ATHEROSCLEROSIS: from Egyptian mummies to immune-mediated intraplaque inflammation

Anton E. Becker, MD, PhD

Emeritus Professor of Cardiovascular Pathology - Academic Medical Center
University of Amsterdam - THE NETHERLANDS

Atherosclerosis has plagued humankind since ancient times, and its understanding has much evolved over the centuries. For Rokitansky, a proponent of the ancient humoral theory, the thickening of the arterial wall was due to deposits derived from the blood. For Virchow, the father of cellular pathology, thickening resulted from a cellular reaction. For both, fat deposition was the result of secondary degeneration. Anitschkow showed that atherosclerotic lesions resulted from the combined effect of hypercholesterolemia (toxic injury) and hypertension (mechanical injury). Other landmarks include the discovery of the scavenger receptors and of the cellular cholesterol cycle, and the identification of platelet-derived growth factor, which gave rise to the response-to-injury hypothesis. With some modifications, this concept still prevails today.

Keywords: atherosclerosis; hypercholesterolemia; inflammation; hypertension; scavenger receptor; platelet-derived growth factor

Address for correspondence:
Anton E. Becker, MD, PhD, Emeritus Professor of Cardiovascular Pathology, IJsselmeerdijk 13, 1473 PP Warder, The Netherlands
(e-mail: aebecker@prettel.nl)

Dialogues Cardiovasc Med. 2006;11:116-122

"Angeiosclerosis is the nemesis through which Nature exacts retributive justice for the violation of her laws." This quotation is from the works of Sir William Osler (1849-1919) and—although I am not certain whether or not he was jesting at the time—it is humbling to realize that today the essence of this statement is still very much in line with the practical consequences of our current understanding of what causes the disease.

HOW DID IT ALL START? A LOOK AT THE TREE'S ROOTS

Atherosclerosis has its roots many thousand years ago: the earliest documented evidence comes from Egyptian mummies. The term arteriosclerosis, however, was first coined by Lobstein in the 1820s, a professor of pathological anatomy at the University of Strasbourg and—to the best of my knowledge—the first-ever appointed professor of pathological anatomy. In his *Treatise of Anatomical Pathology* (Paris, 1829), he not only introduced the name, but also justified it by describing the disease as *"composé d'artère et de sclérose, d'épaississement avec induration"* (freely translated as: composed of rigid arteries with wall thickening and hardening). If Lobstein failed to attribute an inflammatory cause to the disease, this was because he lacked the technology to detect inflammation in the postmortem studies that he performed. In his opinion, the disease started as a nutritional disorder of the wall of the artery, followed by

Figure 1. *Portrait of Karl Freiherr von Rokitansky (1804-1878).*

Reproduced from:
Deutsche Medizinische Wochenschrift 1906, No. 16. © BIUM.

abnormal tissue proliferation of the inner layers. The cause of all these processes, however, remained obscure. Nevertheless, Lobstein's point of view is of major interest because it departed from the classic humoral theory that had held sway over medical thinking for centuries as an explanation for disease processes. His views clashed with those of the celebrated Austrian pathologist and one of the founders of modern pathological anatomy, Baron Karl von Rokitansky (1804-1878), who invoked the old humoral theory to explain the development of atherosclerosis, ***(Figure 1).*** An exponent of the Viennese School of Medicine and the author of the great *Manual of Pathological Anatomy* (1842-1846), Rokitansky stated, in his classic work on arterial diseases, published in 1853, that arteriosclerosis consisted in a thickening of the vascular wall that he attributed to the action of the circulating blood mass:

> ...einer exzedierenden Anbildung und Auflagerung von innerer Gefäßhaut aus der Blutmasse und stellt gleichsam eine Hypertrophie der inneren Gefäßhaut dar [an excess buildup and deposition of inner vessel wall originating in the blood, representing a hypertrophy of the inner vessel wall].

