Infections and their role in atherosclerotic vascular disease

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ABSTRACT

Background. Complications of atherosclerosis are the leading cause of mortality in developed countries, and infections may play a role in the pathogenesis. Numerous studies have addressed this issue in the past decade.

Types of Studies Reviewed. The author examined peer-reviewed studies and reviews on the role of microbes or infections in atherosclerosis, cardiovascular disease and cerebrovascular disease. He included selected articles on epidemiology, pathology, in vitro experiments, animal models and clinical studies.

Results. Cross-sectional and retrospective studies have shown an association between *Chlamydia pneumoniae* antibodies and cardiovascular disease, but prospective studies have not been as convincing. Studies on the association between cardiovascular disease and periodontal disease or loss of teeth have produced conflicting results. Cytomegalovirus infection is associated mainly with accelerated arteriosclerosis after cardiac transplantation. Infectious agents can induce biological mechanisms important for atherogenesis. Mice and rabbit studies have indicated that *C. pneumoniae* is capable of initiating or accelerating the progression of atherosclerosis. Limited studies on cytomegalovirus also suggest the ability to induce early changes of atherosclerosis in a rodent model. Preliminary clinical trials of treatment for *C. pneumoniae* infection suggest a possible short-term benefit, but larger randomized trials for longer periods are in progress.

Conclusion and Clinical Implications. Infectious agents may play an important role in atherogenesis, but currently the jury is not in. Further management of cardiovascular disease could change radically if this concept were proven.

Atherosclerosis, the main underlying disease responsible for cardiovascular and cerebrovascular morbidity and mortality in developed countries, results from multifactor etiology. Traditional factors such as obesity, hyperlipidemia, diabetes, hypertension and cigarette smoking account for only 50 to 60 percent of cases of cardiovascular disease.

For more than a century, it has been postulated that infectious agents are responsible for atherosclerotic diseases.[1] The association between atherosclerosis and infections seems to be a rational one, as the process of development of atherosclerosis involves a chronic low-grade inflammation.[2] Moreover, systemic markers of inflammation (that is, C-reactive protein, leukocyte count, fibrinogen, cell-adhesion molecules and proinflammatory cytokines) are predictors of present and future cardiovascular events and disease.[3]

Infectious agents may play an important role in atherogenesis, but currently the jury is not in.

Renewed interest in this topic has taken place since the late 1980s when Saikku and colleagues[4] noted an association between patients who had had acute myocardial infarctions and the presence of *Chlamydia pneumoniae* antibodies. Since then, several studies have addressed this issue with a number of microorganisms in clinical epidemiologic and in vitro studies, and to a lesser extent in animal models and clinical trials.

EPIDEMIOLOGIC EVIDENCE OF AN ASSOCIATION

Most studies examining a potential association between infections and cardiovascular disease or cerebrovascular disease have used a single serology for the definition of past infection. This is a suboptimal investigative tool

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and usually cannot distinguish between past and persistent infection. Thus, it is not surprising that most of these studies' results have been controversial and conflicting. For an infectious agent to play a role in the pathogenesis of atherosclerosis, it most likely would require a chronic and persistent active but low-grade infection. Unfortunately, in many of the infections being studied (for example, *C. pneumoniae),* there is no established and reliable method of distinguishing past infection from persistent active infection. Also, there is no serology for assessing gingivitis or periodontal disease. Retrospective or case-control (cross-sectional) studies usually rely on indirect measures such as loss of teeth and radiologic evidence of bone loss to diagnose periodontal disease. A few well-designed controlled, prospective studies used periodic periodontal examinations with strict, accepted criteria to make the diagnosis.

