The Multiple Mechanisms by Which Infection May Contribute to Atherosclerosis Development and Course

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Among the initial studies suggesting that infection contributes causally to atherogenesis were investigations of human atherosclerotic tissue demonstrating the presence of pathogen in the diseased vessel wall and classical seroepidemiological studies in which associations were demonstrated between atherosclerosis and prior pathogen infection (evidenced by anti–pathogen antibodies). Appropriately, however, questions were raised relating to the biological meaning of such associations. A pathogen resident in an atherosclerotic vessel, for example, may be just an “innocent bystander,” rather than a causally relevant agent; alternatively, atherosclerotic vessels might just be more vulnerable to infection. And although multiple seroepidemiological studies demonstrated associations between atherosclerosis and antibodies targeted to individual pathogens, other studies did not.1

In addition to these studies providing associative evidence linking infection to atherosclerosis, mechanistic investigations demonstrated important links (reviewed in Epstein et al2). For example, in vitro infection of cells of the vascular wall caused changes that could predispose to atherosclerosis: infection stimulates smooth muscle cell (SMC) proliferation; inhibits apoptosis; increases SMC migration; increases lipid accumulation; produces procoagulant effects; and increases expression of cytokines, chemokines, and cellular adhesion molecules. Infection also increases lesion development in animal models of atherosclerosis and of restenosis.

The nagging question persisted, however: Why, if infection were an important contributor to the development of atherosclerosis, was there such disparity among different epidemiological studies, and why were some individuals who had been infected with a candidate pathogen free of atherosclerosis, while others had extensive disease? Several tentative answers have been forthcoming, all pointing to the conclusion that if a causal relationship exists between infection and atherosclerosis, it is complex.

Individual Variations in the Infection-Induced Persistent Inflammatory Response

One factor contributing to this complex relationship is that the atherosclerotic risk posed by prior infection appears to depend partly on the host’s ability to suppress pathogen-induced inflammatory activity, an attribute for which there is considerable interindividual variability.3 Thus, (1) only about half of individuals who are seropositive to cytomegalovirus (CMV) have a persistent inflammatory response, as evidenced by elevated C-reactive protein (CRP) levels (a marker of inflammation) and (2) the highest prevalence of coronary artery disease (CAD) occurs in the subgroup with combined CMV seropositivity and elevated CRP levels. When adjusted for CAD risk factors, the odds ratios for CAD were 1.3 in the subgroup with CMV seropositivity alone (P=0.7), 2.3 in the subgroup with elevated CRP levels alone (P=0.2), and 4.3 in the subgroup with combined CMV seropositivity and elevated CRP levels (P=0.01). The biological explanation for this undoubtedly derives from the fact that inflammation importantly contributes to the atherogenic process. (It should also be noted that the relation between infection, markers of inflammation, and CAD is importantly influenced by gender. In men, prior CMV infection is not an independent marker of CAD; however, it is strongly associated with elevated CRP levels, which, in turn, are strongly associated with CAD prevalence. In contrast, in women, although prior CMV infection is associated with elevated CRP levels, this trend is not significant, whereas CMV infection is independently predictive of CAD.5)

The findings reported in this issue of Circulation Research by Mach et al6 in which Helicobacter pylori infection of mice did not increase atherosclerotic lesions need to be considered in the context of the above paragraph. Lesions were measured only 6 or 12 weeks after infection, a time considerably less than that required for chronic gastric inflammation to develop in the exact experimental model used by the investigators.6 If the influence infection has on atherogenesis is critically determined by the induction of chronic inflammation, then serious questions can be raised as to whether the hypothesis examined by Mach and coworkers was definitively tested by their experimental design, a limitation acknowledged by the authors.

Effect of Pathogen Burden on Atherosclerosis

A second confounding factor influencing the relation between infection and atherosclerosis derives from what we have termed “pathogen burden.” Until recently, all studies focusing on the role of infection in atherosclerosis examined a single pathogen. We thought, however, that if infection does play a role in atherogenesis, it would be unlikely that any one of these was the causal agent. We therefore postulated that multiple pathogens are causally involved, and that CAD risk relates to the aggregate pathogen load (to “pathogen burden”).
This hypothesis was first tested in a group of individuals being evaluated for CAD by coronary angiography. The relationship of the aggregate number of a panel of five pathogens to which an individual had been exposed (as determined by seropositivity) to CAD risk was determined. The pathogens (CMV, Chlamydia, hepatitis A virus, herpes simplex virus [HSV] 1, and HSV 2) were selected because each was either an obligate intracellular pathogen known to establish a persistent lifelong infection and/or elicited a persistent lifelong immune response (as manifested by increased antibody levels). As the number of seropositive responses increased, CRP levels and CAD prevalence increased. These results have been extended by several prospective studies in which pathogen burden was found to predict cardiovascular event rate. Increasing pathogen burden also leads to increased endothelial dysfunction, one of the earliest manifestations of atherosclerosis. In total, these results suggest that infection may not only contribute to the development of atherosclerosis but can also trigger plaque rupture and acute thrombotic occlusion, the major factors responsible for acute myocardial infarction and for sudden death in patients with CAD.