THE EMERGING TRUNK: SEMINAL DISCOVERIES IN ATHEROSCLEROSIS

This concept was seriously challenged by Rudolf Virchow, the father of cellular pathology, ***(Figure 2),*** who in 1858 published his landmark work, entitled *Cellular Pathology and its Foundation on Physiological and Pathological Histology.* In his opinion, cell damage was the key factor involved in basically all forms of diseases. As for arteriosclerosis, he considered the thickened inner wall of the arteries as resulting from chronic inflammation, analogous to the thickened endocardium in chronic endocarditis. He thus termed the process leading to arteriosclerosis or endarteritis chronica deformans. It is important to realize that in his view inflammation was basically a nutritional disorder. Endarteritis started as a thickening of the inner arterial wall, in which connective tissue cells also participated. These cells accumulated more nutrients than other components, re-

Figure 2. *Portrait of Rudolf Virchow (1821-1902).*

Reproduced from: *Deutsche Medizinische Wochenschrift 1906, No. 16. © BIUM.*

sulting in proliferation, on the one hand, and fatty transformation, on the other. In addition, according to Virchow, a second mode of fatty change existed, not associated with inflammation, which he considered "a pure passive process of degeneration." As a consequence, Virchow made a distinction between superficially located fatty deposits, including the "fatty wear and tear"—in other words, the lipid-rich ruptured plaque in our current terminology—and the more deeply situated atheroma related to the endarteritis. The former condition, in his opinion, should not be classified as arteriosclerosis. Virchow's concepts on arteriosclerosis remained popular for a considerable length of time. In subsequent years the concept that "inflammation" was the key factor in the genesis of arteriosclerosis became widely accepted, even though the original insights gradually shifted, in particular because of the work of Cohnheim (1877). For Cohnheim, the development of connective tissue in the intima was interpreted as the expression of proliferative inflammation with little exudation. Questions were raised as to whether or not white blood cells could be involved in the proliferative process and whether the inflammation could originate in the adventitia and from there spread along the vasa vasorum into the intima (an opinion upheld by Koester, around 1875-1876). Moreover, Cohnheim stated that there could be no inflammation without vessels, and this created a problem in explaining the origin of arteriosclerosis in small arteries without vasa vasorum.

These were fascinating speculations for the time, and certainly so when put in perspective with our present understanding. The most important development at the time, however, was that, with the shift in the concept of inflammation, the opinion about the accumulation of fat also changed. The latter came to be considered as secondary fatty regression of the poorly vascularized connective tissue layers in the intima. The main focus of interest in the genesis of arteriosclerosis, therefore, concentrated on proliferative inflammation with connective tissue thickening of the intima as a result.

Another development of interest during that period was the observation by Langhans in 1866 that dilated and tortuous arteries showed diffuse thickening of the intima, like that seen in arteriosclerotic arteries with localized intimal thickening, which he called "arteriosclerosis diffusa et nodosa."

Thoma, in the late 19th century–early 20th century, while agreeing that the main abnormality in arteriosclerosis was intimal thickening, had noted in neonates that the disease occurred preferentially between the site of attachment of the ligament of Botal and the origin of the umbilical arteries—an observation shown by subsequent investigators to be only partially true. However, it led Thoma to formulate a new pathogenetic concept. In his view, the intimal thickening compensated for a mismatch between postnatal vessel size and the amount of blood transported. For him, the trigger for intimal thickening was the low velocity of the blood. By introducing this concept, Thoma was among the first to ascribe functional significance to mechanical factors in the genesis of arteriosclerosis. It is fascinating also to realize that already at that time he convincingly showed that luminal diameter remained unchanged in spite of the presence of local intimal thickening, unless the disease was far advanced: a concept attributed to Glasgow and colleagues, following their publication in 1987, and now widely known as positive remodeling.