An acute episode of an infectious disease may play a role in precipitating an acute cardiovascular event without being involved in the development or progression of atherosclerosis. For instance, greater cardiovascular mortality has been observed during the influenza season than during other times of the year,[5] with fewer myocardial events in subjects who received influenza vaccinations.[6] Those subjects who had acute ischemic attacks after an influenza infection probably had critical or unstable underlying atherosclerotic plaques. The acute infection theoretically may precipitate an acute thrombus on a vulnerable plaque, leading to an acute myocardial infarction. It is well-recognized that many infections have the potential to produce an environment in the bloodstream favorable for thrombosis, a procoagulant state. Infections can cause thrombocytosis, platelet aggregation, increased fibrinogen and activation of blood clotting Factor X or proinflammatory cytokine stimulation, which upregulates tissue factors and can stimulate the "clotting cascade."[7-9]

The table provides a summary of the epidemiologic evidence associating various infections with coronary artery disease and atherosclerosis, as well as the rank order of the strength of associations (from weakest to strongest: absent, weak, fair, good, very good). The infection with the largest body of evidence supporting an association with cardiovascular and cerebrovascular disease is *C. pneumoniae*.[10] The majority of more than 30 retrospective and cross-sectional studies showed an association between *C. pneumoniae* antibodies and cardiovascular disease (odds ratio, or OR, = 2).[11] A recent meta-analysis of 15 prospective studies, however, found no significant association[12]; the mean OR was 1.59 (95 percent confidence interval, or CI, 1.17 to 2.16) in favor of an association with cardiovascular disease when it was adjusted for the traditional risk factors and adult socioeconomic status, but the OR was only 1.22 (95 percent CI, 0.82 to 1.82) when it was adjusted to include socioeconomic status during childhood. This raises the issue of overadjustment,[13] as most infectious diseases are associated with lower socioeconomic status and overcrowding. Thus, by adjusting for socioeconomic status during childhood, a real association with any infectious diseases may be masked.

With respect to other bacterial infections, most of the data do not support a significant link between cardiovascular disease and *Helicobacter pylori*.[10,14] Several retrospective and cross-sectional studies,[15-20] and a few prospective studies[21-24] found an association between periodontal disease and cardiovascular disease. However, a recent large prospective study of baseline oral examinations of 8,032 dentate subjects with periodontal disease and gingivitis followed up over 21 years found no association between any periodontal inflammatory process and cardiovascular or cerebrovascular disease, after adjusting for traditional risk factors and for adult socioeconomic status.[25]

The major viruses implicated in or associated with complications of atherosclerosis are the herpes viruses, particularly cytomegalovirus. The evidence for association, however, is weak for natural coronary artery disease, fair for restenosis after coronary angioplasty and good for accelerated coronary arteriosclerosis in cardiac transplant recipients.[10]

PATHOLOGICAL EVIDENCE OF ASSOCIATION

The detection of microorganisms or their antigens or of nucleic acid in atherosclerotic lesions is another method of establishing an association with, but not causality of, vascular disease. Studies have been performed comparing arteries with atheroma with normal vessels to detect differences in the presence of microorganisms.[10,14,26] The bulk of the studies involved *C. pneumoniae* or herpes viruses and used different techniques such as immunohistochemistry; polymerase chain reac-

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TABLE

STRENGTH OF THE EVIDENCE ASSOCIATING CONDITIONS OR INFECTIONS WITH CORONARY ARTERY DISEASE AND ATHEROSCLEROSIS.*†

CONDITION/ INFECTIOUS AGENT	EPIDEMIOLOGIC EVIDENCE	PATHOLOGICAL EVIDENCE	BIOLOGICAL PLAUSIBILITY	ANIMAL MODEL EVIDENCE	CLINICAL EVIDENCE
Chlamydia pneumoniae	Good	Very good	Very good	Good	Fair
Cytomegalovirus in					
Native atherosclerosis disease	Weak	Weak	Good	Fair	Absent
Restenosis after angioplasty	Fair	Fair	Fair	Absent	Absent
Cardiac transplant	Good	Fair	Good	Good	Absent
Periodontitis	Fair	Weak	Good	Fair	Absent
Helicobacter pylori	Weak	Weak	Weak	Absent	Absent

* Adapted from Fong[10] with permission of the Canadian Medical Association.
† Rank order of strength from weakest to strongest: absent, weak, fair, good, very good.

tion, or PCR; in situ hybridization; electron microscopy; and culture.

