Current Perspective

Infection and Atherosclerosis
Emerging Mechanistic Paradigms

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Abstract—Although definitive proof of a causal role of infection contributing to atherogenesis is lacking, multiple investigations have demonstrated that infectious agents evoke cellular and molecular changes supportive of such a role. Moreover, both Chlamydia pneumoniae and cytomegalovirus exacerbate lesion development in animal models of atherosclerosis and restenosis. The fact that multiple pathogens have been associated with atherosclerosis implies that many "atherogenic" pathogens exist, and recent data suggest that the risk of atherosclerosis conveyed by infection relates to the number of atherogenic pathogens with which an individual is infected. It also is evident that variability in host susceptibility to the atherogenic effects of pathogens exists; this variability appears to be related at least in part to whether the host can generate an immune response that successfully controls pathogen inflammatory activity and in part to the specific pattern of immune response—humoral or cellular. The latter may relate to host capacity to control pathogen activity and to a pathogen-induced autoimmune component of the atherogenic process. Additional animal and human studies are necessary to further test the validity of the infection/atherosclerosis link and to provide more insight into the mechanisms by which infection may contribute to atherosclerosis, information critical for devising strategies to reduce or eliminate any contribution to atherosclerosis caused by infection. (Circulation. 1999;100:e20–e28.)

Key Words: cytomegalovirus □ Chlamydia pneumoniae □ immune system □ risk factors □ coronary disease □ atherosclerosis

As many as 50% of patients with atherosclerosis lack currently identified risk factors (such as hypertension, smoking, hypercholesterolemia, and diabetes), an observation indicating that additional factors predisposing to atherosclerosis are as yet undetected. Discovery of such factors, along with their accompanying mechanisms of action, would have profound implications for the development of new therapeutic strategies that could reduce the devastating impact this disease is having in the western world and is beginning to have in countries where the populations are adopting western lifestyles.

Injury to the vessel wall and the associated inflammatory response to injury are now generally recognized as the essential components of atherogenesis.1–10 However, the triggers that initiate and sustain the inflammatory process have not been definitively identified, a fact that has fueled efforts to discover the inflammatory triggers. Among the candidate triggers are oxidized LDL and heat shock proteins (HSPs). These components of the atherosclerotic wall are believed by some investigators to elicit an inflammatory response.11–14 Antibodies to these proteins also develop, and although controversial, some studies suggest that these antibodies may play a role in causing autoimmune-induced damage to the vessel wall.15 Another candidate trigger of both inflammatory and autoimmune responses—the subject of this perspective—is infection.

Several excellent reviews on the roles of infection16–24 and autoimmunity25 in atherogenesis have appeared recently. The purpose of the present article is not to repeat these extensive efforts but to focus more on the cellular, molecular, and immune mechanisms by which infection might contribute to atherosclerosis. We also explore the concept that if autoimmunity is involved in the development of atherosclerosis, the stimuli eliciting the autoimmune responses are probably triggered, at least in part, by infection.

Infection as a Contributor to Atherosclerosis
Evidence began accumulating >25 years ago suggesting that herpesviruses may possibly play a role in the development of atherosclerosis. It was found that Marek’s disease virus, an avian herpesvirus, caused typical atherosclerotic lesions in chickens,26 and when smooth muscle cells (SMCs) were infected with the virus in vitro, cholesterol accumulated.27

Presence of Pathogens in the Vessel Wall and Seroepidemiological Studies
Evidence in humans suggesting that infection predisposes to atherosclerosis derived from studies demonstrating that infectious agents reside in the wall of atherosclerotic vessels (initially, cytomegalovirus [CMV] and later Chlamydia pneumoniae), and seroepidemiological studies demonstrating an association between the pathogen-specific IgG antibodies and
atherosclerosis.74,28–31 The first such seroepidemiological associations published related to CMV, which were later followed by the publication of similar associations between atherosclerosis and herpes simplex virus (HSV) types 1 and 2, *C pneumoniae, Helicobacter pylori,* and most recently, hepatitis A virus.52–43 Moreover, prospective studies of the relation between CMV seropositivity and the subsequent development of restenosis after coronary angioplasty revealed increased incidence of restenosis in patients seropositive for CMV.44,45

The data demonstrating a serological or anatomic association between each of these pathogens and atherosclerosis are not entirely consistent. Although some studies show a significant association with coronary artery disease (CAD) or restenosis,24,32–45 others do not.24,39,43,46–53 Moreover, a pathogen resident in an atherosclerotic vessel may be just an “innocent bystander” rather than a causally relevant agent.