Amidst these constantly evolving changes in concepts and insights, Marchand, in 1904, by coining the term atherosclerosis, introduced a change in semantics that signaled a major conceptual shift. This was because he considered the soft atheroma rather than the hardened artery as the most characteristic pathologic feature of the disease. Although it was widely recognized that Marchand was right, the fact that the origin of the fatty debris remained controversial urged most authorities to continue using the term arteriosclerosis until better understanding had been achieved. As a consequence, it took a long time before the term atherosclerosis eventually became accepted and even today there remains some debate as to the precise definition of either term. Personally, I have used the term arteriosclerosis as a generic term referring to any diseased artery characterized by sclerosis, ie, hardening of its wall, such as Mönckeberg sclerosis. The term atherosclerosis I have used only for those disease processes in which intimal lesions resulted from a chronic proliferative inflammatory process associated with intracellular and extracellular lipid accumulation. In my experience, this approach works well in daily practice, although I accept that every now and then it may be impossible to be certain. But then, the precise naming of things often tends to become a bit arcane!

Jores, professor of pathological anatomy in Kiel in the early 1900s, should be credited for his contribution to the understanding of the development of the "elastic hyperplasia of the intima," which we nowadays know as the musculoelastic layer, and its potential role in the genesis of arteriosclerosis *(Figure 3)*. He considered this layer as responsible for the physiologic adaptation to hemodynamic changes, but at the same time pointed out that it could contribute to the development of "regenerating connective tissue proliferation of the intima." Indeed, Jores showed that arteriosclerotic lesions consist of a hyperplastic elastic layer sandwiched between the media and a connective tissue layer on the luminal site. In his opinion, the hypertrophy of the intima provided the substratum for arteriosclerosis: elastic-hyperplastic intimal thickening tends to fatty degeneration and this leads to connective tissue proliferation of the intima.

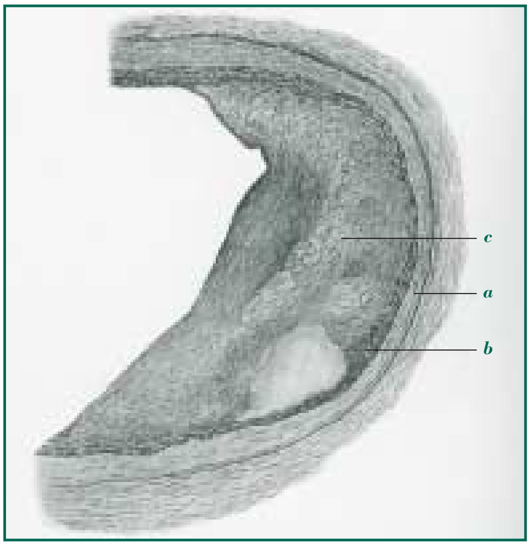

Figure 3. *Drawing of a localized intimal thickening with "atheromatose," which we now would call an atherosclerotic plaque, in a coronary artery.* ***(a)*** *Media.* ***(b)*** *Hyperplastic-elastic layer (musculoelastic layer).* ***(c)*** *Collagen-rich part of the plaque. The light areas are sites of lipid degeneration.*

Reproduced from: *Jores L. Arterien. In:* Henke und Lubarsch. Handbuch der speziellen Pathologischen Anatomie und Histologie. Zweiter Band. Herz und Gefässe. *Berlin, Germany: Verlag von Julius Springer; 1924. Copyright © 1924, Springer-Verlag.*

Although Jores still believed that the lipids that had accumulated in arteriosclerotic lesions resulted from a degenerative process, his attribution of a pathogenetic role to a physiological adaptive process was an important breakthrough. Unaware of Jores' statements at the time, I wrote in 1985 that the musculoelastic layer, because of its smooth muscle cell content, provided a fertile soil for the development of an atherosclerotic lesion, and I still consider this an important prerequisite in the genesis of the disease.

Like Marchand and Thoma, Jores also concluded that intimal thickening did not fulfill the criteria for an inflammatory reaction. They considered the genesis of the disease as a nutritional disorder of the artery wall, in the sense that the densely packed architecture of the elastic-hyperplastic layer interfered with the adequate supply of nutrients, subsequently resulting in intimal proliferation. Adopting this view, Jores also challenged the distinction between superficial (primary degeneration) and deep lipid deposition (related to endarteritis chronica), as introduced by Virchow. It is from this time that Virchow's concept of arteriosclerosis as an inflammatory disease was gradually challenged, and eventually abandoned.

