Editorial

Infectious Serology and Atherosclerosis
How Burdensome Is the Risk?

Joseph B. Muhlestein, MD; Jeffrey L. Anderson, MD

In recent years, atherosclerosis has come to be recognized as active and inflammatory, rather than simply a passive process of lipid infiltration.1 Inflammation occurs in response to vascular oxidative stress and injury through known and unknown stimuli. Inflammatory triggers undoubtedly include oxidized and glycosylated products (eg, modified lipoproteins). Given their association with inflammation, infectious agents also are being explored as potential inciters of vascular inflammation and promoters of atherosclerosis.2,3

The role of infection in human atherosclerosis remains elusive. However, the ability of infectious agents to induce several (if not all) of the inflammatory mechanisms active in atherothrombosis has been demonstrated experimentally. Several direct and indirect cellular and molecular mechanisms by which vascular and selected extra-vascular infections may promote atherosclerosis are listed in Table 1 and are discussed elsewhere.2 In response to pathogens, pathogen-induced products (eg, reactive oxygen species, oxidized low-density lipoprotein) or cross-reacting, autologous molecules, local and systemic (circulating) inflammatory mediators are induced (including chemokines, cytokines, and adhesion molecules), inflammatory cells are recruited and proliferate (monocyte/macrophages, T lymphocytes, smooth muscle cells), and proinflammatory, prothrombotic, and matrix-degrading molecules are expressed. Endothelial dysfunction ensues, lipid accumulation is promoted, and plaque growth and, subsequently, destabilization and thrombosis occur.

Potential atherogenic mechanisms have been most extensively explored for Chlamydia pneumoniae (Cpn) and cytomegalovirus (CMV) (and other Herpesviridae). Cpn and CMV infect vascular wall cells (and selected nonvascular cells) and may provoke or accelerate atherosclerosis by a variety of these mechanisms (Table 1). Relative to CMV, an intriguing proposed molecular mechanism is the binding and inhibition of the tumor suppressor gene p53 by an early CMV gene product (IE2-84).2 This mechanism may explain how abortive CMV infection leads to, among other effects, increased expression of growth factors and growth-factor receptors and to smooth muscle cell proliferation, migration, and inhibition of apoptosis.2

Animal models provide further experimental support for a potential role of infection in atherosclerosis. Indeed, the association of Marek’s disease herpes-virus and atherosclerosis in chickens was made more than a half-century ago, providing a first “proof-of-principle” for the infectious theory of atherosclerosis.3 We and others have shown the ability of Cpn to accelerate atherogenesis in predisposed rabbit and murine models; antibiotic therapy (azithromycin) inhibited this response.5

Other Evidence for Infection as a Human Risk Factor

The sources of evidence used to link infection with atherosclerosis are summarized in Table 2. Perhaps the most provocative evidence is the finding of infectious agents or their products within human atherosclerotic plaque. We and others have found evidence for Cpn in coronary and aortic atheromas from autopsy or atherectomy materials in a high percentage of cases (>70%).3,6 Recently, infectious exposure also was demonstrated to predict coronary endothelial dysfunction in patients undergoing angiography.7

Negative association studies also have been reported, however, and it has been difficult to culture viable Cpn organisms and to extract Cpn DNA from tissue specimens. CMV and other herpes viruses or antigens also have been reported within atherosclerotic arteries, although the database is less robust and less consistent. However, CMV (and other agents) also could play a role from distant, nonvascular sites of infection.8

Most associations between candidate pathogens and cardiovascular disease (CVD) have been based on seroepidemiological observations. CVD risk has been associated with seroprevalence to Cpn and, subsequently, to Helicobacter pylori (Hpyl), CMV, other Herpesviridae, and other pathogens. However, early studies often were limited by relatively small size and incomplete adjustment for confounding variables (such as age, smoking, and socioeconomic status). Subsequent studies have suggested either more modest or absent associations. For Cpn, a meta-analysis of 15 prospective trials yielded a combined odds ratio for coronary events of only 1.15 (95% confidence interval, 0.97 to 1.36) for immunoglobulin G (IgG) seropositivity.9 Meta-analyses of studies of Hpyl serostatus also yielded small, non-significant odds ratios (OR) of 1.1 (0.9 to 1.4) and 1.15 (0.96 to 1.37).10,11 CMV seropositivity did not predict primary coronary risk in our database12 or in 3 prospective prevention...
Investigations found an association between the number of interleukin-6. 15 Suppression of atherogenesis with antimicrobials involved in atherogenesis

