Available online at www.annclinlabsci.org

Review and Hypothesis: Vulnerable Plaque Formation from Obstruction of *Vasa Vasorum* by Homocysteinylated and Oxidized Lipoprotein Aggregates Complexed with Microbial Remnants and LDL Autoantibodies

Uffe Ravnskov[1] and Kilmer S. McCully[2,3]
[1]Independent Investigator, Magle Stora Kyrkogata 9, 22350 Lund, Sweden; [2]Pathology and Laboratory Medicine Service, Boston Veterans Affairs Healthcare System, West Roxbury, MA; [3]Department of Pathology, Harvard Medical School, Boston MA, USA.

Abstract. Little attention has been paid to the function of lipoproteins as part of a nonspecific immune defense system that binds and inactivates microbes and their toxins effectively by complex formation. Because of high extra-capillary tissue pressure, aggregates of such complexes may be trapped in *vasa vasorum* of the major arteries. This complex formation and aggregation may be enhanced by hyperhomocysteinemia, because homocysteine thiolactone reacts with the free amino groups of apo-B to form homocysteinylated low-density lipoprotein (LDL), which is subject to spontaneous precipitation in vitro. Obstruction of the circulation in *vasa vasorum*, caused by the aggregated complexes, may result in local ischemia in the arterial wall, intramural cell death, bursting of the capillary, and escape of microorganisms into the intima, all of which lead to inflammation and creation of vulnerable plaques. The presence of homocysteinylated LDL and oxidized LDL stimulates production of LDL autoantibodies, which may start a vicious circle by increasing the complex formation and aggregation of lipoproteins. The content of necrotic debris and leukocytes and the higher temperature than its surroundings give the vulnerable plaque some characteristics of a micro-abscess that by rupturing may initiate an occluding thrombosis. This suggested chain of events explains why many of the clinical symptoms and laboratory findings in acute myocardial infarction are similar to those seen in infectious diseases. It explains the presence of microorganisms in atherosclerotic plaques and why bacteriemia and sepsis are often seen in myocardial infarction complicated with cardiogenic shock. It explains the many associations between infections and cardiovascular disease. And it explains why cholesterol accumulates in the arterial wall. Some risk factors may not cause vascular disease directly, but they may impair the immune system, promote microbial growth, or cause hyperhomocysteinemia, leading to vulnerable plaques.

Keywords: vulnerable plaque, lipoprotein aggregates, *vasa vasorum*, hyperhomocysteinemia, microbial remnants, autoimmunity, oxidized LDL

Introduction. There is general agreement that atherosclerosis begins as an inflammatory process in the arterial wall, and also that rupture of a vulnerable plaque is the starting point for the creation of the occluding thrombus in myocardial infarction and ischemic stroke [1,2]. Therefore, any hypothesis about the cause of atherosclerosis and its consequences must necessarily be able to point to the origin of the inflammation and to explain how a vulnerable plaque is created [3].

According to the current view, the first step is endothelial dysfunction or damage caused by hypercholesterolemia, hyperhomocysteinemia, or other toxic factors in the circulation, allowing the migration of LDL, cholesterol, and monocytes into the arterial wall. LDL is modified by oxidation,

Address correspondence to Kilmer S. McCully, M.D., Veterans Affairs Medical Center, West Roxbury, MA 02132, USA; tel 857 203 5990; fax 857 203 5623; email kilmer.mccully@med.va.gov.

leading to an accumulation of T-cells and the production of LDL autoantibodies. Modified LDL is taken up by macrophages that are converted to lipid-laden foam cells, considered as the early lesion of atherosclerosis. The inflammatory process, probably aggravated by antigens from microbes such as *Chlamydia, Herpes simplex* and *Cytomegalovirus*, is followed by smooth muscle cell proliferation and the synthesis of extracellular matrix. The macrophages may become overloaded with lipids and die, resulting in the creation of a vulnerable plaque that by rupturing initiates the formation of an occluding thrombus [4].

This suggested chain of events is based mainly on epidemiological observations and experimental models, where vascular changes similar to human atherosclerosis have been produced in rodents with inherited or dietary hypercholesterolemia. However, it conflicts with many clinical, epidemiological, pathological, and experimental observations. There are in particular six disturbing facts:

1. The concept that high LDL cholesterol causes endothelial dysfunction is unlikely because there is no association between the concentration of LDL cholesterol in the blood and the degree of endothelial dysfunction [5].
2. The concept that endothelial damage leads to influx of LDL cholesterol is unlikely as well, because the atherosclerotic plaques seen in extreme hyperhomocysteinemia caused by inborn errors of methionine metabolism do not contain any lipids in spite of pronounced endothelial damage [6,7].
3. No study of unselected individuals has found an association between the concentration of LDL or total cholesterol in the blood and the degree of atherosclerosis at autopsy [8].
4. In studies of women and the elderly, hypercholesterolemia is a weak risk factor for cardiovascular disease, or, in most cases, not a risk factor at all [9], although the large majority of cardiovascular deaths occur in people above 65 years of age.
5. Among individuals with familial hypercholesterolemia (FH) there is no association between LDL-cholesterol and the prevalence or the progress of cardiovascular disease [10-15]. The higher coronary mortality in young people with FH may instead be due to inherited abnormalities of the coagulation system, often seen in FH and a strong risk factor for coronary heart disease in this population [15,16].
6. With one exception [17], an occluding coronary thrombus has never been produced experimentally in rodents by hypercholesterolemia alone [3], indicating that the pathological process in these models may differ from that in human beings.

Origin of vulnerable plaques. In the following discussion we present a new interpretation of the origin of vulnerable plaques that we think is in better agreement with presently available evidence. This interpretation is based on the fact that the lipoproteins function as a nonspecific immune system that binds and inactivates microorganisms and their toxins by complex formation. In the case of a massive microbial invasion, these complexes may aggregate, in particular in the presence of hyperhomocysteinemia, because homocysteine thiolactone causes aggregation and precipitation of thiolated LDL [18]. Complex formation and aggregation may also be enhanced by autoantibodies against thiolated LDL and oxidized LDL. Because of high extra-capillary tissue pressure, the aggregates may be trapped in arterial *vasa vasorum*, resulting in local vascular ischemia, intramural cell death, and the creation of vulnerable plaques.

Such plaques have many characteristics of a micro-abscess, which, by rupturing, initiates the occluding thrombosis and releases its content of infectious material into the circulation and the myocardium. This suggested chain of events explains why many of the clinical symptoms and laboratory findings in acute myocardial infarction are similar to those seen in infectious diseases. It also explains the frequent presence of microbial remnants in atherosclerotic plaques, the many associations between infections and cardiovascular disease, the similarities between myocarditis and myocardial infarction, and why cholesterol accumulates in the arterial wall.

The microbial hypothesis. A century ago, bacteria and viruses were considered as the main cause of atherosclerosis, a view that was based mainly on post-mortem observations. Thus, Thayer reported a high frequency of arterial lesions in patients who died from typhoid fever and a high prevalence of

hardened radial arteries in those who survived [19]. Wiesel found an association between the degree of atherosclerosis in people who had died from an infectious disease and the length of the preceding infection [20], and Osler described the vulnerable plaque as an atherosclerotic pustule [21]. The following statement by Klotz and Manning is typical for the general view at that time: "There is every indication that the production of tissue in the intima is the result of a direct irritation of that tissue by the presence of infection or toxins" [22]. The molecular mechanisms were unknown and because of the chemical composition of advanced atherosclerotic plaques, more recent research has instead focused on cholesterol.

However, in addition to and in accordance with the older findings, much epidemiological, clinical, laboratory, and experimental evidence has more recently been reported, suggesting that infectious processes may play a role in cardiovascular disease [23-27]. Cardiovascular mortality increases during influenza epidemics [28]. A third of patients with acute myocardial infarction or stroke have had an infectious disease immediately before onset [29]. Bacteriemia and periodontal infections are associated with an increased risk of cardiovascular disease [30,31]. Serological markers of infection are often elevated in patients with cardiovascular disease and are also risk factors for such diseases [32]. A role of infectious agents is suggested by the narrowing of the coronary arteries seen in children who died from an infectious disease [33] and from thickening of carotid intima-media on high-resolution ultrasound in those who survived [34].