C. pneumoniae usually is absent or rarely is found in normal arteries; however, it has been detected in an average of 50 percent, but up to 80 percent, of atherosclerotic vessels, depending on the study.[10,27,28] In pathological studies, *C. pneumoniae* is associated with atherosclerosis (OR = 20).[12] Viable *C. pneumoniae* has been recovered from atheromas in a few studies,[29-32] but the highest recovery rate of *C. pneumoniae* reported was only in 10 (38.5 percent) of 26 abdominal aortic aneurysms.[32] No other bacteria have been confirmed to be present in atherosclerotic plaques by culture, and no other microorganisms have been cultured from human atheromas.

Cytomegalovirus and herpes simplex virus type 1 and type 2 have been detected in arterial samples with varying success.[10] Using immunohistochemistry, researchers found cytomegalovirus, but not herpes simplex virus type 1, more frequently in atheromatous vessels than in normal vessels.[33] With PCR methods, however, cytomegalovirus has been detected in diseased arteries and in normal arteries at a similar rate.[34]

Although a recent study was able to detect *H. pylori* DNA in 37 percent of human endarterectomy specimens,[35] two other studies failed to find any evidence of *H. pylori* in vascular tissues.[36,37] Similarly, in a study that used PCR assays to detect bacterial 16s ribosomal deoxyribonucleic acid, the authors reported that 44 percent of 50 atheromas were positive for one of several target periodontal pathogens *(Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus* and *actinobacillus actinomycetemcomitans).*[38] Using a polyclonal antibody for immunostain, Chiu[39] detected at least two oral bacteria in the periphery of plaques from carotid endarterectomy specimens: *P. gingivalis* in 42 percent of the plaques and *Streptococcus sanguis* in 12 percent of the plaques. These data are considered unproven until other independent studies confirm them, and monoclonal antibodies should be used in immunostains of future studies instead of polyclonal antibodies.

BIOLOGICAL PLAUSIBILITY

Infectious agents may play a role in the pathogenesis of atherosclerosis by several mechanisms of action and at different stages. Microorganisms could infect vascular endothelial cells directly, initiating the inflammatory response needed for the initial process of inducing atherosclerosis. Furthermore, even if the induction or initial injury to the endothelium was caused by another inciting agent or factor (for example, hypercholesterolemia or hypertension), infectious agents could accelerate or enhance the process through several mechanisms of action. They include further recruitment and stimulation of proinflammatory cytokines and tissue growth factors in the

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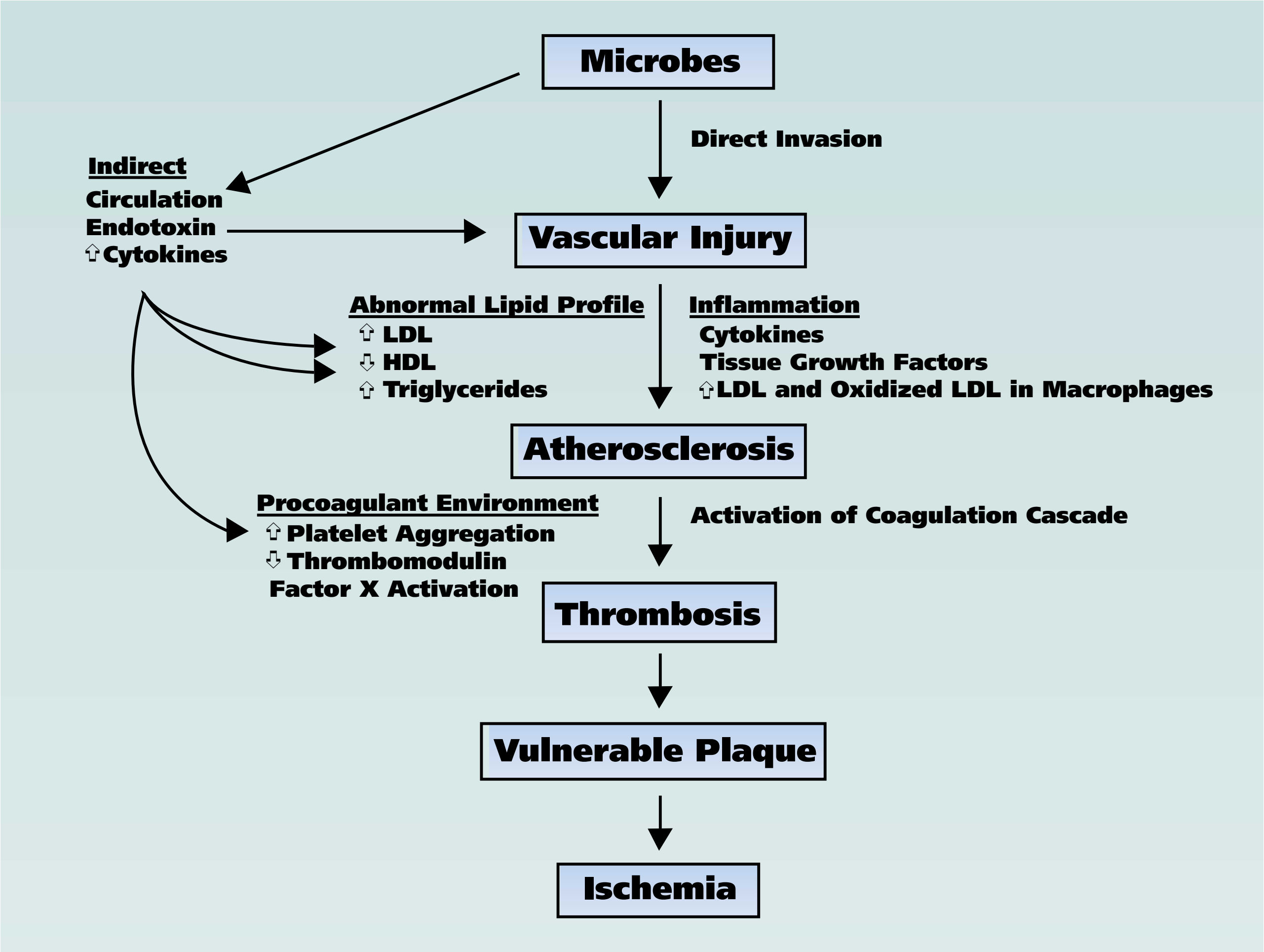