Experimental induction of cellular, molecular mechanisms known to be evidence for atherogenesis in animal models

Table 1. Cellular and Molecular Mechanisms by Which Infections May Promote Atherosclerosis

<table>
<thead>
<tr>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provokes inflammation and its mediators</td>
</tr>
<tr>
<td>↑ local, systemic (circulating) inflammatory mediators (chemokines, cytokines, adhesion molecules, matrix-degrading proteases [MMPs], etc)</td>
</tr>
<tr>
<td>↑ immune responses directed at pathogens, pathogen-products</td>
</tr>
<tr>
<td>↑ host-directed immune responses (autoimmune, mimickry)</td>
</tr>
<tr>
<td>Induce endothelial dysfunction</td>
</tr>
<tr>
<td>↓ vasodilator function</td>
</tr>
<tr>
<td>↓ anticoagulant, ↑ procoagulant phenotype</td>
</tr>
<tr>
<td>Promote lipid accumulation</td>
</tr>
<tr>
<td>↑ scavenger receptor activity (SMCs, macrophages)</td>
</tr>
<tr>
<td>↓ cholesteryl esterase activity</td>
</tr>
<tr>
<td>Promote smooth muscle cell accumulation</td>
</tr>
<tr>
<td>↑ smooth muscle cell proliferation (p53 inhibition), migration,</td>
</tr>
<tr>
<td>↓ apoptosis</td>
</tr>
<tr>
<td>↑ growth factor/receptor expression</td>
</tr>
<tr>
<td>Destabilize plaque: ↑ MMP expression</td>
</tr>
<tr>
<td>Promote thrombosis: ↑ tissue factor expression, ↑ endothelial dysfunction/platelet activation</td>
</tr>
</tbody>
</table>

Studies (OR 0.91, 0.69 to 1.19). 13 In contrast, CMV did predict secondary risk (death/myocardial infarction) in patients with preexisting coronary artery disease (CAD), especially in those with elevated C-reactive protein14 or interleukin-6. 15

Total Pathogen Burden as an Aggregate Serological Risk Factor

Given the modest and variable predictive value of most tested pathogen candidates, Epstein and colleagues2 proposed the sum of relevant infectious exposures, expressed as a total pathogen burden, as an improved prognostic seromarker of risk. Exposure to a panel of 5 pathogens was tested and found to improve prediction of angiographic CAD in a cross-sectional study and incident events among CAD patients in a succeeding prospective study. 17 Subsequently, European investigators found an association between the number of exposures to a panel of 8 pathogens and cardiovascular mortality in CAD patients in the Atherogene Study. 18 Both studies further found that risk was attributable primarily to seropositivity to the viral pathogens tested (Figure). Neither Cpn nor Hpyl IgG serostatus was individually predictive or contributed to “pathogen burden” significance in either study. Indeed, Rupprecht et al18 proposed “Herpes Burden” (aggregate seropositivity to CMV, herpes simplex virus-1, herpes simplex virus-2, and Epstein-Barr virus) as a more efficient cardiovascular seromarker.

Contributions of the Present Study

To these prior observations, Smieja et al19 present in this issue of Circulation their results of serological associations with CVD risk from a relatively large, prospectively studied cohort from the Heart Outcome and Prevention Evaluation (HOPE) Study. As with the 2 previous studies,17,18 it tests a primarily secondary risk population. Outcomes included nonfatal myocardial infarctions and strokes, in addition to CV deaths. After 4.5 years of follow-up, only CMV serostatus of 4 pathogens tested (Cpn, Hpyl, hepatitis A virus [HAV], CMV) was predictive of CV events (adjusted hazard ratio [HR], 1.24, 1.01 to 1.53). Total pathogen score did not add to predictive value (adjusted HR for 4 versus 1/0: 1.16, 0.83 to 1.62).

Superficially, this latter result seems at odds with those of Zhu et al17 and Rupprecht et al18. However, closer inspection suggests more commonality than difference. All 3 studies failed to find seropositivity to Cpn and Hpyl to individually predict risk or to add to aggregate risk prediction. Each study found predictive value to be carried by viral serologies. Specifically, each found CMV seropositivity to carry significant secondary risk (although relative risk was least in HOPE).

HOPE is limited in testing the concept of viral pathogen burden in that only 2 (compared with 4) serologies were tested. Hence, additional studies should be performed to
assess this proposed aggregate marker of risk. The one qualitative difference in HOPE compared with the study by Zhu et al17 was lack of predictive value of HAV. Given that HAV is the least studied of the viral candidates, this new result indicates the need for additional validation.