The lipoprotein immune system. A normal serum factor is able to neutralize the hemolytic effects of streptolysin-S, and, for this reason, the factor was named antistreptolysin-S and was previously considered to be an antibody. However, this concept was questioned in 1939 by Todd et al, who found that this serum factor did not behave as a normal antibody because its titer fell below normal values in patients with rheumatic fever at the peak of the clinical symptoms [35]. A few years later, Stollerman and Bernheimer also found that, in contrast to the antistreptococcal antibodies, the antistreptolysin-S titer did not rise above its normal level during convalescence [36]. At the same time, Humphrey discovered that antistreptolysin-S was located within the lipid fraction of the blood [37]. Stollerman et al identified antistreptolysin-S as a phospholipoprotein complex [38]. Since then, at least a dozen research groups have established that antistreptolysin-S is identical with the lipoproteins and constitutes a nonspecific host defense system, able to bind and inactivate not only streptolysin-S, but also other endotoxins and several virus species [39-55] (Table 1). In rodents, cholesterol is mostly transported by high-density lipoprotein (HDL), and in these species HDL has the main protective effect [42,43], whereas human studies have generally found that all lipoproteins participate in the nonspecific defense system.

Most investigators have identified the immunoprotective role of the lipoproteins by demonstrating inhibition of the biological effects of various microorganisms and endotoxins, such as hemagglutination, hemolysis, the cytokine response of human monocytes, and virus replication. Skarnes first suggested that the lipoproteins also form complexes with microbial products [39]. By using immunodiffusion with anti-endotoxin and serum from various rodents that had been injected with *Salmonella enteridis* endotoxin, he demonstrated lipoprotein-positive staining and esterase activity on the precipitation lines.

Using crossed immunoelectrophoresis, Freudenberg et al found that the HDL peak of rat plasma changed position after injection with various lipopolysaccharides (LPS); they concluded that the effect was due to the formation of a complex between LPS and HDL [42]. By separating a mixture of rabbit plasma and LPS from *Salmonella minnesota* by column chromatography with sepharose linked with LPS antibody, Ulevitch et al found that the eluate from the bound material contained both LPS and apoprotein A1, the major protein of rabbit HDL [43]. There is strong evidence that human lipoproteins complex with microbial components as well. By electron microscopy (EM) Bhakdi et al found that the inactivation of *Staphylococcus aureus* alpha-toxin by purified human LDL led to oligomerization of 3S native toxin molecules into ring structures of 11S hexamers that adhered to the LDL molecules [44].

Table 1. Binding of microbial products by lipoproteins.

Ref.	Microbial product	LDL	HDL	VLDL	All lipoproteins	Source of lipo-proteins	Methods used to demonstrate inactivation and/or binding of the microbial products by the lipoproteins
37	Streptolysin S				++	human	Inhibition of streptolysin S
38	Streptolysin S	++	++			human	Inhibition of streptolysin S
39	LPS; *S. enteritides*				++	rodents	Immunodiffusion
40	*Togavirus*	++	+	+++		human	Inhibition of hemagglutination
41	*S. aureus* δ-hemolysin	++	++			human	Inhibition of δ-hemolysin
42	*S. abortus equi;*		++			rat	Crossed
	S. minnesota	0	++	0		rat	immunoelectrophoresis
43	LPS; *S. minnesota*	0	++	0		rabbit	Binding of LPS to apoA1
44	*S. aureus* α-toxin	++	0			human	Hemolytic titration; EM
45	*Rhabdovirus*	++	(+)	++		human	Inhibition of hemagglutination
46	LPS; *E. coli*	++	++	++		human, rabbit	Inhibition of scavenger receptor
47	*Herpes simplex*	++	++	++		human	EM
48	LPS; *E. coli*				++	human	Inhibition of endotoxin activation of human monocytes
49	LPS; *E. coli*	++	+	++		rabbit	Inhibition of cytokine-response of human monocytes
50	LPS (?)	++	++	0		human	Inhibition of cytokine-response of human monocytes
51	SA *Rotavirus*	++	++	++		human	Inhibition of viral hemagglutination and replication; EM
52	LPS; *S. typhi*	++				human	Inhibition of endotoxin production
53	LPS; *S. typhi*	++	(+)	0		human	Inhibition of endotoxin production
54	LPS; *E. coli*				++	human	Endotoxin sensitivity
55	LPS; *E. coli*				++	mouse	LD_{50} after experimental infection

A semiquantitative review presents the binding and inhibitory effects of low-density (LDL), high-density (HDL), and very low-density (VLDL) lipoprotein on various microbes and bacterial toxins. In 5 studies the total effects of all lipoproteins together were examined. Abbreviations: electron microscopy (EM); lethal dose 50% (LD_{50}); lipopolysaccharide (LPS); apolipoprotein A1 of high-density lipoprotein (ApoA1).

Lipoproteins also form complexes with viruses. Huemer et al found that all lipoprotein subclasses were able to bind purified *Herpes simplex* virus, as demonstrated by EM, enzyme-linked immuno-absorbence assay, and column chromatography [47]. Superti et al confirmed that all human subclasses of lipoproteins were able to inhibit the infectivity and hemagglutination by SA-11 rota-virus, and complex formation was visualized by EM [51].

The lipoprotein immune system may be particularly important in early childhood as, in contrast to antibody-producing cells, this system works immediately and with high efficiency. For instance, human LDL inactivated up to 90% of *Staphylococcus aureus* alpha-toxin [44], and it inactivated an even larger fraction of bacterial lipopolysaccharide (LPS) [48]. In agreement with these findings, hypocholesterolemic rats injected with LPS had a markedly increased mortality compared with normal rats, which could be ameliorated by injecting purified human LDL [54]. On the other hand, hypercholesterolemic mice challenged with LPS or live bacteria had an eightfold increase of LD_{50}, compared with normal mice [55].

Hudgins et al demonstrated that high-molecular weight lipoproteins not only bind LPS, but lipoproteins disappear from the general circulation in infected human beings [56]. They injected a small dose of LPS in normal volunteers and demonstrated the expected rise of the usual inflammatory markers and a fall of total cholesterol, LDL-cholesterol and apo-B, whereas concentrations of HDL-cholesterol and apo-A1 were unchanged.

The formation of complexes between lipoproteins and microbial products may lead to aggregation of lipoprotein particles. In case of a

massive invasion of microorganisms, the size of such aggregates, especially those composed of the high-molecular weight VLDL and LDL, may impede their passage through capillary networks, in particular the *vasa vasorum* of the artery walls, because of high extra-capillary tissue pressure. Indeed, aggregated lipid structures similar to the size of LDL have been demonstrated by electron microscopy in the extracellular space beneath fatty streaks [57].

Recent reviews [58,59] summarized the evidence that both LPS and lipoteichoic acid (the Gram-positive counterpart of LPS) form aggregates in solution. In addition, sphingolipids interact with bacterial toxins, and all lipoproteins isolated from animals treated with LPS contain high levels of sphingolipids (ceramide), which promote lipoprotein aggregation.

An unsettled question concerns the nature of the process that converts macrophages into lipid-laden foam cells, one of the main factors in production of atherosclerotic lesions. Normally excess cellular uptake of cholesterol is counteracted by down-regulation of the LDL receptor, indicating that another pathway must be responsible for foam cell formation. According to the current view, oxidized LDL cholesterol in the arterial wall is taken up by the scavenger receptor of macrophages, allowing an unlimited uptake of cholesterol, independent of the LDL receptor. However, macrophages also take up aggregated LDL by phagocytosis after modification by vortexing or by digestion with phospholipase C [60]. LDL that is modified by complex binding with microbial products is also taken up by the same process, because in vitro experiments have shown that LPS from *Chlamydia pneumoniae* [61] and also from several periodontal pathogens [62] is able to convert macrophages to foam cells in the presence of human LDL.

A direct attack of microorganisms or their products on the endothelium, as often suggested, seems unlikely, as demonstrated by Madjid et al [63]. In a post-mortem study of 27 patients with coronary atherosclerosis, 14 of whom had had a systemic infection within two weeks before death, luminal coronary thromboses and myocardial infarction were found in 5 of the infected patients. They found that the number of macrophages in the infected group was much greater in the adventitia than around the plaques, whereas no difference was noted in the uninfected control group, which suggests that the microbes arrive via *vasa vasorum*. In agreement with this view, Guyton et al found that extracellular lipid deposits are almost entirely located deep within the intima, close to the *vasa vasorum* and well below most of the foam cell lipid [57]. This finding opposes the view that the lipid-rich core region of plaques originates primarily from the debris of dead intimal foam cells, but the finding agrees with the spontaneous atherothrombosis observed in genetic double knockout mice [64]. These thrombi were demonstrated on the surface of atherosclerotic lesions similar to human vulnerable plaques, accompanied by marked medial degeneration and invasion of inflammatory cells into the adventitia.