Aschoff, a contemporary of Jores, and his pupils, largely endorsed the opinions of Jores. They looked upon arteriosclerosis as intimal proliferation starting at birth and progressing with age. They described three periods in this process: a first period immediately after birth, characterized by the development of elastin fibers in the intima; a second period during which the histological picture did not change much and very little actually happened; and a third period characterized by the development of a connective tissue layer in the intima. This stage could be complicated by fatty degeneration, leading to a condition that Aschoff called senile sclerosis. According to Aschoff, the cause of this disease, including the presenile and juvenile forms, was "wear and tear" of the elastic inner layers of the artery. Again, this represented a mechanical approach to the pathogenesis of arteriosclerosis. A negative consequence of Aschoff's work was that for a very long time the pathogenesis of arteriosclerosis was considered by many to be an inevitable result of aging.

EARLY ANIMAL EXPERIMENTS: THE ATHEROSCLEROSIS TREE'S BRANCHES

In the early 20th century, several experiments were performed in an attempt to shed light on the development of arteriosclerosis. Of these experiments, those performed by Russian investigators (Ignatowski, Chalarow, and Anitschkow) were the most promising. In a series of publications (1913, 1914, 1922), Anitschkow showed that feeding cholesterol to rabbits (later also guinea pigs) resulted in an accumulation of fat in the intima of the aorta, very much alike that seen in humans.

However, the problem with the initial experiments was that the amount of cholesterol necessary to induce these changes was exceedingly high and could in no way be compared with the situation in humans. In subsequent experiments, Anitschkow was able to show that by raising the blood pressure through ligation of the aorta, the same result could be obtained with much lower doses. He observed a swelling of the subendothelial layer and the appearance of lipid-laden (xanthoma) cells. He concluded that cholesterol from the blood plasma had infiltrated into the vessel wall and proposed that arteriosclerosis resulted from the combined effect of a toxic insult (hypercholesterolemia causing vessel wall damage) and a mechanical insult (hypertension).

Although Anitschkow's hypotheses met with much skepticism—which remains to this very day—his experimental observations nevertheless had a major impact upon our appraisal of the significance of the lipid deposits, which are widely considered as the histological hallmark of arteriosclerosis, and at the same time as an epiphenomenon (secondary degeneration) of the disease process. I believe it is fair to state that the confrontation between the experimental animal studies carried out by Anitschkow and the findings from detailed morphologic studies in humans was instrumental in promoting further studies into the genesis of arteriosclerosis, in particular the role of cholesterol, and that this was much aided by the explosive development of novel laboratory techniques arising hand in hand with the birth of new disciplines (immunology, molecular biology, genetics, etc).

HOW DID IT ALL CONTINUE? THE TREE BEARS ITS FRUITS

Following World War II, the emphasis of atherosclerosis research initially shifted from Western Europe to North America. In this era also some misconceptions originated as to the early pathogenetic concepts promoted by Rokitansky and Virchow (vide supra). Certainly, Rokitansky, as the protagonist of the humoral theory, had proclaimed that the intimal thickening was derived from the blood, but he tried to reconcile the ancient concepts with modern anatomical knowledge. It goes a bit far, therefore, to consider Rokitansky the father of the thrombotic or encrustation theory as envisioned today. The same applies to the contributions regarding the pathogenesis of arteriosclerosis promoted by Virchow. Certainly, Virchow, as the father of cellular pathology, considered arteriosclerosis as a cellular reaction, which at the time was named "inflammation," but which in its initial context had very little to do with the use of this term nowadays. Moreover, Virchow did not really promote the concept that influx of plasma constituents, with subsequent metamorphosis, caused the appearance of lipoid material. And so, it is a misconception to attribute the so-called filtration theory to Virchow. These comments are not intended to devalue the significant contributions by either man, but merely to put the development of our insight into the genesis of atherosclerosis in a correct historic perspective. It is beyond the scope of this review to provide a comprehensive overview of the developments of the past few decades regarding the pathogenesis of atherosclerosis. I have therefore selected a few aspects, which, in my opinion, have had a major impact on our current understanding.