Unanswered Questions and Future Directions

Taking these new observations into account, one might ask of infectious serology and atherosclerosis: how burdensome is the risk? A spectrum of response still can be justified. However, some role for infection, even if complex or subtle (acting in concert with traditional risk factors), still appears likely to us, with several caveats.

First, lack of serological association (eg, for Cpn) does not prove lack of a role in atherogenesis. Persistent infection may not be distinguished from resolved infection with persistent antibody. Second, the whole is no better than its parts. Future panels testing total pathogen burden may be improved by emphasizing assays that may more appropriately reflect the extent of ongoing chronic infection. Cpn and Hpyl serologies, although of historic interest, appear to contribute little if any to secondary risk assessment. These may be more appropriately evaluated through direct assessment of organism persistence. In contrast, expanding serology panels to include additional viral pathogens appears promising for future research (eg, prospective testing of a “Herpes Burden”). A third caveat relates to infectious causes versus inflammatory propensity. Serological associations neither prove causality nor indicate mechanisms. Indeed, persistent serological response may actually identify individuals who are more immunologically active rather than more infection-burdened. “Immune reactors” may be at an advantage in an environment where infectious risk is prevalent but at a disadvantage where atherosclerosis dominates.

A fourth issue is the need for better markers of active (if subclinical) or latent (inducible) infection. The Bruneck Study tested directly the association of clinically evident, common subclinical) or latent (inducible) infection. The Bruneck Study but at a disadvantage where atherosclerosis dominates.

First, lack of serological association (eg, for Cpn) does not prove lack of a role in atherogenesis. Persistent infection may not be distinguished from resolved infection with persistent antibody. Second, the whole is no better than its parts. Future panels testing total pathogen burden may be improved by emphasizing assays that may more appropriately reflect the extent of ongoing chronic infection. Cpn and Hpyl serologies, although of historic interest, appear to contribute little if any to secondary risk assessment. These may be more appropriately evaluated through direct assessment of organism persistence. In contrast, expanding serology panels to include additional viral pathogens appears promising for future research (eg, prospective testing of a “Herpes Burden”). A third caveat relates to infectious causes versus inflammatory propensity. Serological associations neither prove causality nor indicate mechanisms. Indeed, persistent serological response may actually identify individuals who are more immunologically active rather than more infection-burdened. “Immune reactors” may be at an advantage in an environment where infectious risk is prevalent but at a disadvantage where atherosclerosis dominates.

A fourth issue is the need for better markers of active (if subclinical) or latent (inducible) infection. The Bruneck Study tested directly the association of clinically evident, common infections (acting at a distance) and atherosclerosis progression.29 The presence of chronic respiratory, urinary tract, dental, or other infection amplified by over 4-fold the risk of developing new carotid lesions.

Where infection is not clinically evident, the presence of both seropositivity and inflammatory markers (eg, C-reactive protein or interleukin-6) may better define smoldering, risk-associated vascular infection.14,15 Clinically measurable, circulating products marking active infection would be useful. Cpn heat shock protein, produced during chronic, persistent infections and present in atherosclerotic plaque, deserves further evaluation in this regard.

Finally, what about therapeutic trials targeting CAD patients with inflammatory and infectious risk factors (including seropositivity)? Despite initial enthusiasm, antibiotics targeting Cpn have not proven universally beneficial. Nevertheless, the large (n = 7747) secondary prevention study of azithromycin (Weekly Intervention with Zithromax for Atherosclerosis and Related Disorders [WIZARD]) offers a ray of hope: Treatment for 3 months reduced death/myocardial infarction rates for 6 months (although not for 3 years). Clearly, further studies are needed. As with serostatus, antimicrobial and vaccine trials by themselves cannot prove or disprove the infectious hypothesis. Thus, a large number of questions remain unanswered regarding the infectious theory of atherosclerosis. Nevertheless, progress is gradually occurring. In this context, the present study is a useful, incremental addition to our understanding of this most prevalent yet far from completely understood disease.

References


Key Words: Editorials  □ atherosclerosis □ risk factors
Infectious Serology and Atherosclerosis: How Burdensome Is the Risk?
Joseph B. Muhlestein and Jeffrey L. Anderson

_Circulation_. 2003;107:220-222
doi: 10.1161/01.CIR.0000043909.78380.A0

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/107/2/220

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located,
click Request Permissions in the middle column of the Web page under Services. Further information about
this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/