During the oxidative breakdown of microbial material inside macrophages, cholesterol is partially oxidized and returned to the liver by HDL, and the cholesterol content of fibrous plaques is not higher than in normal arterial tissue [65]. Indeed, several HDL processes that are able to convert oxidized LDL cholesterol to free cholesterol have been identified [66]. Also, esterified cholesterol may be converted to free cholesterol by microbial processes [67] and deposited as extracellular cholesterol crystals found deep within the intima [57].

Hyperhomocysteinemia and autoimmunity. Homocysteine thiolactone, the reactive cyclic anhydride of homocysteine, reacts with free amino groups of protein to form peptide-bound homocysteine [68]. The process of homocysteinylation of proteins is termed thiolation, because this reaction produces a free sulfhydryl group within the peptide-bound homocysteine molecule. Homocysteine thiolactone reacts with the free amino groups of apoB protein of LDL [69]. When an increased concentration of homocysteine thiolactone reacts with human LDL, the thiolated LDL becomes aggregated and subject to spontaneous precipitation [18]. LDL aggregates are phagocytosed by cultured human macrophages, forming foam cells with greatly increased cholesterol and cholesterol ester content.

It was suggested [18] that thiolation of LDL would also alter its antigenic properties and lead to autoantibody formation. Ferguson et al showed that thiolated LDL is immunogenic in rabbits, producing a polyclonal antibody recognizing thiolated LDL [70]. Antibodies to N-thiolated serum albumin were demonstrated in patients with coronary heart disease [71,72]. Thiolated LDL is present in human serum at low concentration (0.04-0.1%), but autoantibodies to human thiolated LDL have not been reported [73].

The possibility that autoantibodies against thiolated LDL may play a role in the creation of atherosclerosis is suggested by other observations. Hyperhomocysteinemia, a potent risk factor for atherosclerosis, is found in autoimmune diseases, such as lupus erythematosus, rheumatoid arthritis, Behcet's disease, inflammatory bowel disease, and myelodysplastic syndrome [74]. These diseases all are characterized by increased susceptibility to vascular disease and activation of immunity and inflammation. Homocysteine activates cytokines and pro-inflammatory molecules, such as IL-1beta, IL-6, IL-12, IL-18, IL-1 receptor antagonist, C-reactive protein (CRP), adhesion molecules (P-selectin, E-selectin, ICAM-1), and metallo-proteinases (MMP-9). Homocysteine up-regulates reactive oxygen species, leading to NF-kappaB activation [74]. CRP binds oxidized LDL and oxidized phospholipids, enhancing phagocytosis to form foam cells [75].

Oxidized LDL and autoimmunity. Oxidized LDL (OxLDL) has long been considered as the main culprit in atherosclerosis. OxLDL stimulates the production of autoantibodies, but the role of anti-OxLDL has been controversial because its titer does not reflect or predict cardiovascular disease [76-80]. We envision that anti-OxLDL antibodies may aggregate and participate in the obstruction of *vasa vasorum*. Therefore, the reason the titer of anti-OxLDL does not reflect cardiovascular disease may be that the expected increased level of anti-OxLDL in patients with cardiovascular disease is counteracted by a decrease in anti-OxLDL level because of the accumulation and aggregation of circulating anti-OxLDL within *vasa vasorum* of arteries. In support of this concept, Schumacher et al found that patients with acute myocardial infarction and a marked elevation of plasma creatine kinase had a significant decrease of anti-OxLDL during the acute phase, whereas this phenomenon was not seen in patients with only a minor elevation of creatine kinase [81]. Su et al found an inverse association between the concentration of anti-OxLDL and progress of atherosclerosis in hypertensive patients, measured as change of the maximum carotid intima-media thickness, suggesting that anti-OxLDL is protective against atherogenesis [82]. This interpretation may be correct in healthy, non-infected people without hyperhomocysteinemia. However, the association may also be explained by the disappearance from the circulation of anti-OxLDL immune complexes by their aggregation with LDL within *vasa vasorum*, because the association was significant for IgM subclasses only, and the much larger size of such complexes may render them more susceptible to aggregation. This interpretation may also explain the recent finding that low levels of IgM antibodies against phosphorylcholine, a component of inflammatory phospholipids known to cause OxLDL-related immune reactions, are associated with a greater risk of ischemic stroke [83].

Creation of the vulnerable plaque. Obstruction of the *vasa vasorum* by aggregated lipoprotein complexes may increase the vulnerability of the cells that they nourish and lead to cell death because of localized ischemia of the vascular wall. *Vasa vasorum* may rupture, and the aggregated LDL particles with their load of microbial products will enter the arterial wall. These products may include living microorganisms, because viable *Chlamydia pneumoniae* have been cultured from atherosclerotic plaques by Ramirez [84] and Jackson et al [85]. Probably this is a common phenomenon, because Maass et al identified viable *Chlamydia pneumoniae* in 11 of 70 atheromas, whereas none was present in 17 non-atherosclerotic control samples [86]. The presence of *Chlamydia pneumoniae* in human coronary plaques was confirmed by electron microscopy [87,88]. These organisms were also demonstrated within adventitia by immunohisto-chemical staining and polymerase chain reaction (PCR) for microbial DNA, presumably arriving via

