Infections May be Causal in the Pathogenesis of Atherosclerosis

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Abstract: There is a universal lack of exposure response between degree of lipid lowering and the outcome in clinical and angiographic trials questioning the current view on atherogenesis. However, there are numerous observations and experiments suggesting that microorganisms may play a causal role. A clue is the fact that the lipoproteins constitute an innate immune system by binding and inactivating microorganisms and their toxic products through formation of circulating complexes. Their size may increase in the presence of hyperhomocysteinemia because homocysteine reacts with low-density lipoprotein (LDL) to form homocysteinylated LDL aggregates. Autoantibodies against homocysteinylated or oxidized LDL may also enhance the aggregation. Because of the high extracapillary pressure, such aggregates may obstruct arterial vasa vasorum producing ischemia and cell death within the arterial wall leading to the creation of a vulnerable plaque. The many epidemiological observations, clinical findings and laboratory experiments that conflict with the cholesterol hypothesis are in good accordance with ours.

Key Indexing Terms: Atherosclerosis; Cholesterol; Lipoproteins; Hypothesis; Infections; Microorganisms; Vulnerable plaque; Homocysteine. **[Am J Med Sci 2012;0(0):1–4.]**

CONTRADICTIONS TO THE CURRENT VIEW

According to the cholesterol hypothesis, atherosclerosis is initiated by endothelial dysfunction caused by hypercholesterolemia, hyperhomocysteinemia or other toxic factors. Endothelial dysfunction is said to allow LDL-cholesterol and monocytes to enter the arterial wall, where LDL-cholesterol becomes oxidized and taken up by the macrophages. These events are considered to cause inflammation and to be the starting point of atherosclerosis. There are obvious contradictions to this interpretation:

1. There is no association between the concentration of LDL cholesterol in the blood and the degree of endothelial dysfunction.[1]
2. The atherosclerotic plaques seen in extreme hyperhomocysteinemia caused by inborn errors of methionine metabolism do not contain any lipids despite pronounced endothelial damage.[2]
3. No autopsy study of unselected, adult individuals has found an association between serum cholesterol and the degree of atherosclerosis.[3] Moreover, there is no association between serum cholesterol and the degree of coronary calcification measured by electron beam tomography.[4] To quote the authors: "There were no significant differences in the calcium scores throughout the entire range of all lipid parameters; calcium percentiles were virtually identical within lipid value subgroups."
4. High cholesterol is not a risk factor for coronary heart disease in women or in old individuals. In fact, more than a dozen studies have found that old people with high cholesterol live the longest.[5–8]
5. According to the 30-year follow-up study from Framingham, both heart and total mortality were the highest among those whose cholesterol had decreased.[9]
6. Trials with anti-inflammatory drugs have demonstrated an increase of cardiovascular death in the treatment groups questioning that inflammation is the inciting cause.[10,11]

According to Karl Popper, the idea that high cholesterol causes atherosclerosis is a true scientific hypothesis because it is falsifiable. As the cited contradictions have effectively falsified the cholesterol hypothesis, there are reasons to consider other ideas.

In a previous article, we presented a new hypothesis suggesting a crucial role of infections.[12] Since its publication, we have identified more studies in the past which are in support, and several new studies have been published presenting data that are in accordance with our hypothesis.

CARDIOVASCULAR DISEASE IS ASSOCIATED WITH INFECTION

Most investigators consider the association between infections and cardiovascular disease as a secondary phenomenon, but by several reasons it may not be that simple. Cardiovascular mortality increases during influenza epidemics, and about a third of patients with acute cardiovascular disease have had an infection immediately before onset.[12] Bacteremia and periodontal infections are associated with an increased risk of cardiovascular disease,[12] and Piconi et al[13] found that treatment of periodontal infections improved endothelial function and reduced the intima-media thickening of the carotid arteries to a much higher degree than seen in any cholesterol-lowering trial. Furthermore, serological markers of infection are increased in patients with cardiovascular disease,[6] and bacteremia and sepsis are found frequently in patients with cardiogenic shock due to myocardial infarction.[12]

One hundred years ago, bacteria and viruses were considered as the main cause of atherosclerosis. The main arguments were the high frequency of arterial lesions in patients who died from typhoid fever and the high prevalence of arteriosclerotic radial arteries in those who survived,[12] and the association between the degree of atherosclerosis in people who had died from an infectious disease and the duration of the preceding infection. Said by Klotz and Manning: "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,"[12] and William Osler described the vulnerable plaque as an atherosclerotic pustule.[12]

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Submitted October 19, 2011; accepted in revised form January 13, 2012.

