

Overview of infections and cardiovascular diseases

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Microbes have been proposed as inciting agents of tissue injury and inflammation, both of which underlie the pathogenesis of atherosclerosis. Viruses, including the herpes simplex virus and cytomegalovirus, as well as bacteria such as *Chlamydia pneumoniae*, have been implicated in the process. In vitro, these agents promote a proinflammatory and a procoagulant phenotype in vascular cells. Viruses augment cell accumulation through alterations of apoptosis. Infectious agents may play a role in pathogenesis of atherosclerosis by triggering an autoimmune response due to microbial molecular mimicry. It is unlikely that a single agent is the sole cause or modulator of this heterogeneous disease. Contradictory epidemiological studies may be reconciled with a new construct suggesting that multiple pathogens infecting an individual in aggregate may promote an inflammatory and procoagulant environment that underlies the pathogenesis of atherosclerosis. (J Allergy Clin Immunol 2001;108:S117-20.)

Key words: Atherosclerosis, microbes, myocardial infarction, viruses

Cardiovascular disease remains the number one killer in the US. Despite Nobel Prize-winning insights, enormous public health education programs, and cutting-edge technologies to treat disease, the etiology of this plague of modern man remains obscure. Well-defined risk factors have been identified including hypercholesterolemia, diabetes mellitus, smoking, and hypertension. Significant data support the roles of genetics and metabolic, lifestyle, and emotional factors. Yet young men and women are dying of myocardial infarctions in their 30s and 40s without having these common risk factors. New concepts to understand the pathophysiology of atherosclerosis, therefore, are demanded. Two recently popularized ideas, inflammation and infection, are neither new nor fully delineated. This is the subject of this presentation and has been recently reviewed.¹

As early as the 1800s, inflammation was noted in the atherosclerotic vessel wall.^{2,3} In the late 1800s and the

Abbreviations used

CMV: Cytomegalovirus

HSV: Herpes simplex virus

early 1900s, heart disease following salmonella or streptococcal infection was suggested.⁴ Osler recognized the possible causative role of infections in atherosclerosis and included it in his 1908 edition of Modern Medicine.⁵ He commented on early atherosclerosis in young people. During the next 60 to 70 years, however, the focus of theories of atherogenesis shifted away from inflammation and infection hypotheses, except for occasional citations in the literature.^{6,7} Ross championed the hypothesis that atherosclerosis is a response to injury.⁸ Most researchers today accept the paradigm that atherosclerosis is an inflammatory process, confirming the microscopic findings of early 19th century pathologists.

MODERN VIEWS ON INFECTION AND ATHEROSCLEROSIS

In 1973, Benditt and Benditt⁹ revived interest in the infection hypothesis. Benditt et al¹⁰ showed evidence of herpes simplex virus (HSV) by in situ hybridization in the atherosclerotic vessel wall. Fabricant's animal models demonstrated that a herpesvirus, Marek's disease, induced arterial lesions similar to those in human atherosclerosis.¹¹ Other epidemiological studies and pathology studies demonstrated that cytomegalovirus (CMV) could be found in the vessel wall.^{12,13} In vitro studies by Hajjar et al¹⁴ found that herpes infections of smooth muscle cells affected cholesterol metabolism, with excessive cholesterol ester accumulation in infected cells.

CMVs were associated with accelerated atherosclerosis following cardiac transplantation.¹⁵ Patients with a previous CMV infection had a high independent risk of restenosis after coronary angiography.^{16,17} Not all studies have found an association or positivity for viruses in atherosclerotic disease.¹⁸⁻²⁰ A variety of other infectious agents have been implicated in the pathogenesis of atherosclerosis^{21,22} (Table I). Dental infections, especially caries and periodontal disease, have been associated with coronary heart disease.^{23,24} The bacterial agent that has attracted the most attention, however, is *Chlamydia pneumoniae*. In 1988, Saikku showed an increased IgG and/or IgA titer to *C pneumoniae* in Finnish patients with acute myocardial infarction or chronic coronary artery disease.^{25,26} *C pneumoniae* has been found within the atherosclerotic lesion by PCR, immunotechniques and

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Presented at the IVIG Advisory Meeting 2000, Bayer Biologicals, October 26-29, 2000, Santa Barbara, Calif.

The author has no significant financial relationship or interest in Bayer Corporation. He has prepared this report to present factual, unbiased information and attests that no commercial association has influenced this report, nor does this publication constitute a commercial or personal conflict of interest.

