Bacteria and Coronary Atheroma
More Fingerprints but No Smoking Gun
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For the better part of 2 decades, scientists from many disciplines have explored the role of innate and acquired immunity in mediating vascular atherosclerosis. Remarkably, many of the distal mechanisms involved in the chronic inflammatory process that is atherosclerosis are mediated by the same cellular mechanisms that are “primed” to protect the body from microbial invasion. This recognition against bacterial pathogens is embodied in the innate immune system that encodes more than 100 germ line–derived pattern recognition receptors (PRRs) designed to recognize highly conserved pathogen-associated molecular patterns. These receptors trigger effector mechanisms designed to phagocytize the foreign antigens and to call in reinforcements (memory B and T cells) provided by the adaptive immune system. Critical to the understanding of the role of the innate immune system in the pathogenesis of vascular atherosclerosis is the growing evidence that PRRs recognize “neoantigens” through the process of molecular mimicry. There are at least 4 candidate neoantigens that have been implicated in the atherosclerotic process. These include heat shock proteins, β2-glycoprotein-I, and, most notably, oxidized low-density lipoprotein (LDL) and related phospholipids. Oxidized LDL and phospholipids stimulate both natural immunoglobulin M antibodies, such as EO6, or other secreted PRRs, such as C-reactive protein, that have been identified as markers and mediators of chronic inflammation in atherosclerosis. Equally important has been the understanding of the role of a specialized group of PRRs known as scavenger receptors (CD36), which are present on monocytes, macrophages, and neutrophils that mediate the uptake of oxidized LDL and the generation of classic foam cells within atherosclerotic plaque. More recently, a third set of PRRs has been identified as central in the atherosclerosis process, known as Toll-like receptors (TLRs). These transmembrane signaling receptors are highly expressed on professional antigen-presenting cells, endothelial cells, and the phagocytic natural killer cells. TLRs recognize both oxidized LDL and lipoteichoic acid in the cell wall of gram-positive organisms, flagellin, and bacterial and viral RNA and DNA remnants. Specific receptors, such as TLR4, are activated naturally by microbial ligands such as lipopolysaccharide and heat-shock protein 60. Notably, lipopolysaccharide is a potent inhibitor of the KLP-2 family of transcription factors implicated to confer an atheroprotective role in the endothelium. This raises the possibility that bacterial infection may “condition” as opposed to infect the vasculature. TLR signaling involves upregulation of interleukin-1β and interleukin-8, resulting in increased adhesion molecule expression (vascular cell adhesion molecule/monocyte chemotactrant protein-1) as well as upregulation of interferon-γ by natural killer cells, leading to macrophage activation. As such, either exogenous microbial pathogen-associated molecular patterns or endogenous neoantigens or both have been shown to activate innate immune responses characteristic of the atherosclerotic process.

Placing Bacterial Fingerprints at the Crime Scene
It comes as little surprise that chronic microbial infection is implicated in the pathogenesis of coronary atherosclerosis. In addition to the aforementioned body of evidence linking the innate immune system in the pathogenesis of atherosclerosis, there is also considerable experimental and epidemiological evidence that provides a probable cause for this consideration. The search for additional triggers to the atherosclerotic process has been further fueled by the growing recognition that the incidence of atherosclerosis is not fully explained by conventional risk factors. To date, the experimental evidence of direct infection of cellular constituents of the vessel wall has been strongest for cytomegalovirus, a herpes virus that can directly infect endothelial cells and also stimulate accumulation of oxidized LDL in smooth muscle cells. However, epidemiological observations support a stronger role for Chlamydia pneumoniae and, more recently, Helicobacter pylori and periodontal bacteria in the pathogenesis of atherosclerosis. A causative association between C pneumoniae and atherogenesis gains some plausibility as the result of the organism’s unique ability to persist in tissue in a dormant phase for prolonged periods, unlike the majority of prokaryotic bacteria.

In this issue of Circulation, the article by Ott and colleagues adds to the growing number of observational studies linking bacterial antigens to atherosclerotic plaque. Using human specimens obtained during coronary atherectomy, these authors identified the “fingerprints” of more than 50 different bacteria species including common organisms such as Staphylococcus and Streptococcus, as well as gram-
negative organisms including *Proteus* and *Klebsiella* from more than 1500 clones. The bacterial diversity in the atheroma was strikingly high, with a range of 5 to 22 bacterial signatures present in a single specimen. Notably, bacterial pathogens previously implicated in coronary atherosclerosis such as *C pneumoniae* were present in 51% of the samples, whereas other commonly implicated pathogens (*Mycoplasma* and *Helicobacter*) were not observed.

A major strength of the study was the use of several powerful molecular techniques to corroborate the findings, including clone libraries of bacterial DNA, denaturing gradient gel analysis, and fluorescent in situ hybridization using DNA riboprobes. In addition, the study was controlled by careful attention paid to the elimination of blood contaminants from the catheter-derived samples and the examination of artefactual tissue from postmortem and donor hearts in which clinical atherosclerosis was excluded.