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The classical study of early atherosclerosis in young American soldiers killed in Korea is frequently cited as proof that atherosclerosis starts in early adulthood.[14] In that study, 77.3% had gross evidence of coronary disease and 15% had more than 50% luminal narrowing. However, such severe changes have never been observed in autopsy studies of young people who have died from other causes. The explanation may be that many of these soldiers had severe, infected wounds before they died. As the author stated, "thrombosis occurred especially in cases in which extensive trauma and shock exerted their influence."

In a postmortem study of several thousand victims in the concentration camp in Dachau, Blaha[15] found extensive atherosclerosis in individuals younger than 35 years. Many had severe infections, and the degree of arteriosclerosis was related to the duration of internment in the camp. Other than severe stress, there was no dietary cholesterol or saturated fat, no smoking, no lack of exercise, no obesity or other risk factors for arteriosclerosis.

Much evidence indicates that atherosclerosis starts in childhood and is associated with infectious diseases. Liuba et al[16] studied infected children by high-resolution ultrasound and found narrowing of the coronary arteries in those who died and thickening of the carotid intima-media layer in those who survived.

EXPERIMENTAL EVIDENCE

Fabricant et al[17] induced visible atherosclerotic changes in chickens by infecting them with Marek's disease herpes virus, and Damy et al[18] worsened atherosclerosis in hypercholesterolemic mice by *Chlamydia pneumoniae* or *Mycoplasma pneumoniae.* Recently, Birck et al[19] produced signs of early atherosclerosis in normo- and hypercholesterolemic minipigs by infecting them with *C pneumoniae*, alone or together with influenza virus. Vascular damage and endothelial dysfunction were most prominent in the coinfected animals but less pronounced in the hypercholesterolemic than in the normocholesterolemic pig, also a contradiction to the current view, but in accordance with our idea that the lipoproteins may be protective due to their antimicrobial properties.

If atherosclerosis is caused by microorganisms, vaccination or antibiotics should be able to prevent cardiovascular disease. Some randomized controlled trials have indeed shown benefit, either from influenza vaccination or from short-term antibiotic treatment, but just as many have failed. These results are not contradictory, because Ott et al[20] have identified remnants of more than 50 bacterial species within atherosclerotic plaques and other investigators have found various virus species as well.[21] It is unlikely that a single antibiotic used during a few weeks should be able to eliminate more than 50 different bacterial or viral species.

ROLE OF THE LIPOPROTEIN IMMUNE SYSTEM

Despite many associations between infections and cardiovascular disease, little attention has been paid to the lipoproteins as mediators of the immune system. In 1939, Todd, Coburn and Bradford Hill found that a serum factor named antistreptolysin-S was not an antibody as previously thought, because its titre fell in rheumatic fever at the peak of the clinical symptoms and during convalescence.[12] Ten years later, Humphrey located antistreptolysin-S within the lipoprotein fraction of the blood. Since then, a dozen research groups have documented that antistreptolysin-S is identical with the lipoproteins and constitutes a nonspecific host defense system that is able to bind and neutralize not only streptolysin-S but also other endotoxins and a large number of bacterial and viral species.[12] *In vitro* studies have shown that human LDL inactivates up to 90% of *Staphylococcus aureus* α-toxin and an even larger fraction of bacterial lipopolysaccharide (LPS). Compared with normal rats, hypocholesterolemic rats injected with LPS have a markedly increased mortality, which can be ameliorated by injecting purified human LDL, and hypercholesterolemic mice challenged with LPS or live bacteria have an 8-fold higher LD50 compared with normal rats.[12]

Studies on human beings are in support as well. For instance, a review of 19 cohort studies found that low serum cholesterol is a risk factor for infectious diseases.[22] It has been argued that low cholesterol is a secondary phenomenon. However, in a 15-year follow-up study of more than 100,000 healthy people those with low cholesterol had been admitted significantly more often to hospital because of an infectious disease.[22] Obviously, the low cholesterol could not be secondary to a disease that they had not yet developed. Also in agreement is that before 1900, when infectious diseases were the commonest cause of death, the lifespan of people with familial hypercholesterolemia was longer than that of the general population.[23]