The cellular composition of the atherosclerotic plaque

In the early 1960s, several investigators (eg, Haust, Geer, Wissler) pointed out that smooth muscle cells constituted a major component of the atherosclerotic lesion. The presence of a high proportion of smooth muscle cells in the elastic-hyperplastic intimal layer was also confirmed, justifying the introduction of the term musculoelastic layer. The recognition of smooth muscle cells, rather than fibroblasts, as the principal cell type opened new avenues for the study of the proliferative response underlying plaque formation. The smooth muscle cells were initially thought to originate in the media, which in essence is correct, but, in the setting of atherosclerosis, the musculoelastic layer could well be the main contributor; hence my above comment on the fertile soil. It also appeared that smooth muscle cells could exhibit different phenotypes, ranging from the mature contractile type to the synthetic type. The latter variety refers to a cell poor in contractile elements, but rich in endoplasmic reticulum and capable of producing extracellular matrix components—this is the type of smooth muscle cell involved in proliferative processes. This provided the basis for the understanding of the occurrence of cellular lesions with little collagen as well as of those almost solely composed of collagen.

It was also discovered that the vast majority of lipid-containing cells within the atherosclerotic lesions are macrophages rather than smooth muscle cells. The introduction of immunohistochemical techniques provided a firm footing for this observation while at the same time contributing in a major way to the identification of other cell types. From all this emerged new pathogenetic concepts based on the potential of cell-to-cell interactions. Thus, the identification of lymphocytes led to a breakthrough in our current understanding of the disease, as discussed in the section on intraplaque inflammation. The prominent role of macrophages in the atherosclerotic process became clear following the identification of the scavenger receptors and of the intracellular cholesterol cycle by Brown and Goldstein in 1983. The discovery of oxidatively modified lipoproteins in atherosclerotic lesions led to the hypothesis that uptake via the scavenger receptors resulted in intracellular lipid accumulation and foam cell formation, ultimately contributing to cell death and atheroma formation. It was found that macrophages function not only as scavengers, but also produce enzymes, cytokines, and growth-regulating molecules. Macrophages were shown to derive from blood monocytes, recruited via the expression of endothelial leukocyte adhesion molecules (eg, vascular cell adhesion molecule–1, intercellular adhesion molecule–1, E-selectin), a phenomenon shown in animal studies to be enhanced by hypercholesterolemia. The functional importance of endothelial cells had only recently been shown by Furchgott and Zawadski (1980), revealing the obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. This was the beginning of the understanding that endothelial cell dysfunction could interfere with the delicate balance between the mitogenic and vasomotor effects of endothelial cells and thus upset vascular homeostasis. The notion that endothelial cells do not just line the inner vessel wall, but play a pivotal role in maintaining the proper balance between the blood and tissue compartments, has had a major impact on our current understanding of the pathogenesis of atherosclerosis.

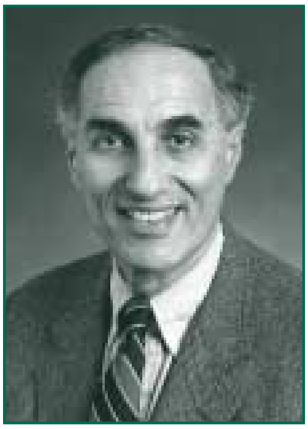

Figure 4. *Russell Ross (1929-1999). Photograph courtesy of the University of Washington.*