As with previous observational studies in this experimental domain, there are several potential confounders. Prominent among them are the possible interactions between bacterial infections and common atherosclerotic risk factors. An example would be the fact that smoking is associated with both greater risk of respiratory tract infections with *Staphylococcus*, *Streptococcus*, and *Chlamydia* species as well as of atherosclerosis and may thus confound the association. We know little about the conventional risk factors in the current study population and so confounding factors remain a concern in the association. In at least 1 study, the prevalence of *C pneumoniae* DNA in carotid plaques was 96% in smokers but only 36% in nonsmokers. A second concern is selection bias in that atherectomy samples are usually taken from proximal, eccentric, and complex coronary lesions. It would be important to examine less complex coronary atheromata in diverse segments of the coronary artery to confirm similar association with such diverse bacterial fingerprints.

However, a curious finding from the control samples may shed important light as to whether bacterial fingerprints in vascular plaques are a cause or a consequence of atherosclerosis. In the samples taken from potential heart donors and postmortem samples from patients with malignancy who may have been immunocompromised, no bacterial DNA was observed. Although the control samples were screened for clinical coronary artery disease, it nonetheless seems likely that these patients would have fatty streaks and at least mild coronary atherosclerosis, given their age range (30 to 70 years). These data suggest that bacterial infections are not involved in early atherosclerotic disease and probably are evident only after significant vascular perturbations related primarily to atherosclerosis. Recent evidence confirms a greater prevalence of implicated microbes in complicated or advanced lesions. As such, it seems highly unlikely that bacterial infections are either necessary or sufficient to cause coronary atherosclerosis but more probably may participate or promote aspects of atherogenesis in conjunction with conventional triggers such as oxidized LDL.

**Implications**

The finding of more than 50 bacterial fingerprints in these atherectomy samples raises several new considerations in unraveling the pathogenesis of bacterial infections in atherosclerosis. First, the findings suggest that a “conspiracy” of bacterial pathogens as opposed to a single infection is involved in atherogenesis, which may help to explain the inefficacy of antibiotics, such as macrolides or fluoroquinolones, in clinical trials. It may be that the burden of bacterial infections rather than a single pathogen is the key to the progression of coronary atherosclerosis. We have no knowledge in the present study as to whether the subjects were particularly prone to bacterial infections. This would place bacterial infections in the category of risk factors for rather than pathological agents in coronary atherosclerosis. Second, it seems highly improbable that as many as 12 different bacteria actively infected the coronary vasculature, leading to inflammation and atherosclerosis. Rather, the plethora of bacterial fingerprints raises the possibility that these sensitive molecular probes are picking up pathogen-associated molecular pattern signals emanating from natural killer cells and macrophages carrying their refuse (phagocytized bacterial DNA) from distant skirmishes with a bacterial invader in a noncardiac site such as the gingiva, the skin, or the respiratory tract. This scenario has been borne out in the unraveling of the pathogenesis of human immunodeficiency “infection” in human myocardium. In situ hybridization studies that used both DNA and RNA riboprobes have consistently demonstrated SIV remnants in the myocardium from infected rhesus macaques, raising the possibility that these retroviruses were infecting cardiomyocytes. Using colocalization studies and confocal microscopy, our laboratory determined that when present, SIV viral remnants always colocalized to CD68+ macrophages or CD4+ T lymphocytes, trafficking through the myocardium. Thus, the presence of bacterial DNA in coronary atheroma does not confer pathogenesis and underscores the need to understand the nature of the cellular company that they keep. Bacterial DNA in coronary endothelial cells would suggest primary infection, but localization to macrophages or T cells, particularly memory T cells, could simply represent the “Trojan horse.” Therefore, colocalization studies would shed important light on the potential pathogenic role by identifying the cellular constituents in which bacterial infection resides.

Finally, the diversity of bacteria reported in atherectomy samples in the present study and their association with mature or advanced as opposed to early lesions raise the possibility that atherosclerotic plaques may secondarily form functional biofilms. A biofilm is an assemblage of microbial cells that are associated with a surface in a matrix of polysaccharide material. Biofilm-associated organisms differ from their planktonic counterparts with respect to gene transcription, nutritional needs, secretory protein products, and reproductive rates. Biofilms develop attachments to specific surfaces based on properties of the surface and aqueous medium interaction. Mature atheroma are just such surfaces because of their eccentricity and the perturbed flow characteristics in the microenvironment. These unique characteristics would explain the lack of efficacy of antibiotics in clinical trials to date but represent a persistent source of antigenemia fueling a chronic inflammatory state and leaving a plethora of bacterial fingerprints. In the end, it may matter not “who done
it” in atherosclerosis. Rather, the inflammation mediated by innate and acquired immune responses is the common link—the smoking gun—and its modulation should be the target of ongoing investigation.

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**Disclosures**

None.

**References**


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