in the pathogenesis of occlusive coronary thrombosis. (a) Postmortem angiogram showing total occlusion of the left anterior descending artery at the arrow (original magnification ×0.6). (b) The thrombosed vascular segment is cut transversely at 2–3 mm intervals (white contrast medium in the non-thrombosed vascular lumen). Section no. 2 shows the disrupted fibrous cap (arrow), and it is seen that both a cap fragment and fatty atheromatous substance have been lost. The thrombotic process has started at the site of rupture (Section no. 2–4) and attains its occlusive property just distal to the rupture (Section no. 5). (c) Microscopy of Section no. 4 shows direct communication between the vascular lumen and the atheromatous "gruel" (big arrow) and mural thrombosis at one of the free edges of the torn fibrous cap which projects into the lumen (small arrow) (original magnification ×13). (d) Microscopy of Section no. 5 shows a detached fragment of the fibrous cap (small asterisk) and atheromatous material with cholesterol clefts (big asterisk) intimately mixed with aggregated platelets in the vascular lumen (original magnification ×15). (e) Histological section from the perfusion area of the left anterior descending artery showing an intramyocardial artery occluded by a platelet embolus containing atheromatous plaque material with cholesterol clefts from the ruptured plaque proximal in the artery (original magnification ×33).*

Weaknesses

A relatively small series. Thrombosis occurring at sites of less severe atherosclerotic lesions may have been less stable and more likely to be dislodged or lysed by the time histological examination was performed. Thus the conclusion that occlusive thrombosis occurs only at sites of severe underlying stenosis may have been overstated.

Title 6

Compensatory enlargement of human atherosclerotic coronary arteries

Author

Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ

Reference

N Engl J Med 1987; **316**: 1371–1375

Abstract

Whether human coronary arteries undergo compensatory enlargement in the presence of coronary disease has not been clarified. We studied histologic sections of the left main coronary artery in 136 hearts obtained at autopsy to determine whether atherosclerotic human coronary arteries enlarge in relation to plaque (lesion) area and to assess whether such enlargement preserves the cross-sectional area of the lumen. The area circumscribed by the internal elastic lamina (internal elastic lamina area) was taken as a measure of the area of the arterial lumen if no plaque had been present. The internal elastic lamina area correlated directly with the area of the lesion (r = 0.44, p less than 0.001), suggesting that coronary arteries enlarge as lesion area increases. Regression analysis yielded the following equation: Internal elastic lamina area = 9.26 + 0.88 (lesion area) + 0.026 (age) + 0.005 (heart weight). The correlation coefficient for the lesion area was significant (p less than 0.001), whereas the correlation coefficients for age and heart weight were not. The lumen area did not decrease in relation to the percentage of

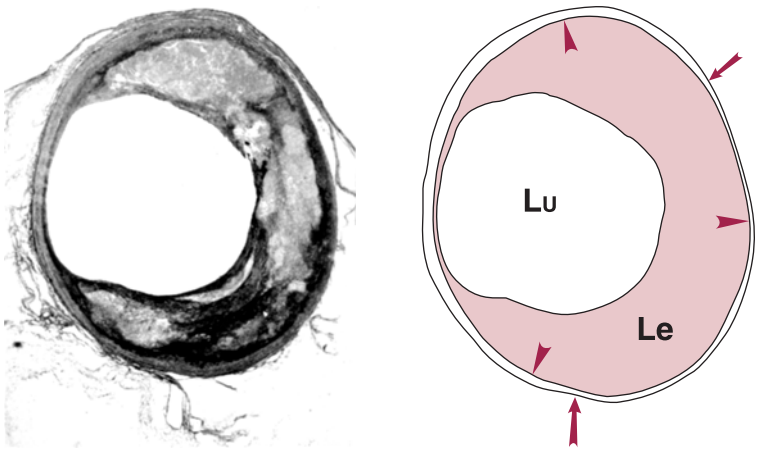

Figure 1 *Photograph of a cross section of a typical left main coronary artery with an advanced atherosclerotic plaque (left panel), and a corresponding contour tracing of the artery (right panel). The lumen (Lu) is clearly demarcated by the intimal surface. The internal elastic lamina (arrowheads) is readily discernible for most of the vessel circumference and almost always beneath the plaque as well, despite underlying atrophy of the media where the plaque and arterial wall tend to bulge outward (between the arrows). The area occupied by the lesion (Le) is shaded. The cross-sectional stenosis is defined as the extent to which the area encompassed by the internal elastic lamina (i.e. the potential lumen area if no plaque were present) is occupied by the lesion (percentage of stenosis, lesion area/internal elastic area × 100). In this vessel, the cross-sectional stenosis is 46% (magnification, × 7.4).*

stenosis (lesion area/internal elastic lamina area × 100) for values between zero and 40 percent but did diminish markedly and in close relation to the percentage of stenosis for values above 40 percent ($r = -0.73$, p less than 0.001). We conclude that human coronary arteries enlarge in relation to plaque area and that functionally important lumen stenosis may be delayed until the lesion occupies 40 percent of the internal elastic lamina area. The preservation of a nearly normal lumen cross-sectional area despite the presence of a large plaque should be taken into account in evaluating atherosclerotic disease with use of coronary angiography.