Figure. Potential mechanism of infectious agents in atherosclerosis. LDL: Low-density lipoprotein. HDL: High-density lipoprotein.

arterial wall, as well as enhancement of lipid (low-density lipoprotein, or LDL) accumulation through stimulation of macrophage scavenger or LDL-receptors. Microbes could indirectly influence the development and progression of atherosclerosis by a systemic effect without directly invading the arterial endothelium. Release of endotoxin or lipopolysaccharide into the circulation could indirectly damage vascular endothelium or the immune response, and systemic cytokine release could result in lipid profile predisposing to atherosclerosis or could predispose the arterial environment to a procoagulant state, resulting in acute thrombus on a preexistent unstable or critical plaque, thus causing an acute ischemic event (Figure).

C. pneumoniae is capable of infecting vascular endothelial cells, smooth muscle cells and macrophages of human origin in vitro.[40] Moreover, *C. pneumoniae* and its lipopolysaccharide can enhance LDL accumulation in human-derived monocytes to form foam cells,[41] which are a key component of atherosclerosis. Furthermore, viable *C. pneumoniae* or its heat shock protein 60 can induce oxidation of LDL intracellularly[42]; oxidized LDL is considered to be the main toxic element leading to the process of atherosclerosis. In vitro studies also have demonstrated that *C. pneumoniae* or its components can stimulate proinflammatory cytokines and metalloproteinase production in tissues—factors important in atherogenesis.[43]

Similarly, in vitro studies have demonstrated that herpes viruses (particularly cytomegalovirus) and oral bacteria *(P. gingivalis)* can invade cells of vascular origin.[10,44] These microorganisms also are capable of initiating the inflammatory cascade through proinflammatory cytokines. Oral bacteria such as *S. sanguis* and *P. gingivalis* can induce platelet aggregation in vitro and may

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increase the risk of developing acute thrombosis.[10] *P. gingivalis* also can stimulate the coagulation cascade by activation of Factor X.[45] Furthermore, *P. gingivalis* and several other oral bacteria recently were shown to induce foam cell formation in the murine macrophage line.[46]