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0091-6749/2001 \$35.00 + 0 1/0/117976

doi:10.1067/mai.2001.117976

TABLE I. Infectious agents implicated in atherogenesis

Viruses
CMV ¹²
HSV ¹⁰
HIV ⁵⁶
Coxsackie B ⁵⁷
Influenza A ⁵⁸
Hepatitis A ⁵⁴
Bacteria
Chlamydia pneumonia ²⁵
Helicobacter pylori ⁵⁹
Dental infections: Streptococcus mutans ²³
Porphyromonas gingivalis, etc.
Enterobacteria ⁶⁰

electron microscopy.²⁷⁻²⁹ Grayston showed a strong link between *C pneumoniae* infection and atherosclerosis.³⁰ Antibiotic interventions also have been reported. Treating survivors of myocardial infarction with azithromycin suggests a 5-fold reduction in adverse cardiovascular events compared with patients receiving placebo.^{31,32} Animal studies have demonstrated that *C pneumoniae* and a high-cholesterol diet given to rabbits induced more intimal thickening, which can be prevented by antibiotics.³³ Other trials have not supported the early positive effect of antibiotics in patients with coronary artery disease.³⁴ There are some seroepidemiological studies that do not find evidence of an association between the presence of infection and coronary artery disease.^{35,36}

IN VITRO EVIDENCE SUPPORTING THE HYPOTHESIS

In vitro studies have modeled the potential proinflammatory and prothrombotic effects of infections in the vessel wall.³⁷ Both HSV and CMV can promote inflammation in a variety of ways.³⁸ For example, adhesion molecules can be induced when endothelial cells are infected with HSV or CMV, including P-selectin, E-selectin, vascular cell adhesion molecule, intercellular adhesion molecule, and others.³⁹⁻⁴⁰ We have shown that CMV immediate early genes act as promoters for intercellular adhesion molecule-1 expression.⁴¹ CMV infection can up-regulate the cellular transcription of nuclear factor κ B.⁴² Our laboratory and others demonstrated that viral infection of the endothelium enhances thrombin generation and platelet binding on the endothelial surface.⁴³⁻⁴⁵ Vascular endothelial cells enhance tissue factor expression, in addition to down-regulating anticoagulant protein molecules such as prostacyclin and thrombomodulin.^{46,47} Speir and Epstein⁴⁸ demonstrated that CMV infection could be found in human coronary arteries that had restenosed following angioplasty. There was a significant correlation between p53 immunoreactivity and the presence of CMV DNA.

CMV immediate early genes may inhibit the function of p53, contributing to smooth muscle proliferation and accumulation by blocking p53's inhibition of cell-cycle progression. We have shown that CMV infection of endothe-

lial cells blocks serum starvation-induced apoptosis.⁴⁹ CMV infected cells showed elevated p53 levels. Trafficking of p53 was altered, however, with p53 localizing in the cytoplasm rather than the nucleus. CMV infection of fibroblasts caused p53 to localize in the viral proliferation center.⁵⁰ We speculate that the ability of human CMV to prevent apoptosis plays a role in postangioplasty restenosis, transplantation arterio-obliteration, and atherosclerosis.

CONCLUSIONS

Although attractive, the microbial pathogenesis theory for atherosclerosis remains unproven. Over the past century, microbiologists have invoked fulfillment of Koch's postulates to determine pathogen causality. It is unlikely that a multifactorial disease process such as atherosclerosis will be due to a single microbial agent, to fulfill Koch's postulates such that a single agent when transferred to another host always will induce atherosclerosis. Conflicting epidemiological data also do not support a single causative agent. Considerable in vitro, animal, and human epidemiological data support the theory that infectious agents can promote a proinflammatory, procoagulant, and proatherogenic environment in the vessel wall, however. Microbial genes and molecules can catalyze these processes and foil normal cellular events.

Molecular mimicry is a popular hypothesis for how infections promote the inflammatory response.⁵¹ Must intact microbes enter the vessel wall or can they incite immune responses from afar? A new focus on pathogen-induced autoimmunity toward vasculature has been suggested. For example, microbes contain molecules that mimic host cellular components.⁵² An immune response to a pathogen may cross-react with vessel wall cellular structures.⁵² For example, *C pneumoniae* HSP60, which is homologous to human HSP60, promotes activation of endothelium and enhances an inflammatory response.⁵³ Epstein et al⁵⁴ have championed the concept of pathogen burden in support of this autoimmune theory. Individuals infected with multiple pathogens such as HSV-1, HSV-2, CMV, *Helicobacter pylori*, and hepatitis A, have high C-reactive and protein levels (markers of inflammation) and the greatest relative risk for coronary artery disease.⁵⁵ Pathogens might contribute to the atherosclerotic process, therefore, by promoting inflammatory responses.

Do microbes and inflammation play a role in the pathogenesis of atherosclerosis? Infection may contribute to the process promoting vessel wall injury initiated by oxidized lipids, smoking-derived oxidants, hypertensive shear, or diabetes gly-oxidized molecules. Inflammation and immune reactions in response to infection may exacerbate and act synergistically with all of the aforementioned vasculotoxic moieties. Further research, both in vitro and in vivo, is required to answer these questions.

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