The evolution of the response-to-injury concept

In 1974, Russell Ross *(Figure 4)* and coworkers identified, in vitro, a platelet-derived serum factor that stimulated the proliferation of arterial smooth muscle cells. This observation prompted Ross and Glomset in 1976 to propose their response-to-injury theory for the pathogenesis of atherosclerosis. One of the important aspects of this hypothesis is that it linked, for the first time, a specific molecule (platelet-derived growth factor) to atherosclerosis. At the same time, Ross and Glomset considered endothelial denudation a prerequisite, causing platelet adhesion and the release of growth factor *(Figure 5).*

Proliferation of smooth muscle cells then leads to the formation of an intimal lesion. With restoration of the endothelial lining, the lesion could regress, but in case of persistent or repeated injury—eg, due to hypercholesterolemia—the lesion progresses to the classic plaque composed of smooth muscle cells and lipid. In 1986, Ross updated his original response-to-injury concept, recognizing the fact that endothelial cell adhesion and invasion of monocytes were a critical step in the early pathogenesis of atherosclerosis. The concept that the endothelial lining had to be denuded in order to start the response was abandoned and replaced by dysfunction. Despite subsequent refinements, the "response-to-injury" concept as defined by Ross still prevails today.

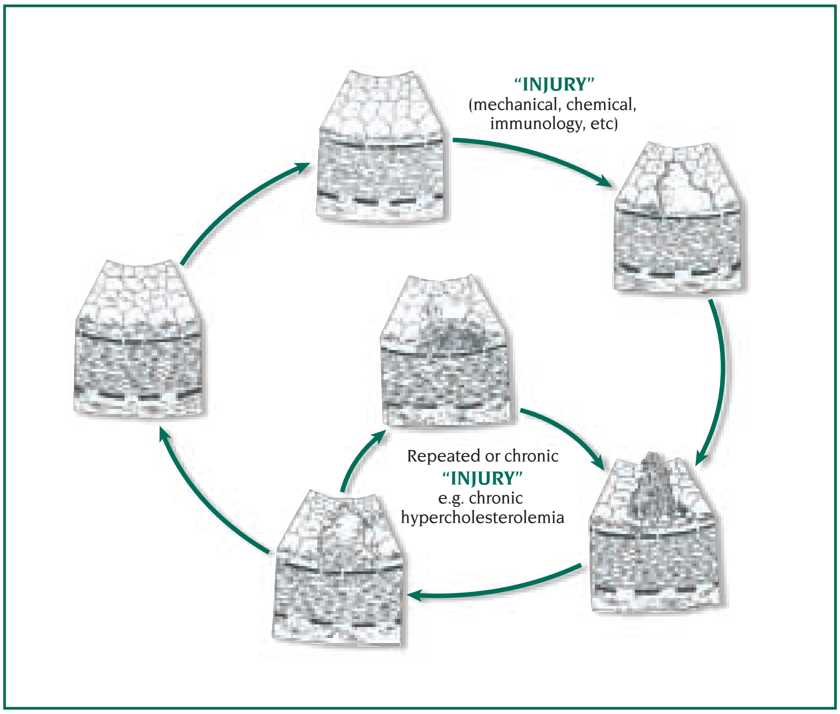

***Figure 5.** The original diagram illustrating the "response-to-injury" concept explaining the genesis of atherosclerosis.*

***Reproduced from:** Ross R, Glomset JA. The pathogenesis of atherosclerosis.* N Engl J Med. *1976; 295:420-425. Copyright © 1976, Massachusetts Medical Society.*

Atherosclerosis: an inflammatory disease

Clinical-pathological studies, in particular the meticulous autopsy-based studies performed by Michael Davies in London, had shown that the vast majority of atherosclerotic plaques that had led to clinical complications (myocardial infarction, stroke, etc) exhibited a particular morphology. These lesions contained a large lipid core—the atheroma—covered on the luminal side by a thin fibrous cap, which often showed fissuring or surface erosion. This morphology was considered unstable (or associated with unstable clinical conditions), which led to the name unstable (or vulnerable) plaque. The clinical significance of these observations was confirmed by the advent of imaging techniques, which identified the large lipid pool in these lesions.