**Direct Effects of Infectious Agents on Cellular Components of the Vessel Wall That Could Predispose to Atherosclerosis**

Additional evidence favoring causality has derived from investigations demonstrating that infection of cells residing in the vessel wall cause cellular and molecular changes conducive to atherogenesis.

**SMC Accumulation**

**Stimulation of SMC Proliferation**

Because SMCs in vitro are permissive for CMV replication, which is followed by cell death, it has not been possible to demonstrate any direct effect of infection on SMC proliferation. However, many of the important cellular effects of CMV derive from expression of its immediate-early (IE) gene products in the absence of early and late gene expression and of viral replication. In this type of infection, called an abortive infection, death of the host cell does not occur. It is likely that abortive infections are of biological relevance, providing a mechanism whereby intracellular pathogens contribute to disease progression.

In vitro models of abortive infection can be achieved by infecting cells of 1 species with CMV derived from another. (There are several related types of CMV, and the ability of the different types of CMV to replicate in cells is specific for each host species.) For example, Albrecht and associates54 demonstrated that when hamster embryo fibroblasts are infected with human CMV (causing an abortive infection), the rate of DNA replication and mitotic activity is increased. Moreover, it recently has been shown that infection of rat SMCs with human CMV (also causing an abortive infection) leads to SMC replication.55 Evidence that CMV infection in vivo leads to cellular proliferation was suggested by studies of a rat model of aortic allograft transplantation. CMV infection was associated with a doubling of the proliferation rate of SMCs in the allograft neointima.56

One molecular mechanism by which CMV may increase cellular proliferation is through inhibition of the tumor suppressor gene, p53; the p53 gene product inhibits cell cycle progression57 and therefore cell proliferation.58 IE2–84, 1 of the IE gene products of CMV, is capable of binding to p53 and inhibiting its transcriptional activity.59,60 The inhibitory effect of CMV infection on p53 activity appears to be mediated, at least in part, by the exclusion of p53 from the nucleus by cytoplasmic sequestration.61

Another mechanism by which CMV infection could lead to cellular proliferation is stimulating the secretion of growth factors or increasing the expression of growth factor receptors. Human fibroblasts, when infected with CMV, release a factor or factors that stimulate DNA synthesis of BALB/c 3T3 cells62 and proliferation of human endothelial cells.63 In the rat model of aortic allograft transplantation referred to above,56 it was found that CMV infection is associated with an almost doubling of basic fibroblast growth factor (bFGF; FGF2) mRNA expression, and with increased expression of mRNAs of acidic fibroblast growth factor (aFGF; FGF1), platelet-derived growth factor-BB (PDGF-BB), and epidermal growth factor. CMV infection of SMCs has recently been shown to increase PDGF receptor expression.55

**Inhibition of Apoptosis**

CMV infection inhibits apoptosis in human endothelial cells61 and in HeLa cells, an effect caused at least partly by the IE gene products of the virus.64 One of the mechanisms by which this occurs is probably inhibition of the p53-modulated apoptotic program.57 Thus, 1 of the IE gene products of CMV, IE2–84, binds to and inhibits the transcriptional activity of p53.59,60 Consistent with this activity was the finding that overexpression of IE2–84 (with an adrenoviral vector used for gene transfer) protected human coronary artery SMCs from p53-induced apoptosis.65 That such an effect is not limited to CMV was demonstrated by the finding that Chlamydia-infected cells are resistant to apoptosis induced by a wide variety of agents.66 If inhibition of apoptosis occurred in infected SMCs of the vessel wall, such an effect could lead to excessive accumulation of these cells, thereby contributing to an increase in the mass of restenosis/atherosclerosis lesions.