Infectious diseases cause dyslipidemia,[24,25] but to call the lipid pattern atherogenic may be misleading. The altered lipid profile may instead reflect the body's response to infections. A recent report by Pletcher et al[26] showed that time-averaged cumulative nonoptimal lipid levels in young adults were associated with an increased coronary calcium score later in life. The periodic dyslipidemia was interpreted as the first sign of atherosclerosis. As no previous study of unselected individuals has shown an association between the blood lipids and degree of atherosclerosis,[3,4] a more likely interpretation is that the increased calcium score may have been the result of spontaneously resolved infections and that the calcified lesions later in life may be scars after healed infections.

CREATION OF THE VULNERABLE PLAQUE

We suggest that in the case of chronic or severe acute infections, arterial vasa vasorum may be obstructed by complexes formed between lipoproteins, microorganisms and their toxic products. Their size may increase in the presence of antibodies against oxidized or homocysteinylated LDL. Homocysteine reacts with LDL, and homocysteinylated LDL aggregates are phagocytozed by macrophages to form foam cells.[27] In addition, hyperhomocysteinemia causes endothelial dysfunction, narrowing the lumen of capillaries and leading to trapping of lipoprotein aggregates within vasa vasorum in areas of high tissue pressure.[28] Because vasa vasorum are end arteries, their blockage by this process may cause ischemic cell death of the arterial wall and lead to the creation of a vulnerable plaque.

It is generally accepted that rupture of a vulnerable plaque is the main cause of most arterial thromboses. If the vulnerable plaque is a pustule, as suggested by Osler, its temperature should be higher than its surroundings, which was found to be the case.[12] Moreover, the symptoms and the laboratory findings in acute myocardial infarction are similar to those of an infectious disease.

PATHOLOGICAL EVIDENCE

If the starting point of atherosclerosis is in the vasa vasorum, inflammation should be most pronounced in the adventitia. In accordance, Higuchi et al[29,30] found medial thinning and 4 times more lymphocytes and monocytes and more microvessels in the adventitia beneath vulnerable plaques than beneath stable ones, and in the vasa vasorum, monocytes

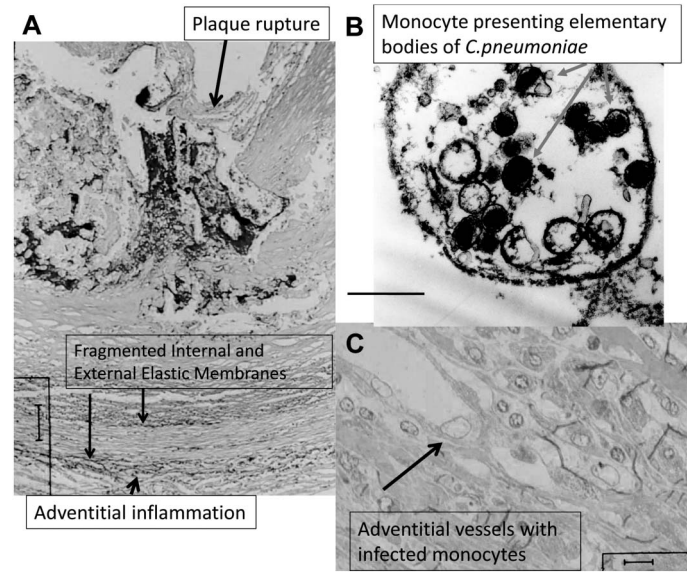

FIGURE 1. Microscopic evidence. (A) Light microscopy of a ruptured vulnerable plaque demonstrates adventitial inflammation, injured media and fragmented internal and external elastic membranes. Note that the inflammation is most pronounced in the adventitia. Movat; scale bar: 1 mm. (B) Electron microscopy of a capillary in the adventitia demonstrates a monocyte containing cytoplasmic elementary bodies of *Chlamydia pneumoniae*, characterized by the typical pear shape because of expansion of the external membrane. Araldite and OsO_4; scale bar: 0.5 μm. (C) Light microscopy of semithin section of a block before electron microscopy analysis demonstrates adventitial capillaries containing many monocytes and surrounding macrophages. Electron microscopy demonstrates cytoplasmic elementary bodies of *C pneumoniae*. Araldite and Toluidine Blue: scale bar: 10 μm.

containing elementary bodies of *C pneumoniae* were seen (Figure 1).