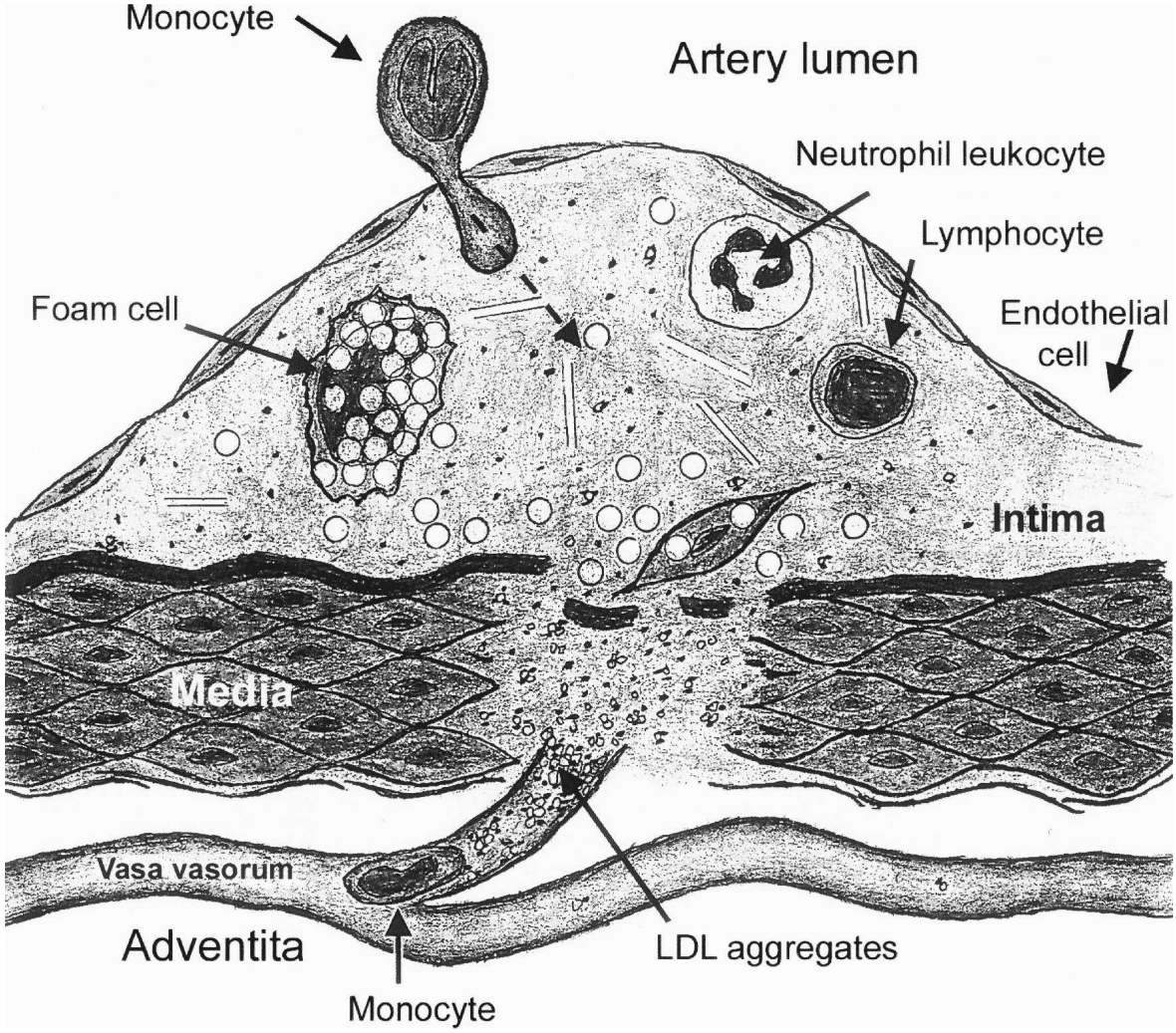

Fig. 1. Development of the vulnerable plaque. The small globules inside the *vasa vasorum* and in the vulnerable plaque represent lipoproteins; the black dots represent microorganisms, endotoxins, anti-OxLDL autoantibodies, and anti-thiolated-LDL autoantibodies; the large globules at the basal part of the vulnerable plaque and inside the macrophages represent lipid droplets. The right capillary represents the situation in a normal healthy artery; there are only a few microbes and the lipoproteins are able to traverse the capillary lumen without adherence or obstruction. The left capillary represents the situation in an artery with a severe microbial invasion; microbial products and autoantibodies stick to the lipoproteins, which aggregate and obstruct the capillary lumen, leading to local ischemia, microbial growth, and inflammation. A monocyte enters the plaque from the arterial lumen by diapedesis between endothelial cells; another monocyte enters the plaque via *vasa vasorum*, leading to formation of foam cell macrophages within the plaque. In the case of an intact immune system, the inflammatory area heals and becomes converted to a fibrous plaque. In the case of an insufficient immune system, microorganisms escape into the tissue and create a microabscess, the vulnerable plaque.

monocytes migrating from *vasa vasorum* [89]. Other living microorganisms may be present as well, but to our knowledge no successful isolations from human plaques have been reported.

Indirect evidence of a role of living microorganisms in the creation of vulnerable plaques was presented by Grattan et al [90]. They found graft failure because of accelerated atherosclerosis in two-thirds of 91 cardiac transplant patients infected with *Cytomegalovirus*, but only in one-third of 209 non-infected patients.

With a healthy immune system, the microorganisms may be eliminated, new capillaries will enter the lesion, and reparative processes will convert the dead tissue into a stable, fibrous plaque. But in case of an insufficient clearing of the

Flow chart for development of the vulnerable plaque

Microorganisms and spores continually invade the body through the airways, skin and gastro-intestinal system, and some of them or their toxic products are bound and inactivated by complex formation with lipoproteins. In the case of a major microbial invasion, the complexes may aggregate. Hyperhomocysteinemia may increase the complex formation and aggregation through thiolation of LDL. Autoantibodies against oxidized and thiolated LDL, aggregated LPS, and lipoteichoic acid, and complexes between sphingolipids and bacterial toxins may further increase the size of the accumulated lipoprotein complexes. Because of their size and because of high extra-capillary tissue pressure, the aggregated complexes are trapped within *vasa vasorum* of the major arteries.

↓

Monocytes entering either via the endothelium or via *vasa vasorum* are converted to macrophages, which take up the aggregates by phagocytosis, forming foam cells.

↓

Foam cells

Normal immune system

The foam cells probably move back to the circulation. Before re-entering the arterial lumen, they are seen as fatty streaks just beneath the endothelium. The micro-organisms and their products are destroyed inside the macrophages by oxidation. By this process both cholesterol and LDL are oxidized as well. In case of a massive microbial invasion, some of the foam cells may die, but more macrophages arrive, new capillaries are formed, and the surrounding tissue is strengthened by proliferation of smooth muscle cells and fibrous tissue.

↓

Stable, fibrous plaque

Disturbed immune system

Vasa vasorum become obstructed, leading to ischemia of the arterial wall, foam cell death, and release of their content into artery wall. Microorganisms and endotoxins enter the dead tissue, the capillaries are damaged, erythrocyte extravasation may occur, and a micro abscess is created.

↓

Vulnerable plaque

↓

The vulnerable plaque ruptures. Cholesterol and microbial products are emptied into the coronary artery and transported to the heart and the general circulation.

↓

A thrombus is built at the margins of the burst plaque.

Partial occlusion — ↓ — **Unstable angina**

Total occlusion — ↓ — **Myocardial infarction**

microorganisms and the ensuing inflammatory response, cell death may accelerate and impede repair processes, creating a vulnerable plaque, the preferential site for occluding thrombi [91]. The suggested chain of events is illustrated in Fig. 1 and in a flow chart (page 10).

Clinical and pathological observations. According to our hypothesis, LDL-cholesterol does not enter the artery through the endothelium as suggested previously, but via the capillary web of *vasa vasorum* in and around the arterial walls. Oxidation of LDL does not take place before LDL has entered the macrophage but occurs after phagocytosis, as part of a normal physiological process explaining why attempts to prevent cardiovascular disease by antioxidants have been largely unsuccessful.

Some reasons for considering the vulnerable plaque to be a type of micro-abscess are that more than one plaque may occur simultaneously [91,92], and their temperature is higher than that of the surrounding tissue [93]. Whereas neutrophilic polymorphonuclear leukocytes, the hallmark of pyogenic infections, are rare in stable plaques, they are always found in and around the core of vulnerable plaques, and there are just as many neutrophils in the intact as in the ruptured plaques [94], contradicting the assumption that their presence is secondary to rupture.

Our interpretation explains the clinical and laboratory similarities between myocardial infarction and myocarditis [95], and it explains the frequent occurrence of bacteriemia and sepsis in myocardial infarction complicated with cardiogenic shock [96]. It explains why fever, diaphoresis, leukocytosis and elevation of inflammatory markers in the blood, including CRP, the classical symptoms of an infectious disease, are common findings in myocardial infarction. Chronic elevation of CRP in patients with atherosclerosis is a risk factor for myocardial infarction. Our interpretation agrees with the almost constant finding of polymorphonuclear leukocytes in the myocardium in acute myocardial infarction, as well as in infarctions of other organs. It also explains a recent report of *Chlamydia pneumoniae* antigens within cardiomyocytes of patients with fatal myocardial infarction [97], an observation needing corroboration from future studies.

Fatty streaks are not necessarily the precursors of atherosclerotic plaques. Fatty streaks are present in the fetus and are more frequent in early than late childhood [98,99], presumably reflecting a normal and reversible response to infections.

Hydrodynamic pressure is usually cited as the reason that atherosclerosis is localized only within systemic arteries. This explanation is probably correct, not because the arterial pressure damages the endothelium, but because the lipoprotein complexes are trapped more easily in *vasa vasorum* of the systemic arteries where the tissue pressure is much higher than within *vasa vasorum* around the veins and the pulmonary arteries. By the same reasoning, atherosclerotic plaques are localized to areas of the intimal surface where the hydrodynamic forces, turbulence of blood flow, and tissue pressure are especially high, namely at the branching points of arteries, within tortuous arteries, and within coronary arteries that are compressed by myocardial contractions. Whereas normal pulmonary arteries are generally free of atherosclerosis, they develop atherosclerotic intimal plaques in various conditions that lead to pulmonary hypertension. Current concepts of the anatomy and physiology of *vasa vasorum* [100] emphasize that these vessels are functionally end arteries, supplying the media to a depth where blood flow and patency are compressed by pressure transmitted from the arterial lumen.

The predilection for plaques within systemic arteries also contradicts the idea that microbes attack the endothelium directly, because if this were so, atherosclerosis would be just as common in veins. Also the focal occurrence of atherosclerotic lesions is in better accordance with a microbial genesis, because if elevated LDL cholesterol were the most important cause, atherosclerosis should be a more generalized disease.