**Increased SMC Migration**

One mechanism contributing to increasing neointimal mass in both atherogenesis and restenosis is SMC migration from the media and adventitia to the developing neointima. Using a standard in vitro assay system for cell migration, we previously found that infection of rat SMCs with human CMV (to produce an abortive infection) increases SMC migration.55 This was associated with enhanced SMC PDGF receptor expression, a finding compatible with the demonstration that 1 mechanism responsible for SMCs migrating toward the lumen after vascular injury involves the PDGF system.1

**Lipid Accumulation**

In the 1970s, Minick and associates26 and Fabricant et al27 demonstrated that infection of chickens with Marek’s disease virus (an avian herpesvirus) caused atherosclerosis-like lesions in the coronary arteries and aortas and increased accumulation of cholesterol in both intracellular and extracellular sites. Hajjar et al67 demonstrated a potential mechanism for this effect by showing that HSV infection of human
SMCs decreases lysosomal and cytoplasmic cholesterol ester hydrolytic activity.

Enhanced activity of the scavenger receptor represents an additional mechanism by which pathogens could increase lipid accumulation. We demonstrated that infection of human SMCs with CMV increases uptake of oxidized LDL, an effect mediated, at least in part, by the class A (type I and II) scavenger receptor.68 The increased uptake of oxidized LDL appears mediated by an IE gene product, because this effect was also seen when rat SMCs were infected with human CMV (leading to an abortive infection with expression of only the IE genes of CMV). This conclusion was supported by the finding that 1 of the IE gene products (IE1–72) has the capacity to increase the transcriptional activity of the class A scavenger receptor promoter.68 In addition, C pneumoniae infection of monocyte-derived macrophages incubated with LDL increases the number of foam cells and the accumulation of cholesteryl esters.69 Unlike the CMV findings, it appeared that these Chlamydia-induced changes are not mediated by scavenger receptors, because the response was not attenuated by the scavenger receptor ligand fucoidan.

**Endothelial Dysfunction**

**Procoagulant Effects**

Thrombosis contributes importantly to the development of atherosclerosis and to the precipitation of acute coronary events. Normal endothelium has multiple antithrombotic mechanisms, among which are the capacity of the cells to synthesize heparin sulfate, prostacyclin, nitric oxide, plasminogen activator, and thrombomodulin. Several studies, particularly those from the laboratories of Hajjar et al67 and Vercellotti et al,21 have now demonstrated that infectious agents can alter endothelial cells from a phenotype that is normally anticoagulant to 1 that is procoagulant. For example, HSV infection of endothelial cells increases endothelial cell synthesis of tissue factor, the rate of thrombin generation on the cell surface, and platelet adherence while it decreases prostacyclin and thrombomodulin generation.70–72 CMV infection of endothelial cells also causes procoagulant effects.73

**Inhibition of Vasodilator Function**

A critical function of endothelial cells is to modulate vascular tone, and loss of this function is believed to be 1 of the earliest manifestations of CAD. A recent study has demonstrated that individuals infected with CMV (as evidenced by anti-CMV antibodies) have impaired endothelium-mediated coronary vasodilator responses.74 The endothelial vasodilatory dysfunction seemed to be related to both nitric oxide-- and non–nitric oxide–mediated pathways.

**Increased Expression of Cytokines, Chemokines, and Cellular Adhesion Molecules**

Atherogenesis is believed to develop and progress in response to a sequence of events triggered by the response to vascular injury.1 Various types of injury have been shown to affect the endothelium, causing increased production of reactive oxygen species (ROS), cytokines, chemokines, and cellular adhesion molecules. ROS contributes to oxidation of LDL, a primary player in the development of atherosclerosis, and chemokines and cellular adhesion molecules lead to the attraction and adhesion of monocytes to the vessel wall. The adhering monocytes migrate to the subintimal space, where they differentiate into macrophages and produce their own proinflammatory, proatherosclerotic molecules. The processes leading to the expression of these cytokines, chemokines, and adhesion molecules can be augmented by infection, because CMV and HSV infection of cells causes expression of these molecules.75–77 Moreover, CMV infection of SMCs leads to the development of ROS.78