Summary

The purpose of this study was to determine whether human coronary arteries undergo compensatory enlargement in the presence of atherosclerosis. Histological analysis of left main coronary arteries was performed on 136 human hearts. Among the specimens there was a wide range of lumen area and lesion sizes. Comparisons between specimens revealed that lesion area increased with age at a rate of 0.08 mm^2 per year and lumen area decreased at a rate of 0.37 mm^2 for each mm^2 increase in lesion area. The internal elastic lamina area increased at a rate of 0.60 mm^2 for each mm^2 increase in lesion area. Thus, the vessel wall area increased with growth of the lesion, a relationship independent of age and heart weight. When a plot was made of lumen area vs. percentage of stenosis, there was no relationship of lumen area with stenosis up to 40% stenosis. However, as the stenosis increased above 40%, there was a marked reduction of lumen area. Thus, during early stages of plaque growth there is preservation of lumen size accomplished by expansion of the arterial wall.

Citation Count

1103

Key message

Arterial remodelling occurs with growth of atherosclerotic plaques. Coronary angiograms that use contrast agents to opacify the arterial lumen may markedly underestimate atherosclerotic burden.

Strengths

This was the first clear demonstration of vascular remodelling in humans. The implications of the concept of vascular remodelling with the accompanying necessary matrix turnover have generated an entire new field of study in vascular biology.

Weaknesses

This was not a longitudinal study. Thus a diverse population with varying risk factors could have affected the results. Only the left main artery was studied. Different results may occur in smaller arteries.

Title 7

Evolution and progression of atherosclerotic lesions in coronary arteries of children and young adults

Author

Stary HC

Reference

Arteriosclerosis 1989; **9**(Supp): I-19–I-32

Abstract

In an autopsy study of the evolution of atherosclerotic lesions in young people, we obtained the coronary arteries and aortas of 1160 male and female subjects who died between full-term birth and age 29 years. In this article, we report the light and electron microscopic observations of the coronary arteries of 565 of these subjects in which we fixed the coronary arteries by perfusion with glutaraldehyde under pressure. From birth, the intima was always thicker in the half of the coronary artery circumference opposite the flow-divider wall of a bifurcation (eccentric thickening). In cases where we found lipid in the intima, there was always more in eccentric thickening. Isolated macrophage foam cells in the intima of infants were the earliest sign of lipid retention. These cells occurred in 45% of infants in the first 8 months of life but decreased subsequently. At puberty, more substantial accumulations of macrophage foam cells reappeared in more children. Foam cells were now accompanied by lipid droplets in existing smooth muscle cells and by thinly scattered extracellular lipid. Sixty-five percent of children between ages 12 and 14 years had such lesions. An additional 8% of children had progressed beyond this early stage and had developed advanced preatheroma or atheroma stages. Such advanced lesions, located only in areas of eccentric thickening, were characterized by the addition of massive extracellular lipid that displaced normal cells and matrix and, thus, damaged and weakened the arterial wall.

Summary

This paper describes a large series of coronary histology data from 565 human subjects who died between full-term birth and 29 years of age. At the time of this study, there was controversy regarding the extent of atherosclerosis in the young and at what age dietary interventions to reduce atherosclerosis should be instituted. The left main, proximal left anterior descending and proximal circumflex arteries were analysed. Based on the findings of this series, a classification system was developed to characterize development of atherosclerotic lesions. Type 1 lesions consist of isolated macrophage foam cells in the proteoglycan layer with no extracellular lipid or vascular smooth muscle cells. These lesions were seen in some infants as early as the first week of life and appeared to be decreased after the first year. Type II lesions are fatty streaks composed of layers of cells with lipid droplet-inclusions. More macrophages are present than type I lesions. These lesions were noted beginning from late in the first decade of life. A type III lesion is a preatheroma that is intermediate between the fatty streak and the atheroma. These lesions contain all the components of a fatty streak with a marked increase in the number of extracellular lipid particles. They appear grossly as a small white elevation and are typically seen beginning in the mid second decade of life. Type IV lesions were seen in subjects beginning at the end of puberty