The increased incidence of cardiovascular events found after treatment with rofecoxib and other non-steroidal anti-inflammatory drugs [101] contradicts the idea that atherosclerosis is caused by the inflammation itself, but it is in accord with an infectious origin of atherosclerosis, where inflammation is a necessary step for healing. The ability of HMG-coenzyme A reductase inhibitors

(statins) to prevent cardiovascular disease, in spite of their non-steroidal anti-inflammatory properties, is probably attributable to their other pleiotropic effects, including the enhancement of fibrinolysis and nitric oxide production, and the inhibition of platelet activation.

An apparent contradiction to our interpretation is that prevention of cardiovascular disease by antibiotics has been largely unsuccessful. However, in these trials patients have usually received a single antibiotic, chosen because it was effective against *Chlamydia pneumoniae*, the organism that has been studied most intensively, and the trials have been of relatively short duration.

Chlamydia pneumoniae is not the only microbe that is found in atherosclerotic plaques. Ott et al identified fragments from >50 different microbial species within atherosclerotic plaques, but not a single one in normal arterial tissue [102]. On average, each patient had microbial remnants from 12 different species; some patients had more, some had fewer [102], and other investigators have found various virus species as well [103-105]. It is highly unlikely that a single antibiotic could eliminate >50 different microbial species. It is not even likely that antibiotics could eliminate *Chlamydia pneumoniae*, because this species is able to survive inside living cells, where they are resistant to the effects of antibiotics [106]. Furthermore, antibiotics are generally ineffective against viral infections. Whether the total burden of multiple microbial invasions or the effect of a single pathogen is the key to progression remains to be determined [107].

Evidence that high cholesterol is protective. Since LDL participates in the immune system, high plasma cholesterol concentrations should be an advantage to survival, not a risk. There is much evidence that high cholesterol is protective against infectious diseases. Plasma cholesterol levels have been found to be inversely associated with total mortality in the elderly and with mortality from respiratory and gastrointestinal diseases [9], most of which have an infectious origin. Cholesterol levels are also inversely associated with mortality after post-operative abdominal infections, inversely associated with the risk of being admitted to hospital because of an infectious disease, and inversely associated with the risk of contracting HIV and AIDS [9].

The protective effect of plasma cholesterol levels is also supported by observations in patients with inherited disorders of cholesterol metabolism. Before the year 1900, when infectious disease was the commonest cause of death, the life span of people with 50% risk of having familial hypercholesterolemia (FH) was longer than in the general population [108]. The frequent and severe infections in children with the extremely low cholesterol levels that are found in Smith-Lemli-Opitz syndrome are alleviated by addition of cholesterol to the diet [109].

The lack of an association between the degree of cholesterol lowering and outcome that were found in clinical and angiographic trials [8] could be explained if the benefits from HMG-coenzyme A reductase inhibitors (statins) were due to their pleiotropic effects and not to their inhibition of the cholesterol synthesis. Even if the lowering of LDL cholesterol by these drugs were unimportant, there should have been an exposure-response relationship between LDL-cholesterol and outcome, because both the pleiotropic effects and cholesterol lowering are caused by inhibition of the mevalonate pathway. A more complete blockage of the mevalonate pathway should result in stronger pleiotropic effects and a more pronounced lowering of cholesterol, and vice versa. As this was not the case, the findings imply that high cholesterol is protective and that its lowering therefore counteracts exposure-response. This view is in accordance with the trial findings and our present interpretation of these findings.

Similar events in other arteries. If an imbalance between the microbial burden and the immune system contributes to coronary heart disease, other parts of the artery system should be affected as well, and this seems to be true. Stroke and myocardial infarction commonly occur in the same patient, and vulnerable plaques in the carotid arteries are the starting point of thrombosis in cerebral infarcts [110]. In a consecutive study of the common iliac, common carotid, and renal arteries of 49 patients who died in a hospital, those with a history of cardiovascular events had 2-4 times more intimal macrophages and a denser network of *vasa vasorum*

in all of the arteries than atherosclerotic patients without cardiovascular events [111]. Foam cells have been identified adjacent to Bruch´s membrane of the retina, where their number increases with the age of patients [112]. Foam cells are also found in sclerotic glomeruli [113,114]. In addition, adipose tissue, skin, and muscle specimens from people over age 70 have about 25% more cholesterol than those from people age 30, and tendon specimens have several hundred percent more [115].

Conclusions. Our interpretation of the origin of vulnerable plaques explains the molecular, cellular, and tissue processes resulting in atherosclerosis and cardiovascular disease. Promoting factors may not necessarily act by damaging the arterial wall directly, but rather by inhibiting the immune system, by facilitating microbial growth, by causing hyperhomocysteinemia, and by promoting complex formation and aggregation of homocysteinylated lipoproteins. Our interpretation is in accord with several of the classical risk factors. Hyperhomocysteinemia is found in B vitamin deficiency, smoking, hypertension, hypothyroidism, renal failure, and aging, all classical risk factors for cardiovascular disease [116]. Mental stress, a well-known risk factor for cardiovascular disease, stimulates production of cortisol, and an excess of cortisol, either from Cushing's disease of the adrenal glands or from medical therapy, promotes infections. Furthermore, mental stress, hostility, and anger increase the concentration of homocysteine in blood [117,118], potentially promoting aggregation of LDL particles [18]. Many infectious diseases are more prevalent in smokers and diabetics. The suggestion that excess iron is a risk factor for vascular disease [119] is also in accordance with our interpretation, because bacterial growth is stimulated by the presence of free iron [120]. Therefore, attempts to prevent cardiovascular disease and prolong life may be more successful if we understand the fallacies of the lipid hypothesis [121] and determine what is harmful to the immune system and what may strengthen it.

Our interpretation satisfies Karl Popper's definition of a scientific hypothesis, because it is susceptible to falsification:

1. We anticipate that viable microorganisms and endotoxins in the arterial wall are located within developing vulnerable plaques.
3. We anticipate that arteries of germ-free, normocholesterolemic animals should have fewer foam cells and fatty streaks than their conventionally reared litter mates.
3. A blood culture should be taken in all patients with unstable angina or myocardial infarction, and we anticipate that if it is positive, the course of the disease should be improved with an appropriate antibiotic.

Acknowledgement

We thank Charles F. Foltz, Medical Media Service, VA Medical Center, West Roxbury MA, for assistance in preparing the figure.

References

1. Hansson GK, Nilsson J. Introduction: atherosclerosis as inflammation: a controversial concept becomes accepted. J Int Med 2008;263:462-463.
2. Lusis AJ. Atherosclerosis. Nature 2000;407:233-241.
3. Hansson GK, Heistad DD. Two views on plaque rupture. Arterioscler Thromb Vasc Biol 2007;27:697.
4. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. NEJM 2005;352:1685-1695.
5. Reis SE, Holubkov R, Conrad-Smith AJ, Kelsey SF, Sharaf BL, Reichek N, Rogers WJ, Merz CN, Sopko G, Pepine CJ. Coronary microvascular dysfunction is highly prevalent in women with chest pain in the absence of coronary artery disease: results from the NHLBI WISE study. Am Heart J 2001;141:735-741.
6. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969;56:111-128.
7. McCully KS. Hyperhomocysteinemia and arteriosclerosis: historical perspectives. Clin Chem Lab Med 2005;43:980-986.
8. Ravnskov U. Is atherosclerosis caused by high cholesterol? Q J Med 2002;95:397-403.
9. Ravnskov U. High cholesterol may protect against infections and atherosclerosis. Q J Med 2003;96:927-934.
10. Miettinen TA, Gylling H. Mortality and cholesterol metabolism in familial hypercholesterolemia. Long-term follow-up of 96 patients. Arteriosclerosis 1988;8:163-167.
11. Hopkins PN, Stephenson S, Wu LL, Riley WA, Xin Y, Hunt SE. Evaluation of coronary risk factors in patients with heterozygous familial hypercholesterolemia. Am J Cardiol 2001;87:547-453.
12. Hill JS, Hayden MR, Frohlich J, Pritchard PH. Genetic and environmental factors affecting the incidence of coronary artery disease in heterozygous familial hypercholesterolemia. Arterioscler Thromb 1991;11:290-297.
13. Ferrières J, Lambert J, Lussier-Cacan S, Davignon J. Coronary artery disease in heterozygous familial hypercholesterolemia