Maiellaro and Taylor[31] have also presented arguments for an "outside-in" mechanism that may work in concert with the conventional "inside-out" one. They point out the rich presence in the adventitia of macrophages and T and B lymphocytes and suggest that the latter may generate antibodies against inflammatory antigens. The nature of these antigens is still undetermined; the authors suggest that heat shock proteins, modified lipoproteins and other surface antigens may be responsible. We suggest that microbes, or microbial antigens, released from the lipoprotein complexes in case of tissue anoxia caused by the obstruction of vasa vasorum are causing the inflammatory response. In accordance, Nicolaou et al[32] have shown that 9 bacteria, representing those most frequently reported to be present in human atheroma induced, foam cell formation of monocytes and macrophages.

If the vulnerable plaque is a vascular pustule, it should contain infectious agents, and this is also the case. Using electron microscopy and *in situ* hybridization, Higuchi et al[30,33] have detected *M pneumoniae*, *C pneumoniae* and also archaeal bodies situated in the lipid core of ruptured vulnerable plaques. These microorganisms attract mainly lymphocytes, but because neutrophilic granulocytes are frequently found in and around the vulnerable plaques,[6] other microorganisms may play a role as well, an issue for future research.

CONCLUSIONS

The function of lipoproteins in the immune system has been ignored in the literature about lipids and atherosclerosis, although it may provide the key to understanding the pathogenesis of atherosclerosis. We suggest that aggregates formed between the lipoproteins and microbes and enlarged by antibodies against oxidized or homocysteinylated LDL may obstruct arterial vasa vasorum because of the high extracapillary tissue pressure. By this process, the arterial wall may become anoxic, leading to an accumulation of toxic substances and microorganisms in the arterial wall inciting the inflammatory response. The vulnerable plaque may simply be a microabscess, as first suggested by William Osler 100 years ago.

ACKNOWLEDGMENT

We are indepted to Professor Maria de Lourdes Higuchi for having supplied the figure.

REFERENCES

1. **Reis SE, Holubkov R, Conrad Smith AJ, et al**; **WISE Investigators.** 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;141:735–41.
2. **McCully KS.** Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969;56:111–28.
3. **Ravnskov U.** Is atherosclerosis caused by high cholesterol? Q J Med 2002;95:397–403.
4. **Hecht HS, Superko HR, Smith LK, et al.** Relation of coronary artery calcium identified by electron beam tomography to serum lipoprotein levels and implications for treatment. Am J Cardiol 2001;87:406–12.
5. **Schatz IJ, Masaki K, Yano K, et al.** Cholesterol and all-cause mortality in elderly people from the Honolulu Heart Program: a cohort study. Lancet 2001;358:351–5.
6. **Onder G, Landi F, Volpato S, et al.** Serum cholesterol levels and in-hospital mortality in the elderly. Am J Med 2003;115:265–71.
7. **Ulmer H, Kelleher C, Diem G, et al.** Why Eve is not Adam: prospective follow-up in 149650 women and men of cholesterol and other risk factors related to cardiovascular and all-cause mortality. J Womens Health (Larchmt) 2004;13:41–53.
8. **Schupf N, Costa R, Luchsinger J, et al.** Relationship between plasma lipids and all-cause mortality in nondemented elderly. J Am Geriatr Soc 2005;53:219–26.
9. **Anderson KM, Castelli WP, Levy D.** Cholesterol and mortality. 30 years of follow-up from the Framingham study. JAMA 1987;257:2176–80.
10. **Johnsen SP, Larsson H, Tarone RE, et al.** Risk of hospitalization for myocardial infarction among users of rofecoxib, celecoxib, and other NSAIDs: a population-based case-control study. Arch Intern Med 2005; 165:978–84.
11. **Hippisley-Cox J, Coupland C.** Risk of myocardial infarction in patients taking cyclo-oxygenase-2 inhibitors or conventional non-steroidal anti-inflammatory drugs: population based nested case-control analysis. BMJ 2005;330:1366–72.

12. **Ravnskov U, McCully KS.** Vulnerable plaque formation from obstruction of Vasa vasorum by homocysteinylated and oxidized lipoprotein aggregates complexed with microbial remnants and LDL autoantibodies. Ann Clin Lab Sci 2009;39:3–16.