**Direct Effects of Infectious Agents on Components of the Vessel Wall**

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<tr>
<th>SMC accumulation</th>
<th>Lipid accumulation</th>
<th>Endothelial dysfunction</th>
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<tr>
<td>pS3 Inhibition</td>
<td>Cholesterol esterase hydrolytic activity</td>
<td>Procoagulant effects</td>
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<td>↑ SMC proliferation</td>
<td>↑ Scavenger receptor activity</td>
<td>Impaired vasodilator function</td>
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<td>↓ Apoptosis</td>
<td>↑ Expression of chemokines, cytokines, and adhesion molecules</td>
<td>Targeted to pathogen</td>
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<td>↑ Growth factor expression</td>
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**Delivery of Pathogen to the Vessel Wall**

Both CMV and C pneumoniae are present in atherosclerotic arteries,24,28–31 and CMV DNA has been found in restenosis lesions.59 It is possible that these and other pathogens directly infect the vessel wall and persist in a latent state, produce an abortive infection, or replicate at a low (and possibly intermittent) level. An alternative possibility focuses on the circulating monocyte as a “Trojan horse,” a vehicle for delivery of pathogen to the vessel wall. That this can occur is indicated by the facts that these cells harbor latent CMV79–84 and that C pneumoniae is capable of replicating in a macrophage cell line.85,86

With CMV, it is believed that the virus infects monocyte precursors present in bone marrow and that the viral genome persists in these cells.79,80 Given that CMV infection develops in bone marrow transplant recipients seronegative for CMV but whose donors are CMV seropositive, it is likely that myelomonocytic precursor cells act as a reservoir of CMV and that circulating monocytes then act as a vector, delivering the virus to sites of vascular inflammation or injury. For Chlamydia, it is believed that pulmonary alveolar macrophages act as the vector, picking up the pathogen during the course of a pulmonary infection.

Although Chlamydia is capable of replicating in monocytes/macrophages,85,86 this is not the case with CMV.
Despite the presence of viral genome in circulating monocytes, viral gene products are not expressed in such cells (ie, the virus is in a latent state). Expression of IE viral gene products occurs only after differentiation of monocytes to form macrophages, which happens after the monocytes have entered the subintimal space.\(^\text{52,53}\) In this regard, it has been shown that such constituents of the vessel wall as endothelial cells, SMCs, and oxidized LDL increase the activity of the major immediate early promoter of CMV.\(^\text{84}\) Thus, CMV and perhaps other pathogens may be delivered by circulating monocytes to sites of vascular injury where the vascular milieu contributes to pathogen reactivation, resulting in the induction of pathogen-related effects that contribute to atherogenesis.

**Systemic Alterations Produced by Infectious Agents That Could Predispose to Atherosclerosis Without the Need for Pathogen Residence in the Vessel Wall**

Given the evidence outlined above, it would be reasonable to conclude that a set of mechanisms by which infection contributes to atherosclerosis consists of direct effects of the pathogen on the vessel wall mediated by mechanisms requiring the actual presence of the pathogen locally (the Table). However, other mechanisms not requiring local residence are most likely also operative. For example, infection causes a spectrum of systemic effects, producing fever, malaise, myalgias, and a panoply of signs and symptoms, including alterations in the circulating levels of such factors as cytokines, acute-phase reactants, white blood cells, and responses mediated by the immune system, such as an increase in antibodies targeted to the invading pathogen. These systemic manifestations of infection can be either profound or subtle; importantly, however, these changes provide mechanisms other than local residence-requiring direct vascular effects, whereby pathogens could influence the course of atherosclerosis.

That infection might exert effects on an injured vessel wall that do not necessitate pathogen residence in the vessel is suggested by the results of studies to determine whether CMV infection increases the neointimal response to vascular injury in the rat.\(^\text{57,88}\) Although rats infected with CMV manifested an increased neointimal response to injury, the virus was essentially absent from the injured segment of the vessel; neither replicating virus nor viral proteins could be detected despite the fact that replicating virus could be isolated from such tissues as the spleen and salivary gland.

In the following section, we discuss the mechanisms by which such indirect effects might occur. Because little experimental data exist, the ensuing discussion is meant to be exploratory and hypothesis generating.