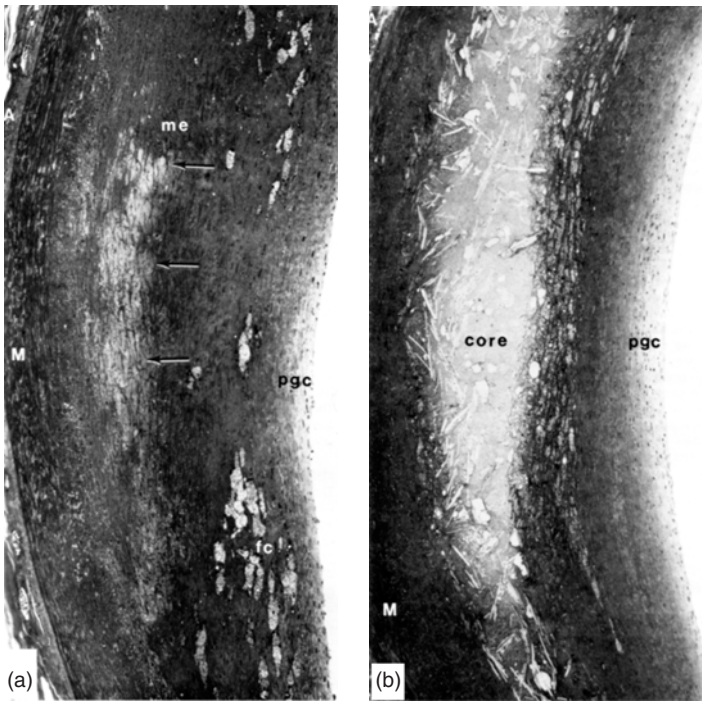

Figure 1 *(a) Outer wall at the level of the left main coronary artery just proximal to the main bifurcation. Extracellular lipid (arrows) is abundant and concentrated in the musculoelastic layer (me) of eccentric thickening and displaces some structural intimal smooth cells. Macrophage foam cells (fc) and lipid-laden smooth muscle cells are layered above the extracellular aggregates. Such lipid deposits were classified as preatheroma (type III lesion). From a 25-year-old white man who died in a motorcycle accident. (b) Outer wall at the LAD 1 level with eccentric thickening now metamorphosed into a lesion classified as atheroma (type IV lesion). Extracellular lipid now forms a confluent core in the musculoelastic layer of eccentric thickening. While this lipid deposit thickens the intima, it also weakens the wall as it displaces structural smooth muscle cells. From a 19-year-old white man who committed suicide. Pgc: proteoglycan intima; M: media; and A: adventitia.*

and consisted of large lipid or necrotic cores. Macrophage foam cells were seen bordering the lipid core on the luminal aspect of the lesion. A type V lesion is a fibroatheroma in which the proteoglycan layer has changed in composition with an increased number of smooth muscle cells embedded in a dense matrix of collagen and capillaries. This layer of smoothmuscle cells becomes a fibrous cap. These lesions were typically seen beginning in the mid third decade of life.

Stary described the frequency of the various lesions at different ages and this data established that vascular lesions are quite common even in young children. This study raised many questions regarding the sequence of events that would lead to a fatty streak at such young ages and subsequent progression of lesion complexity.

Citation Count

15

Key message

Vascular lesions consistent with early atherosclerosis begin very early in life and appear to progress in complexity through a series of defined stages.

Strengths

Detailed, extensive histological examination of very young human subjects. The idea that lesion growth occurs through predictable stages representing various cellular and molecular events set a framework for future investigations.

Weaknesses

Descriptive study. Although this classification system has been useful to facilitate our understanding of atherogenesis, the staging of atherosclerotic lesions may be an oversimplification of the underlying pathophysiology and not take into account the marked heterogeneity of atherogenesis between individuals.