**Indirect Effects of Infection That Could Predispose to Atherosclerosis**

By residing in the vessel wall, pathogens can exert direct effects on atherogenesis. However, infection causes a spectrum of systemic effects, producing alterations in circulating cytokines, acute-phase reactants, white blood cells, and responses mediated by the immune system, such as increased antibody levels. As the number of seropositive responses increased, CRP levels and CAD prevalence increased. These results have been extended by several prospective studies in which pathogen burden was found to predict cardiovascular event rate. Increasing pathogen burden also leads to increased endothelial dysfunction, one of the earliest manifestations of atherosclerosis.

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**Increase in Circulating Cytokines**

Infection-induced increases in circulating cytokines may constitute one specific indirect mechanism by which infection contributes to atherogenesis. For example, serum of CMV-infected apolipoprotein E knockout mice induces monocyte chemoattractant protein-1 (MCP-1) expression in endothelial cells (ECs), an effect due, at least partly, to infection-induced increases in interferon-γ (IFN-γ). MCP-1, which is expressed by macrophages, ECs, and SMCs in human atherosclerotic lesions, is chemoattractant for monocytes as well as for CD4+ and CD8+ T lymphocytes. Monocytes/macrophages constitute the majority of the leukocyte population found in human atherosclerotic plaques and are considered the main inflammatory mediators of atherosclerosis. Thus, this cytokine-mediated change in EC function could exacerbate atherogenesis by recruiting monocytes and T cells into the vessel wall and thereby establish or exacerbate local inflammatory responses, even in the absence of resident virus.

**Immune-Mediated Mechanisms**

Multiple studies suggest that immune responses contribute to the development of atherosclerosis. Although the antigens serving as immune targets are unknown, one major candidate is heat shock protein (HSP). HSPs are highly conserved proteins synthesized in large amounts when cells are exposed to stressful stimuli such as inflammation, infection, and oxidizing agents.

Xu and Wick suggested that although HSPs are normally intracellular proteins, marked stress-induced overexpression may lead to their presentation on the cell surface, stimulating an autoimmune reaction, and thereby contribute to the development of atherosclerosis. The validity of the concept that autoimmunity plays a role in atherogenesis and that HSP is one of the autoantigens was supported by the findings that increased expression of human HSP60 has been observed on ECs, macrophages, and SMCs in human atherosclerotic lesions, and that antibody titers of anti-human HSP60 correlate with both the presence and extent of CAD.
That infection might also contribute to atherogenesis via immune mechanisms targeting HSP has been suggested by several studies. All bacteria encode for HSPs, and in experimental models atherosclerotic lesions can be induced by immunization of animals with mycobacterial HSP65. In addition, antibodies to mycobacterial HSP65 are associated with carotid artery thickening. Because HSP molecules are highly conserved, these associations are compatible with the concept that bacterial infection induces the development of antibodies (such as antibodies against mycobacterial HSP65), which then cross-react with human HSPs that are overexpressed on ECs, thereby provoking an immune contribution to the development of atherosclerosis.

Compelling evidence has accrued over the past decade suggesting that certain infectious agents contribute to the course of atherosclerosis. Nonetheless, definitive proof of the general concept is still lacking, as is proof of a causal role of specific pathogens. What is clear, however, is that if infection does play a causal role, the relationship between infection and atherogenesis is extremely complex. The concept that pathogens exert atherogenic effects solely by infecting the vessel wall and exerting direct effects on vascular wall cells is undoubtedly incorrect; inflammatory and immune responses, not requiring residence of the pathogen in the vessel wall, appear to be important mediators (Figure). It also appears that the aggregate number of certain pathogens with which an individual is infected, rather than any individual pathogen, will determine both the infection-related propensity to develop atherosclerosis and the infection-related likelihood of a patient’s course being punctuated by plaque rupture and acute thrombotic arterial occlusion.

References


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