patients with the same LDL receptor gene mutation. Circulation 1995;92:290-295.
14. Neil HAW, Seagroatt V, Betteridge DJ, Cooper MP, Durrington PN, Miller JP, Seed M, Naoumova RP, Thompson GR, Huxley R, Humphries SE. Established and emerging coronary risk factors in patients with heterozygous familial hypercholesterolaemia. Heart 2004; 90:1431-1437.
15. Jansen AC, van Aalst-Cohen ES, Tanck MW, Cheng S, Fontecha MR, Li J, Defesche JC, Kastelein JJ. Genetic determinants of cardiovascular disease risk in familial hypercholesterolemia. Arterioscler Thromb Vasc Biol 2005;25:1475-1481.
16. Sugrue DD, Trayner I, Thompson GR, Vere TV, Dimeson J, Stirling Y, Meade TW. Coronary artery disease and haemostatic variables in heterozygous familial hypercholesterolaemia. Br Heart J 1985;53:265-268.
17. Calara F, Silvestre M, Casanada F, Yean N, Napoli C, Palinski W. Spontaneous plaque rupture and secondary thrombosis in apolipoprotein E-deficient and LDL receptor-deficient mice. J Pathol 2001;195:257-263.
18. Naruszewicz M, Mirkiewicz E, Olszewski AJ, McCully KS. Thiolation of low-density lipoprotein by homocysteine thiolactone causes increased aggregation and altered interaction with cultured macrophages. Nutr Metab Cardiovas Dis 1994;4:70-77.
19. Thayer WS. On the cardiac and vascular complications and sequels of typhoid fever. Bull Johns Hopkins Hosp 1904; Oct:323-340.
20. Wiesel J. Die Erkrankungen arterieller Gefässe im Verlaufe akuter Infektionen. II Teil. Z Heilkunde 1906; 27:262-294.
21. Osler W. Diseases of the arteries. In: Modern Medicine: its Practice and Theory (Osler W, Ed), Lea & Fibiger, Philadelphia, 1908; pp 426-447.
22. Klotz O, Manning MF. Fatty streaks in the intima of arteries. J Pathol Bacteriol 1911;16:211-220.
23. Grayston JT, Kuo CC, Campbell LA, Benditt EP. *Chlamydia pneumoniae* strain TWAR and atherosclerosis. Eur Heart J 1993;Suppl K:66-71.
24. Melnick JL, Adam E, Debakey ME. *Cytomegalovirus* and atherosclerosis. Eur Heart J 1993;Suppl K:30-38.
25. Nicholson AC, Hajjar DP. *Herpesvirus* in atherosclerosis and thrombosis. Etiologic agents or ubiquitous bystanders? Arterioscler Thromb Vasc Biol 1998;18:339-348.
26. Ismail A, Khosravi H, Olson H. The role of infection in atherosclerosis and coronary artery disease. A new therapeutic target. Heart Dis 1999;1:233-240.
27. Kuvin JT, Kimmelstiel MD. Infectious causes of atherosclerosis. Am Heart J 1999;137:216-226.
28. Madjid M, Miller CC, Zarubaev VV, Marinich IG, Kiselev OI, Lobzin YV, Filippov AE, Casscells SW. Influenza epidemics and acute respiratory disease activity are associated with a surge in autopsy-confirmed coronary heart disease death: results from 8 years of autopsies in 34,892 subjects. Eur Heart J 2007;28:1205-1210.
29. Smeeth L, Thomas SL, Hall AJ, Hubbard R, Farrington P, Vallance P. Risk of myocardial infarction and stroke after acute infection or vaccination. NEJM 2004;351:2611-2618.
30. Valtonen V, Kuikka A, Syrjanen J. Thrombo-embolic complications in bacteremic infections. Eur Heart J 1993;14Suppl K:20-23.
31. Spahr A, Klein E, Khuseyinova N, Boeckh C, Muche R, Kunze M, Rothenbacher D, Pezeshki G, Hoffmeister A, Koenig W. Periodontal infections and coronary heart disease: role of periodontal bacteria and importance of total pathogen burden in the Coronary Event and Periodontal Disease (CORODONT) study. Arch Intern Med 2006;166:554-549.
32. Espinola-Klein C, Rupprecht HJ, Blankenberg S, Bickel C, Kopp H, Victor A, Hafner G, Prellwitz W, Schlumberger W, Meyer J. Impact of infectious burden on progression of carotid atherosclerosis. Stroke 2002;33:2581-2586.
33. Pesonen E. Infection and intimal thickening: evidence from coronary arteries in children. Eur Heart J 1994;15Suppl C:57-61.
34. Liuba P, Persson J, Luoma J, Yla-Herttuala S, Pesonen E. Acute infections in children are accompanied by oxidative modification of LDL and decrease of HDL cholesterol, and are followed by thickening of carotid intima-media. Eur Heart J 2003;24:515-521.
35. Todd EW, Coburn AF, Hill AB. Antistreptolysin S titres in rheumatic fever. Lancet 1939;2:1213-1217.
36. Stollerman GH, Bernheimer AW. Inhibition of streptolysin S by the serum of patients with rheumatic fever and acute streptococcal pharyngitis. J Clin Invest 1950;29:1147-1155.
37. Humphrey JH. The nature of antistreptolysin S in the sera of man and of other species; the lipoprotein properties of antistreptolysin S. Br J Exp Pathol 1949;30:365-375.
38. Stollerman GH, Bernheimner AW, MacLeod CM. The association of lipoproteins with the inhibition of streptolysin S by serum. J Clin Invest 1950;29:1636-1645.
39. Skarnes RC. In vivo interaction of endotoxin with a plasma lipoprotein having esterase activity. J Bacteriol 1968;95:2031-2034.
40. Shortridge KF, Ho WK, Oya A, Kobayashi M. Studies on the inhibitory activities of human serum lipoproteins for Japanese encephalitis virus. Southeast Asian J Trop Med Public Health 1975;6:461-466.
41. Whitelaw DD, Birkbeck TH. Inhibition of staphylococcal delta-hemolysin by human serum lipoproteins. FEMS Microbiology Letters 1978;3:335-339.
42. Freudenberg MA, Galanos C. Interaction of lipopolysaccharides and lipid A with complement in rats and its relation to endotoxicity. Infect Immun 1978;19:875-882.
43. Ulevitch RJ, Johnston AR, Weinstein DB. New function for high density lipoproteins. Isolation and characterization of a bacterial lipopolysaccharide-high density lipoprotein complex formed in rabbit plasma. J Clin Invest 1981;67:827-837.
44. Bhakdi S, Tranum-Jensen J, Utermann G, Fussle R. Binding and partial inactivation of *Staphylococcus aureus* alpha-toxin by human plasma low density lipoprotein. J Biol Chem 1983;258: 5899-5904.
45. Seganti L, Grassi M, Mastromarino P, Pana A, Superti F, Orsi N. Activity of human serum lipoproteins on the infectivity of rhabdoviruses. Microbiologica 1983;6:91-99.
46. Van Lenten BJ, Fogelman AM, Haberland ME, Edwards PA. The role of lipoproteins and receptor-mediated endocytosis in the transport of bacterial lipopolysaccharide. PNAS USA 1986;83:2704-2708.
47. Huemer HP, Menzel HJ, Potratz D, Brake B, Falke D, Utermann G, Dierich MP. *Herpes simplex* virus binds to human serum lipoprotein. Intervirology 1988;29:68-76.
48. Flegel WA, Wölpl A, Männel DN, Northoff H. Inhibition of endotoxin-induced activation of human monocytes by human lipoproteins. Infect Immun 1989;57:2237-2245.
49. Cavaillon JM, Fitting C, Haeffner-Cavaillon N, Kirsch SJ, Warren HS. Cytokine response by monocytes and macrophages to free and lipoprotein-bound lipopolysaccharide. Infect Immun 1990;58:2375-2382.
50. Northoff H, Flegel WA, Yurttas R, Weinstock C. The role of lipoproteins in inactivation of endotoxin by serum. Beitr Infusionsther 1992;30:195-197.
51. Superti F, Seganti L, Marchetti M, Marziano ML, Orsi N. SA-11 rotavirus binding to human serum lipoproteins. Med Microbiol Immunol 1992;181:77-86.