Title 8

Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophage, and smooth muscle cell content

Author

Davies MJ, Richardson PD, Woolf N, Katz DR, Mann J

Reference

Br Heart J 1993; **69**: 377–381

Abstract

OBJECTIVE: To assess the size of the lipid pool and the number of smooth muscle cells and monocyte/macrophages in human aortic plaques that were intact and to compare the results with those in aortic plaques undergoing ulceration and thrombosis. DESIGN: The lipid pool was measured as a percentage of the total cross sectional area of the plaque. Immunohistochemistry was used to identify cell types (monocytes/macrophages (M phi) by EBM11 and HAM56, smooth muscle cells by alpha actin). The area of the tissue occupied by each cell type was measured by quantitative microscopy in the peripheral (shoulder) area of the plaque and the plaque cap. Absolute counts of each cell type were expressed as the ratio of SMC:M phi. MATERIAL: Aortas were obtained at necropsy from men aged less than 69 years who died suddenly (within 6 hours of the onset of symptoms) of ischaemic heart disease. 155 plaques from 13 aortas were studied. Four aortas showed intact plaques only (group A, $n = 31$). Nine aortas showed both intact plaques (group B, $n = 79$) and plaques that were undergoing thrombosis (group C, $n = 45$). RESULTS: In 41 (91.1%) of the 45 plaques undergoing thrombosis (group C) lipid pools occupied more than 40% of the cross sectional area of the plaque. Only 12 (10.9%) of the 110 intact plaques (groups

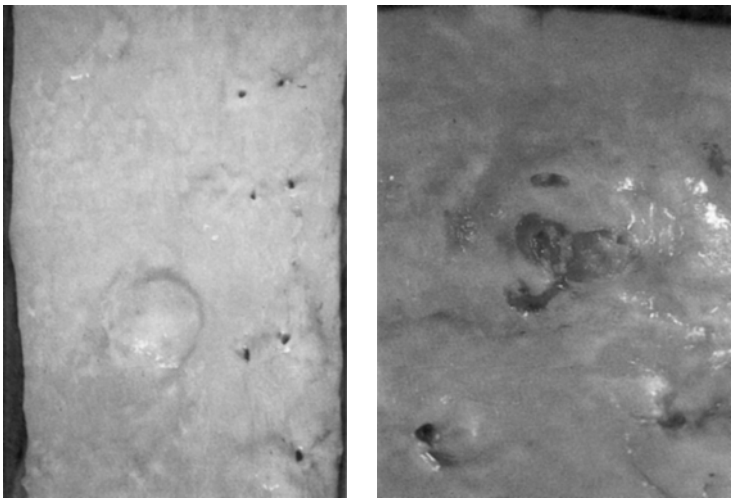

Figure 1 *Contrast between an intact advanced aortic plaque (left), in which the surface is opaque and smooth, and early ulceration (right), in which thrombus has formed over the plaques. However, a large part of the plaque is not covered by thrombus.*

A + B) had lipid pools of this size. The mean size of the lipid pool in plaques of groups A, B, and C was 12.7%, 27.3% and 56.7% respectively. Compared with intact plaques those undergoing thrombosis contained a smaller volume of smooth muscle cells (2.8% v 11.8%) and a larger volume of monocyte/macrophages (13.7% v 2.9%) in the plaque cap. The ratio of the number of smooth muscle cells to monocytes/macrophages was 7.8 in group A plaques, 4.1 in group B plaques, and 1.0 in group C plaques. This gradient was the result of an absolute increase in monocyte/macrophages and an absolute decrease in smooth muscle cells. CONCLUSIONS: In the aorta ulceration and thrombosis were characteristic of plaques with a high proportion of their volume occupied by extracellular lipid, and in which there was a shift toward a preponderance of monocyte/macrophages compared with smooth muscle cells in the cap.

Summary

This study was one of the first to characterize the composition of atherosclerotic plaques at risk for thrombosis. To address this important issue, aortas were obtained at necropsy from adult males who died suddenly of ischaemic heart disease. 155 plaques from 13 aortas were analysed. In 41 of 45 disrupted plaques (91%) with thrombosis, the lipid pool occupied more than 40% of the cross-sectional area of the plaque whereas only 11% of intact plaques had lipid pools of this size. Compared with intact plaques, those with thrombosis also contained a smaller volume of smooth muscle cells (2.8 vs. 11.8%) and a larger amount of monocyte macrophages (13.7% vs. 2.9%) in the cap of the plaque.

Citation Count

397

Key message

Atherosclerotic plaques at risk of thrombotic complications are characterized by large lipid pools, thin fibrous caps and increased macrophage activity.

Strengths

These relatively simple observations have stood the test of time. Systemic indices of inflammation which probably correlate with plaque inflammation (i.e. macrophage activity) have proven potent indicators of cardiac risk. Potent lipid lowering therapies which appear to reduce large lipid pools and inflammatory activity have been shown to reduce cardiovascular events. Additional therapies targeting plaque stabilization are under development.

Weaknesses

Only aortas were analysed in this study which may not exhibit identical pathology to coronary arteries. Since only autopsy specimens were analysed, it is difficult to prove whether the changes in plaque cellular composition observed in the setting of thrombosis were a cause or effect of the thrombus.