Subsequently, our group was able to show, in 1994, that complications of unstable plaques, such as plaque ruptures and erosions, always occurred at sites where macrophages and lymphocytes predominated, rather than smooth muscle cells. We and others, like Hansson et al in Sweden and Libby et al in the USA, had previously shown the presence of intraplaque inflammation within the atherosclerotic plaque, which most likely resulted from an adaptive immune response. As a result of these and subsequent findings, much research today is devoted to the study of potential antigens responsible for triggering the inflammatory response. These include oxidized low-density lipoprotein (oxLDL), as well as other proinflammatory and immunogenic agents such as stress proteins and infectious agents. The significance of the inflammatory concept is further emphasized by the fact that we and others were able to show, using coronary atherectomy specimens, that patients with unstable clinical conditions (unstable angina, acute myocardial infarction) had significantly more intraplaque inflammation than those with stable conditions. Similarly, patients with acute coronary syndromes exhibit signs of systemic immune activation, revealed by raised plasma levels of inflammatory cytokines and acute-phase reactants, such as C-reactive protein (CRP).

The question can be raised, of course, as to whether atherosclerosis is an adaptive immune response from the very start or whether the immune reaction modifies an already existing disease process. Be that as it may, our current insight—at least from my viewpoint—is best summarized by a quote from Ross' last review, published in January 1999 in the *New England Journal of Medicine,* a few months before his death:

> The lesions of atherosclerosis represent a series of highly specific cellular and molecular responses that can best be described, in aggregate, as an inflammatory disease.

FURTHER READING

Becker AE, de Boer OJ, van der Wal AC.
The role of inflammation and infection in coronary artery disease.
Annu Rev Med. 2001;52:289-297.

Brown MS, Goldstein JL.
A receptor-mediated pathway for cholesterol homeostasis.
Science. 1986;232:34-47.

Brown MS, Goldstein JL.
Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis.
Ann Rev Biochem. 1983;52: 223-261.

Davies MJ.
The birth, growth, and consequences of the atherosclerotic plaque.
Dialogues Cardiovasc Med. 1999;4: 115-130.

Hansson GK, Holm J, Jonasson L.
Localisation of T lymphocytes and macrophages in fibrous and complicated human atherosclerotic plaques.
Atherosclerosis. 1988;72:135-141.

Hansson GK.
Cell-mediated immunity in atherosclerosis.
Curr Opin Lipidol. 1997;8:301-311.

Jores L. Arterien.
In: Henke und Lubarsch, eds.
Handbuch der speziellen Pathologischen Anatomie und Histologie. Zweiter Band. Herz und Gefässe. Berlin, Germany: Verlag von Julius Springer; 1924.

Libby P.
Inflammation: a common pathway in cardiovascular diseases.
Dialogues Cardiovasc Med. 2003;8: 59-73.

Libby P, Hansson GK.
Involvement of the immune system in human atherogenesis: current knowledge and unanswered questions.
Lab Invest. 1991;64:5-15.

Ross R, Glomset JA.
The pathogenesis of atherosclerosis.
N Engl J Med. 1976;295:420-425.

Ross R.
Atherosclerosis—an inflammatory disease.
N Engl J Med. 1999;340:115-126.

Ross R.
The pathogenesis of atherosclerosis: a perspective for the 1990's.
Nature. 1993;362:801-809.

Ross R.
The pathogenesis of atherosclerosis—an update.
N Engl J Med. 1986;314:488-491.

van der Wal AC, Becker AE, van der Loos CM, et al.
Fibrous and lipid-rich plaques plaques are part of interchangeable morphologies related to inflammation: a concept.
Coron Artery Dis. 1994;5:463-469.

van der Wal AC, Becker AE, van der Loos CM, et al.
Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterised by an inflammatory process irrespective of the dominant plaque morphology.
Circulation. 1994;89:36-44.

van der Wal AC, Das PK, Bentz van de Berg D, et al.
Atherosclerotic lesions in man. In situ immunophenotypic analysis suggesting an immune mediated response.
Lab Invest. 1989;61:166-170.