**Immune Responses Targeted to Self-Proteins Located in the Vessel Wall: Potential Role of Molecular Mimicry**

Molecular mimicry, resulting in the triggering of an immune response targeted to self-proteins, has been proposed as a mechanism responsible for the development of autoimmune diseases.\(^\text{89–93}\) The concept of molecular mimicry requires infection by a pathogen that contains peptide sequences homologous with host protein. The immune response, although initiated by the invading pathogen and targeted to pathogen antigens, also becomes an autoimmune attack against host tissues containing the cross-reacting peptide sequences. In this model of disease, immune-mediated host tissue injury can occur even if the responsible pathogen is not present in the target tissue, a mechanism confirmed by the elegant studies from Oldstone’s laboratory.\(^\text{94}\) Molecular mimicry, although not definitely proven as a cause of human disease, has been implicated in such autoimmune diseases as type 1 diabetes mellitus, multiple sclerosis, and Guillain-Barré syndrome,\(^\text{95–98}\) and more recently has been implicated in the development of cardiomyopathies.\(^\text{99}\)

The most suggestive evidence to date indicating a role of molecular mimicry-induced autoimmune mechanisms in atherosclerosis derives from the important studies of Wick and associates.\(^\text{25,100–103}\) They pointed out that the vascular wall is subject to various stresses that induce HSP expression. These stresses include hypercholesterolemia, elevated homocysteine levels, cytokines, free radicals, circulating products derived from smoking, and mechanical stresses such as hypertension. They then hypothesized that although the induction of HSPs is a cellular defense mechanism preventing denaturation of cellular proteins caused by perturbations of the cell’s environment, the marked overexpression of HSPs may cause them to act as “cryptic antigens,” inducing an autoimmune reaction and thereby contributing to the development of atherosclerosis.

These investigators also emphasized that HSPs are a family of proteins that are highly conserved across all species, including bacteria.\(^\text{25,100}\) They therefore raised an alternative mechanism by which an autoimmune response to HSPs could occur: The immune response stimulated by infection and targeted to the infecting pathogen could, because of the high degree of amino acid sequence homology between pathogen and host HSPs, also target self-HSPs. Although a series of studies by these and other investigators make a compelling case for a possible role of autoimmunity in atherosclerosis, particularly because the immune response targets HSP,\(^\text{25,100–105}\) there are no direct data implicating infection, via molecular mimicry, as the responsible trigger for this autoimmune response.

Other data indirectly support the molecular mimicry/autoimmunity mechanism as being operative in atherosclerosis. Autoantibodies to certain cytokines have been detected in apparently healthy individuals.\(^\text{106,107}\) That molecular mimicry may be involved in stimulating the expression of these antibodies is suggested by the fact that Epstein-Barr virus contains a peptide sequence homologous to IL-10, and Kaposi’s sarcoma–associated herpesvirus contains an IL-6 homologous sequence.\(^\text{108}\) CMV contains proteins homologous to chemokine receptors.\(^\text{109–111}\) In addition, in preliminary studies from our laboratory, we demonstrated that susceptibility to CMV-related CAD in women occurs in the subgroup with a humoral immune response to CMV infection, whereas those women with an immunodominant cellular response evidenced no such susceptibility.\(^\text{112}\) This finding raises the possibility of an infection-driven, autoimmune-mediated antibody component of disease.
Finally, some studies relate increased prevalence of periodontal disease to increased prevalence of CAD.\textsuperscript{24,113–118} One hypothesis driving these studies is that chronic periodontal infection leads to CAD through the production of a long-term inflammatory response. In this regard, elevated levels of specific salivary IgA antibodies against mycobacterial HSP65 were found to be significantly increased in patients with gingivitis.\textsuperscript{119} Mycobacteria HSP65 is highly homologous to human HSP60. The investigators speculated that the pathogens causing the gingivitis expressed HSP; this then stimulated an IgA antibody response in the host, which they suggested may serve as cross-reactive autoantigens that contribute to the gingivitis. It is equally plausible that the development of IgG antibodies, if present, could contribute to an autoimmune-induced atherosclerotic process.