52. Weinstock C, Ullrich H, Hohe R, Berg A, Baumstark MW, Frey I, Northoff H, Flegel WA. Low density lipoproteins inhibit endotoxin activation of monocytes. Arterioscler Thromb 1992;12:341-347.
53. Flegel WA, Baumstark MW, Weinstock C, Berg A, Northoff H. Prevention of endotoxin-induced monokine release by human low- and high-density lipoproteins and by apolipoprotein A-1. Infect Immun 1993;61:5140-5146.
54. Feingold KR, Funk JL, Moser AH, Shigenaga JK, Rapp JH, Grunfeld C. Role for circulating lipoproteins in protection from endotoxin toxicity. Infect Immun 1995;63:2041-2046.
55. Netea MG, Demacker PNM, Kullberg BJ, Boerman OC, Verschueren I, Stalenhoef AF, van der Meer JW. Low-density lipoprotein receptor-deficient mice are protected against lethal endotoxemia and severe Gram-negative infections. J Clin Invest 1996;97:1366-1372.
56. Hudgins LC, Parker TS, Levine DM, Gordon BR, Saal SD, Jiang XC, Seidman CE, Tremaroli JD, Lai J, Rubin AL. A single intravenous dose of endotoxin rapidly alters serum lipoproteins and lipid transfer proteins in normal volunteers. J Lipid Res 2003;44:1489-1498.
57. Guyton JR, Klemp KF. Transitional features in human atherosclerosis. Intimal thickening, cholesterol clefts, and cell loss in human aortic fatty streaks. Am J Pathol 1993;143:1444-1457.
58. Van Amersfoort ES, Van Berkel TJC, Kuiper J. Receptors, mediators, and mechanisms involved in bacterial sepsis and septic shock. Clin Microbiol Rev 2003;16:379-414.
59. Khovidhunkit W, Kim M-S, Memon RA, Shigenaga JK, Moser AH, Feingold KR, Grunfeld C. Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. J Lipid Res 2004;45:1169-1196.
60. Heinecke JW, Suits AG, Aviram M, Chait A. Phagocytosis of lipase-aggregated low density lipoprotein promotes macrophage foam cell formation. Sequential morphological and biochemical events. Arterioscler Thromb 1991;11:1643-1651.
61. Kalayoglu MV, Indrawati, Morrison RP, Morrison SG, Yuan Y, Byrne GI. Chlamydial virulence determinants in atherogenesis: the role of chlamydial lipopolysaccharide and heat shock protein 60 in macrophage-lipoprotein interactions. J Infect Dis 2000;181Suppl 3:S483-489.
62. Qi M, Miyakawa H, Kuramitsu HK. *Porphyromonas gingivalis* induces murine macrophage foam cell formation. Microb Pathog 2003;35:259-267.
63. Madjid M, Vela D, Khalili-Tabrizi H, Casscells SW, Litovsky S. Systemic infections cause exaggerated local inflammation in atherosclerotic coronary arteries: clues to the triggering effect of acute infections on acute coronary syndromes. Tex Heart Inst J 2007;34:11-18.
64. Welch CL, Sun Y, Arey BJ, Lemaitre V, Sharma N, Ishibashi M, Sayers S, Li R, Gorelik A, Pleskac N, Collins-Fletcher K, Yasuda Y, Bromme D, D'Armiento JM, Ogltree ML, Tall AR. Spontaneous thrombosis and medial degeneration in Apo3-/-, Npc1-/- mice. Circulation 2007;116:2444-2452.
65. Noble NL, Boucek RJ, Kao KY. Biochemical observations of human atheromatosis: analysis of aortic intima. Circulation 1957;15:366-372.
66. Bonnefont-Rousselot D, Therond P, Beaudeux JL, Peynet J, Legrand A, Delattre J. High density lipoproteins (HDL) and the oxidative hypothesis of atherosclerosis. Clin Chem Lab Med 1999;37:939-948.
67. Fabricant CG, Krook L, Gillespie JH. Virus-induced cholesterol crystals. Science 1973;181:566-567.
68. Benesch R, Benesch RE. Thiolation of proteins. PNAS USA 1958;44:848-853.
69. Vidal M, Sainte-Marie J, Philippot J, Bienvenue A. Thiolation of low-density lipoproteins and their interactions with L2C leukemic lymphocytes. Biochimie 1986;68:723-730.
70. Ferguson E, Parthasarathy S, Joseph J, Kalyanaraman B. Generation and initial characterization of a novel polyclonal antibody directed against homocysteine thiolactone-modified low density lipoprotein. J Lipid Res 1998;39:925-933.
71. Undas A, Jankowski M, Twardowska M, Padjas A, Jakubowski H, Szczeklik A. Antibodies to N-homocysteinylated albumin as a marker for early-onset coronary artery disease in men. Thromb Haemost 2005;93:346-350.
72. Yang X, Gao Y, Zhou J, Yang Y, Wang J, Song L, Liu Y, Xu H, Chen Z, Hui R. Plasma homocysteine thiolactone adducts associated with risk of coronary heart disease. Clin Chim Acta 2006;364:230-234.
73. Perla-Kajan J, Twardowski T, Jakubowski H. Mechanisms of homocysteine toxicity in humans. Amino Acids 2007;32:561-572.
74. Lazzerini PE, Capecchi PL, Selvi E, Lorenzini S, Bisogno S, Galezzi M, Pasini FL. Hyperhomocysteinemia, inflammation and autoimmunity. Autoimmun Rev 2007;6:503-509.
75. Chang MK, Binder CJ, Torzewski M, Witztum JL. C-reactive protein binds to both oxidized LDL and apoptotic cells through recognition of a common ligand: phosphoryl choline of oxidized phospholipids. PNAS USA 2002;99:13043-13048.
76. Uusitupa MI, Niskanen L, Luoma J, Vilja P, Mercuri M, Rauramaa R, Ylä-Herttuala S. Autoantibodies against oxidized LDL do not predict atherosclerotic vascular disease in non-insulin-dependent diabetes mellitus. Arterioscler Thromb Vasc Biol 1996;16:1236-1242.
77. Leinonen JS, Rantalaiho V, Laippala P, Wirta O, Pasternack A, Alho H, Jaakkola O, Ylä-Herttuala S, Koivula T, Lehtimäki T. The level of autoantibodies against oxidized LDL is not associated with the presence of coronary heart disease or diabetic kidney disease in patients with non-insulin-dependent diabetes mellitus. Free Radic Res 1998;29:137-141.
78. Wilson PW, Ben-Yehuda O, McNamara J, Massaro J, Witztum J, Reaven PD. Autoantibodies to oxidized LDL and cardiovascular risk: the Framingham Offspring Study. Atherosclerosis 2006;189:364-368.
79. Mayr M, Kiechl S, Tsimikas S, Miller E, Sheldon J, Willeit J, Witztum JL, Xu Q. Oxidized low-density lipoprotein autoantibodies, chronic infections, and carotid atherosclerosis in a population-based study. J Am Coll Cardiol 2006;47:2436-2443.
80. Tsimikas S, Aikawa M, Miller RJ Jr, Miller ER, Torzewski M, Lentz SR, Bergmark C, Heistad DD, Libby P, Witztum JL. Increased plasma oxidized phospholipid:apolipoprotein B-100 ratio with concomitant depletion of oxidized phospholipids from atherosclerotic lesions after dietary lipid-lowering: a potential biomarker of early atherosclerosis regression. Arterioscler Thromb Vasc Biol 2007;27:175-181.
81. Schumacher M, Eber B, Tatzber F, Kaufmann P, Halwachs G, Fruhwald FM, Zweiker R, Esterbauer H, Klein W. Transient reduction of autoantibodies against oxidized LDL in patients with acute myocardial infarction. Free Radic Biol Med 1995; 18:1087-1091.
82. Su J, Georgiades A, Wu R, Thulin T, de Faire U, Frostegård J. Antibodies of IgM subclass to phosphorylcholine and oxidized LDL are protective factors for atherosclerosis in patients with hypertension. Atherosclerosis 2006;188:160-166.
83. Sjoberg BG, Su J, Dahlbom I, Gronlund H, Wikstrom M, Hedblad B, Berglund G, de Faire U, J, Frostegård. Low levels of IgM antibodies against phosphorylcholine - a potential risk marker for ischemic stroke in men. Atherosclerosis 2007, doi:10.1016/j.atherosclerosis.2008.07.009.
84. Ramirez J. Isolation of *Chlamydia pneumoniae* from the coronary artery of a patient with atherosclerosis. Ann Int Med 1996;125:979-982.