ANIMAL MODELS

Using animal models is important in assessing or establishing causality between any putative risk factor and atherosclerosis. The ability to produce disease in a nonprimate animal, however, does not necessarily mean it can be reproduced in humans. *C. pneumoniae* has been shown to enhance or accelerate atherosclerosis in cholesterol-fed rabbits[47] and in gene knockout mice with apolipoprotein E deficiency, or apoE,[48] or LDL-receptor deficiency.[49] Moreover, it has been shown that *C. pneumoniae* can induce early changes of atherosclerosis de novo in nonhypercholesterolemic rabbits.[50] However, *C. pneumoniae* has not been shown to initiate atherosclerosis in nonhypercholesterolemic mice, which raises the issue of species specificity. The early changes in rabbits that were induced by *C. pneumoniae* have not been shown to progress to the mature atheroma with a lipid core as seen in the hypercholesterolemic model; some increase in dietary lipids may be necessary to develop to this stage. Morphologically, however, the preatheroma lesions induced by the infection are indistinguishable microscopically from those produced by giving a very low-cholesterol diet to rabbits—the type of diet that results in blood levels considered normal for humans.[50]

The avian herpes virus (not found in humans) was found to produce atherosclerosis in normocholesterolemic chickens in the 1970s.[10] This was attributed to increased cholesterol uptake in the arterial intima. In addition, cytomegalovirus has been shown to produce endothelial changes resembling early atherosclerosis in rodents.[10] Most recently, repeated systemic inoculation of *P. gingivalis* once a week for 24 weeks has been reported to accelerate atherosclerosis in a heterozygous apoE-deficient murine model.[51]

CLINICAL TRIALS

Preliminary small prospective studies designed to determine proof of concept have shown that antibiotics may reduce early cardiovascular events.[10] Larger retrospective case-control studies have shown similar benefit, but other types have not.[10] Larger controlled prospective studies to determine the true benefits of antibiotics in patients with established cardiovascular disease are ongoing. These trials are designed mainly to show a benefit of antibiotics at the late stage of precipitation of acute thrombus in patients with pre-existing lesions. Therefore, a negative result would not preclude the role of infectious agents in the initiation or the acceleration of progression in the earlier stages of atherosclerosis.

FUTURE DIRECTIONS

The role of infectious agents in the pathogenesis of atherosclerosis will have a major public health implication if it is confirmed. The current state of the art is shrouded in uncertainty and controversy, and a lot more research needs to be conducted to come to a final answer.

Epidemiologic studies can only provide clues to an association between infective agents and atherosclerosis; they do not prove causality. In certain instances, an association has been well-established, if not by epidemiologic data, then by the overwhelming pathological evidence such as in the case of *C. pneumoniae.* Intervention trials are important to confirm the role of microbes in humans, but the current ongoing trials have shortcomings such as selecting patients with known cardiovascular disease, thus having to assess acute precipitating events; in addition, the most appropriate antibiotic regimens have not been defined by animal models. Even a few thousand patients followed up for two to four years may not be enough. Larger, longer-term trials spanning a decade or more for subjects who do not have the clinical complications of atherosclerosis need to be conducted.

Expansion of further research in animal models needs to continue. They must try to simulate as much as possible the natural infections that occur in humans; otherwise, the results may not be applicable to the disease process or the pathogenesis of atherosclerosis in humans. Additional animal models such as minipigs and nonhuman primates that produce diseases similar to those found in humans need to be used. Additionally, the interactions of microbes with other traditional risk factors (such as diabetes, smoking and hypertension) need to be explored in animal models.

All of these suggestions may need to be implemented to provide the burden of evidence needed

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to establish cause and effect between infection and atherosclerosis. It is not likely that Koch's postulate will be fulfilled by any of the putative agents in a chronic disease with so many known existing etiologies and potential risk factors. Koch's postulate says the organism must be present in every case of the disease; the organism must be isolated from the diseased host and grown in pure culture; the specific disease must be reproduced when pure culture is inoculated into a healthy susceptible host; and the organism must be recovered again from the experimentally infected host.

CONCLUSION

Infectious agents may play an important role in atherogenesis, but the jury is not in. Further studies are needed to prove causality of atherogenesis from *C. pneumoniae* and to establish an association between cardiovascular disease and periodontitis. There is, however, sufficient evidence from biological mechanisms and animal models to warrant interventional studies on periodontitis and development of cardiovascular events. ▪

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