**Circulating Pathogen-Derived or Pathogen-Stimulated Factors That Could Induce Changes in the Vascular Wall**

Studies have shown that various circulating pathogen-derived or pathogen-stimulated factors can elicit changes in monocyte/macrophages that could be proatherogenic and that might even contribute to plaque instability. For example, bacterially derived lipopolysaccharide and chlamydial or human HSP induce tissue necrosis factor-α secretion by macrophages,\textsuperscript{120} a cytokine that stimulates endothelial cells to express adhesion molecules. These factors also stimulate endothelial cells and SMCs to express interleukin (IL)-1 mRNA\textsuperscript{121} and IL-6\textsuperscript{122} and preferentially stimulate endothelial cells to express E-selectin and ICAM-1.\textsuperscript{122} In addition, several surface antigens of *Porphyromonas gingivalis*, a Gram-negative bacterium causally related to periodontitis, stimulate BALB/c peritoneal macrophages to secrete IL-1-β.\textsuperscript{123} Tissue necrosis factor-α, IL-1, IL-6, and ICAM-1 have all been related to atherogenesis.

The above responses can be considered as possibly contributing to the development of atherosclerosis. In addition, lipopolysaccharide, chlamydial HSP, and human HSP have also been shown to stimulate the secretion of matrix metalloproteinases.\textsuperscript{120} These enzymes have the capacity to degrade connective tissue and thereby are believed to predispose, by weakening the fibrous cap of atheromatous lesions, to plaque rupture. Thus, pathogens not only may stimulate responses that are proatherogenic but also may contribute to the precipitation of plaque instability and rupture, the most common cause of death from CAD.

Although elevated circulating C-reactive protein (CRP) levels have usually been taken as a systemic marker for inflammation, some evidence also suggests that this acute-phase reactant may play some causal role in atherogenesis. For example, CRP has been shown to colocalize with the terminal complement complex in the intima of early human atherosclerotic lesions.\textsuperscript{124} This finding suggests some causal link between elevated levels of this acute-phase reactant and atherogenesis, given that CRP activates complement and that complement activation has been implicated in the development of the atherosclerotic plaque.\textsuperscript{125}

The potential role of some circulating, infection-related factor predisposing to plaque destabilization received additional support from the finding that acute respiratory-tract infections are associated with an increased risk of acute myocardial infarction. The data revealed a relative risk of 2.7 for acute myocardial infarction occurring in relation to an acute respiratory-tract infection in the preceding 10 days.

**Individual Variations in Host-Pathogen Interactions That Influence Inflammatory Activity and the Susceptibility to Pathogen-Induced Contribution to Vascular Disease**

**Inflammatory Activity**

As noted above, although many epidemiological studies have demonstrated that seropositivity to several infectious agents is associated with increased CAD risk, the data are inconsistent, with other studies showing no increased risk. One possible explanation for this disparity is that whether prior infection, as reflected by seropositivity, contributes to CAD risk may depend, at least in part, on the capacity of the host to suppress pathogen inflammatory activity. The validity of this concept is suggested by a study demonstrating that (1) although some individuals seropositive to CMV have elevated CRP levels, others, CRP is within the normal range and (2) highest CAD prevalence occurs in the subgroup with combined CMV seropositivity and elevated CRP levels.\textsuperscript{126} When adjusted for CAD risk factors, the ORs 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 (\(P=0.01\)).

**Humoral and Cellular Immune Responses**

A second possible explanation for the disparity in seroepidemiological studies may relate to the type of immune response mounted by the host to CMV infection. Thus, CMV appears to be a significant predictor of CAD in women.\textsuperscript{112} Importantly, however, the data showed that the type of immune response to CMV determines whether CMV predisposes to CAD. In women evaluated for CAD by coronary angiography, blood samples were tested for humoral (Ab\(^+\)) and cellular (Tc\(^+\)) responses to CMV. Susceptibility to CMV-related CAD was limited to those women with a humoral immune response to CMV (Ab\(+/Tc^−\) or Ab\(+/Tc^+\)). The prevalence of CAD in those without an antibody response but with a cellular response (Ab\(−/Tc^+\)) was similar to that in women without apparent prior CMV infection (Ab\(−/Tc^−\)).