85. Jackson LA, Campbell LA, Kuo CC, Rodriguez DI, Lee A, Grayston JT. Isolation of *Chlamydia pneumoniae* from a carotid endarterectomy specimen. J Infect Dis 1997;176:292-295.
86. Maass M, Bartels C, Engel PM, Mamat U, Sievers HH. Endovascular presence of viable *Chlamydia pneumoniae* is a common phenomenon in coronary artery disease. J Am Coll Cardiol 1998;31:827-837.
87. Kuo C-C, Shor A, Campbell LA, Fukushi H, Patton DL, Grayston JT. Demonstration of *Chlamydia pneumoniae* in atherosclerotic lesions of coronary arteries. J Inf Dis 1993; 167:841-849.
88. Campbell LA, O'Brien ER, Capuccio AL, Kuo C-C, Wang S-P, Stewart D, Patton DL, Cummings PK, Grayston JT. Detection of *Chlamydia pneumoniae* TWAR in human coronary atherectomy tissues. J Inf Dis 1995;172:285-288.
89. Vink A, Pasterkamp G, Poppen M, Schonfeld AH, deKleijn DPV, Roholl PJM, Fontijn J, Plomp S, Borst C. The adventitia of atherosclerotic coronary arteries frequently contains *Chlamydia pneumoniae*. Atherosclerosis 2001;157:117-122.
90. Grattan MT, Moreno-Cabral CE, Starnes VA, Oyer PE, Stinson EB, Shumway NE. *Cytomegalovirus* infection is associated with cardiac allograft rejection and atherosclerosis. JAMA 1989;261:3561-3566.
91. Falk E. Plaque rupture with severe pre-existing stenosis precipitating coronary thrombosis. Characteristics of coronary atherosclerotic plaques underlying fatal occlusive thrombi. Br Heart J 1983;50:127-134.
92. Buffon A, Biasucci LM, Liuzzo G, D'Onofrio G, Crea F, Maseri A. Widespread coronary inflammation in unstable angina. NEJM 2002;347:5-12.
93. Madjid M, Naghavi M, Malik BA, Litovsky S, Willerson JT, Casscells SW. Thermal detection of vulnerable plaque. Am J Cardiol 2002;90:36L-39L.
94. Naruko T, Ueda M, Haze K, van der Wal AC, van der Loos CM, Itoh A, Komatsu R, Ikura Y, Ogami M, Shimada Y, Ehara S, Yoshiyama M, Takeuchi K, Yoshikawa J, Becker AE. Neutrophil infiltration of culprit lesions in acute coronary syndromes. Circulation 2002;106:2894-2900.
95. Costantini M, Tritto C, Licci E, Sticchi G, Capone S, Montiaro A, Bruno A, Nuzzaci G, Picano E. Myocarditis with ST-elevation myocardial infarction presentation in young men. A case series of 11 patients. Int J Cardiol 2005;101:157-158.
96. Kohsaka S, Menon V, Lowe AM, Lange M, Dzavik V, Sleeper LA, Hochman JS; SHOCK Investigators. Systemic inflammatory response syndrome after acute myocardial infarction complicated by cardiogenic shock. Arch Intern Med 2005; 165:1643-1650.
97. Spagnoli LG, Pucci S, Bonanno E, Cassone A, Sesti F, Ciervo A, Mauriello A. Persistent *Chlamydia pneumoniae* infection of cardiomyocytes is correlated with fatal myocardial infarction. Am J Pathol 2007;170:33-42.
98. Stary HC. Macrophages, macrophage foam cells, and eccentric intimal thickening in the coronary arteries of young children. Atherosclerosis 1987;64:91-108.
99. Stary HC. Evolution and progression of atherosclerotic lesions in coronary arteries of children and young adults. Arteriosclerosis 1989;9(1 Suppl):I19-32.
100. Ritman EL, Lerman A. The dynamic vasa vasorum. Cardiovasc Res 2007;75:649-658.
101. Johnsen SP, Larsson H, Tarone RE, McLaughlin JK, Norgard B, Friis S, Sorensen HR. Risk of myocardial infarction among users of rofecoxib, celecoxib, and other NSAIDs: a population-based case-control study. Arch Intern Med 2005;165:978-984.
102. Ott SJ, El Mokhtari NE, Musfeldt M, Hellmig S, Freitag S, Rehman A, Kuhbacher T, Nikolaus S, Namsolleck P, Blaut M, Hampe J, Sahly H, Reinecke A, Haake N, Gunther R, Kruger D, Lins M, Herrmann G, Folsch UR, Simon R, Schreiber S. Detection of diverse bacterial signatures in atherosclerotic lesions of patients with coronary heart disease. Circulation 2006;113:929-937.
103. Melnick JL, Petrie BL, Dreesman GR, Burek J, McCollum CH, DeBakey ME. Cytomegalovirus antigen within human arterial smooth muscle cells. Lancet 1983;2:644-647.
104. Pampou SY, Gnedoy SN, Bystrevskaya VB, Smirnov VN, Chazov EI, Melnick JL, DeBakey ME. *Cytomegalovirus* genome and the immediate-early antigen in cells of different layers of human aorta. Virchows Arch 2000;436:539-552.
105. Shi Y, Tokunaga O. *Chlamydia pneumoniae* and multiple infections in the aorta contribute to atherosclerosis. Pathol Int 2002;52:755-763.
106. Gieffers J, Füllgraf H, Jahn J, Klinger M, Dalhoff K, Katus HA, Solbach W, Maass M. *Chlamydia pneumoniae* infection in circulating human monocytes is refractory to antibiotic treatment. Circulation 2001;103:351-356.
107. Katz JT, Shannon RP. Bacteria and coronary atheroma: more fingerprints but no smoking gun. Circulation 2006;113:920-922.
108. Sijbrands EJ, Westendorp RG, Defesche JC, de Meier PH, Smelt AH, Kastelein JJ. Mortality over two centuries in large pedigree with familial hypercholesterolaemia: family tree mortality study. Brit Med J 2001;322:1019-1023.
109. Elias ER, Irons MB, Hurley AD, Tint GS, Salen G. Clinical effects of cholesterol supplementation in six patients with the Smith-Lemli-Opitz syndrome (SLOS). Am J Med Genet 1997;68:305-310.
110. Yuan C, Mitsumori LM, Beach KW, Maravilla KR. Carotid atherosclerotic plaque: noninvasive MR characterization and identification of vulnerable lesions. Radiology 2001;221:285-299.
111. Fleiner M, Kummer M, Mirlacher M, Sauter G, Cathomas G, Krapf R, Biedermann BC. Arterial neovascularization and inflammation in vulnerable patients: early and late signs of symptomatic atherosclerosis. Circulation 2004;110:2843-2850.
112. Curcio CA, Millican CL, Bailey T, Kruth HS. Accumulation of cholesterol with age in human Bruch's membrane. Invest Ophthalmol Vis Sci 2001;42:265-274.
113. Schonholzer KW, Waldron M, Magil AB. Intraglomerular foam cells and human focal glomerulosclerosis. Nephron 1992; 62:130-136.
114. Lee HS, Kruth HS. Accumulation of cholesterol in the lesions of focal segmental glomerulosclerosis. Nephrology 2003;8:224-230.
115. Crouse JR, Grundy SM, Ahrens EH. Cholesterol distribution in the bulk tissues of man: variation with age. J Clin Invest 1972; 51:1292-1296.
116. McCully KS. Homocysteine, vitamins, and vascular disease prevention. Am J Clin Nutr 2007;86(Suppl):1563S-1568S.
117. Stoney CM. Plasma homocysteine levels increase in women during psychological stress. Life Sciences 1999;64:2359-2365.
118. Stoney CM, Engebretson TO. Plasma homocysteine concentrations are positively associated with hostility and anger. Life Sciences 2000;66:2267-2275.
119. Sullivan JL. The iron paradigm of ischemic heart disease. Am Heart J 1989,117:1177-1188.
120. Bullen JJ, Rogers HJ, Spalding PB, Ward CG. Natural resistance, iron and infection: a challenge for clinical medicine. J Med Microbiol 2006;55:251-258.
121. Ravnskov U. The fallacies of the lipid hypothesis. Scand Cardiovasc J 2008;42:236-239.