13. **Piconi S, Trabattoni D, Luraghi C, et al.** Treatment of periodontal disease results in improvements in endothelial dysfunction and reduction of the carotid intima-media thickness. FASEB J 2009;23:1196–204.

14. **Enos WF, Holmes RH, Beyer J.** Coronary disease among United States soldiers killed in action in Korea; preliminary report. JAMA 1953;152:1090–3.

15. **Blaha F.** [Observations on the pathogenesis of arteriosclerosis from an experience in a concentration camp.] Ćas Lék Ćes [J Czech Physicians] 1958;97:86–9.

16. **Liuba P, Persson J, Luoma J, et al.** 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;24:515–21.

17. **Fabricant CG, Fabricant J, Litrenta MM, et al.** Virus-induced atherosclerosis. J Exp Med 1978;148:335–40.

18. **Damy SB, Higuchi ML, Timenetsky J, et al.** *Mycoplasma pneumoniae* and/or *Chlamydophila pneumoniae* inoculation causing different aggravations in cholesterol-induced atherosclerosis in apoE KO male mice. BMC Microbiol 2009;9:194–201.

19. **Birck MM, Pesonen E, Odermarsky M, et al.** Infection-induced coronary dysfunction and systemic inflammation in piglets are dampened in hypercholesterolemic milieu. Am J Physiol Heart Circ Physiol 2011; 300:H1595–601.

20. **Ott SJ, El Mokhtari NE, Musfeldt M, et al.** Detection of diverse bacterial signatures in atherosclerotic lesions of patients with coronary heart disease. Circulation 2006;113:929–37.

21. **Shi Y, Tokunaga O.** *Chlamydia pneumoniae* and multiple infections in the aorta contribute to atherosclerosis. Pathol Int 2002;52:755–63.

22. **Ravnskov U.** High cholesterol may protect against infections and atherosclerosis. Q J Med 2003;96:927–34.

23. **Sijbrands EJ, Westendorp RG, Defesche JC, et al.** Mortality over two centuries in large pedigree with familial hypercholesterolaemia: family tree mortality study. BMJ 2001;322:1019–23.

24. **Apostolou F, Gazi IF, Kostoula A, et al.** Persistence of an atherogenic lipid profile after treatment of acute infection with Brucella. J Lipid Res 2009;50:2532–9.

25. **Gidding SS, Stone NJ, Bookstein LC, et al.** Month-to-month variability of lipids, lipoproteins, and apolipoproteins and the impact of acute infection in adolescents. J Pediatr 1998;133:242–6.

26. **Pletcher MJ, Bibbins-Domingo K, Liu K, et al.** Nonoptimal lipids commonly present in young adults and coronary calcium later in life: the CARDIA (Coronary Artery Risk Development in Young Adults) study. Ann Intern Med 2010;153:137–46.

27. **Naruszewicz M, Mirkiewicz E, Olszewski AJ, et al.** Thiolation of low-density lipoprotein by homocysteine thiolactone causes increased aggregation and altered interaction with cultured macrophages. Nutr Metab Cardiovasc Dis 1994;4:70–7.

28. **McCully KS.** Chemical pathology of homocysteine. IV. Excitotoxicity, oxidative stress, endothelial dysfunction, and inflammation. Ann Clin Lab Sci 2009;3:219–32.

29. **Higuchi ML, Gutierrez PS, Bezerra HG, et al.** Comparison between adventitial and intimal inflammation of ruptured and nonruptured atherosclerotic plaques in human coronary arteries. Arq Bras Cardiol 2002; 79:20–4.

30. **Higuchi ML, Sambiase N, Palomino S, et al.** Detection of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in ruptured atherosclerotic plaques. Braz J Med Biol Res 2000;33:1023–6.

31. **Maiellaro K, Taylor WR.** The role of the adventitia in vascular inflammation. Cardiovasc Res 2007;75:640–8.

32. **Nicolaou G, Goodall AH, Erridge C.** Diverse bacteria promote macrophage foam cell formation via toll-like receptor-dependent lipid body biosynthesis. J Atheroscler Thromb 2012;19:137–48.

33. **Higuchi ML, Santos MH, Roggério A, et al.** A role for archaeal organisms in development of atherosclerotic vulnerable plaques and myxoid matrices. Clinics (Sao Paulo) 2006;61:473–8.