**Pathogen Burden as a Determinant of Elevated CRP and CAD Risk**

If infection plays a role in atherogenesis, it would be highly likely that multiple pathogens would be causally involved and, importantly, that CAD risk would relate to the aggregate pathogen load (what we have called pathogen burden). This hypothesis was tested in a group of individuals being evaluated for CAD by coronary angiography. The relationship of pathogen burden (aggregate number of a panel of 5 pathogens to which an individual had been exposed, as determined by seropositivity) to CRP levels and CAD risk was determined.\textsuperscript{127} The pathogens tested were CMV, Chlamydia,
hepatitis A virus, and HSV types 1 and 2. A dose-response relationship was found: As the number of seropositive responses an individual had increased, CRP levels and CAD prevalence increased. Although these results must be confirmed in a prospective study, it appears that insofar as infection does predispose to the development of atherosclerosis, CAD risk is related to the aggregate number of potentially atherogenic pathogens to which an individual has been exposed.

**Animal Models of Atherosclerosis and Infection**

Given that atherosclerosis is a multifactorial disease, Koch’s postulates to establish causality will never be satisfied. These postulates were based on infectious diseases caused by a single pathogen, requiring that all patients with the disease must have evidence of being infected with the casual agent and that all individuals infected with the agent develop the disease. In contrast, the concept developed in this article is that infectious agents are risk factors for atherosclerosis, neither necessary nor sufficient for disease development. With this disease paradigm, proof of causality can be achieved only in terms of probability rather than as certainty.

One way to determine a higher probability of causality is to establish that the cellular and molecular changes induced by infection predispose to atherosclerosis. The studies detailed in this review, in aggregate, present a compelling case. However, the only direct evidence that can prove a particular infectious agent has the capacity to contribute to atherosclerosis is to demonstrate causality in an animal model of atherosclerosis. This has been achieved by several investigations.

*Chlamydia pneumoniae* has been shown to increase atherosclerosis in both a rabbit model and a mouse apoE knockout model of atherosclerosis. Similar results have been demonstrated for CMV infection in the apoE knockout model. In addition, as noted above, in an acute injury model (balloon injury of a rat carotid artery), infection with CMV increases the resulting neointimal response.

**Summary and Conclusions**

In summary, there is an increasing body of information compatible with the concept that infectious agents contribute to the development of atherosclerosis and to plaque instability and rupture. These possibilities are strengthened by the multiple investigations providing cellular and molecular mechanistic support for such roles and by investigations demonstrating that certain pathogens can exacerbate lesion development in animal models. It is equally clear, however, that no simple relationship exists between infection and atherogenesis. As with other infectious diseases, the propensity for a given infectious agent to contribute to the development of atherosclerosis will depend on host-pathogen interactions. This in turn will determine susceptibility to pathogen inflammatory activity and therefore to atherogenic potential. And if additional studies support an important autoimmune component to atherogenesis, the type of humoral and cellular immune responses mounted by the host to infection may determine whether a particular pathogen will contribute to atherosclerotic lesion development in a particular host (the Figure).

As the case for a role of infection grows, the existing paradigms to explain the natural history of the clinical manifestations of CAD can be expanded. For example, it was appreciated many years ago that atherosclerosis does not progress as a slow gradual process; rather, the underlying progressive nature of the disease is punctuated by intermittent acute exacerbations. This has been appreciated both clinically and by morphological investigations of atherosclerotic plaques. The concept that infection plays a role in atherosclerosis fits this model very well. Thus, by producing persistent subclinical infections causing a chronic inflammatory state (leading to activation of inflammatory cells and of immune processes), infectious agents could contribute to gradual plaque enlargement. However, punctuation of this low-grade persistent activity by recurrent acute infections or intermittent reactivation of latent infections, whether clinically overt or silent, could cause acute exacerbations of the atherosclerotic process, including plaque rupture and thrombotic occlusion.

It must be emphasized that evidence definitively proving a causal role of infection in atherosclerosis is lacking. Although the temptation is great to initiate clinical trials testing whether interventions targeted to pathogens will decrease progression of atherosclerosis or its complications, we cannot lose sight of the importance of performing additional animal and human studies that can further test the validity of the concept and provide more mechanistic information as to how pathogens may predispose to atherogenesis. This additional information will be critical for devising intelligent and effective future strategies to reduce or eliminate any contribution to atherosclerosis caused by infection.

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