EDITORIAL COMMENT

Endotoxin: Another Phantom Menace?*

John F. Carlquist, PhD
Salt Lake City, Utah

The microbial world, since its origin, has conducted countless genetic experiments to better ensure adaptation and hence survival in its biologic niche. Through this trial and error process, “smart” parasites have learned that the ability to persistently infect without limiting the host’s survival ensures their own survival. The host is spared immediate demise, but we are just beginning to understand the long-term consequences that arise from chronic infections. Significant causes of morbidity and mortality not previously thought to have an infectious etiology (e.g., cancer and heart disease) are being associated with the residual effects of chronic infections that may have existed unnoticed for years. Thus, attaining a more complete understanding of the mechanisms and potential outcomes of this situation assumes primary importance.

In recent years, much evidence has accumulated that implicates chronic infection and inflammation in the etiology of atherosclerosis. Specifically identified infectious agents include Chlamydia pneumoniae, cytomegalovirus, Porphyromonas gingivalis–caused periodontitis (periodontitis of any etiology is a risk factor) and Helicobacter pylori. Moreover, chronic inflammatory mediators are believed to contribute to the atherogenic process—principally, C-reactive protein (CRP), proinflammatory cytokines and various growth factors (1,2). A direct link between infection and atherosclerosis has not been rapidly forthcoming, although several proposed mechanisms have been supported experimentally. One hypothesis proposes direct vascular invasion. Indeed, chlamydial organisms have been identified in atheromatous plaque and have also been observed in the endothelium and smooth muscle of affected vessels (3,4). However, for these organisms that do not directly invade the vessel wall, an alternative pathologic mechanism must exist. Systemic inflammatory mediators may arise in response to infections at distant sites, but nonetheless promote coronary or carotid plaque development. Consistent with this hypothesis, inflammatory mediators have marked atherogenic effects on endothelium (5), and these effects are augmented within preexisting plaque (6). Thus, it appears probable that some infectious agents contribute to disease by maintaining a heightened state of inflammatory response.

Infection produces a large array of mediators capable of initiating and maintaining the inflammatory/immune response. Not least among these are the products of the microbes themselves. Unmethylated bacterial deoxyribonucleic acid containing the CpG motif is a potent immunostimulant (7); bacterial heat-shock proteins (HSP) can activate macrophages (8) and also stimulate the production of antibodies. These antibodies frequently cross-react with human HSP and are often invoked as etiologic in autoimmune or autoimmune-like conditions. However, the prototypical inflammatory bacterial product is endotoxin. Endotoxins possess a multitude of biologic effects—most notably, the initiation of a proinflammatory cascade subsequent to binding its specific receptor (CD14) on macrophages. This interaction stimulates the production and release of tumor necrosis factor (TNF)–alpha, interleukin (IL)–1, IL–6, IL–8, IL–12, migration inhibitory factor, chemokines, interferon, eicosanoids and reactive oxygen intermediates (9,10). These macrophage mediators, in turn, stimulate the production of a second wave of chemokines, cytokines, adhesion molecules and signal molecules in a variety of cell types. Among these are intercellular adhesion molecule, vascular cell adhesion molecule–1, nitric oxide and acute-phase reactants (CRP, serum amyloid A). Thus, endotoxin is one of the most potent biologic response modifiers currently recognized.

In view of the association between inflammation and atherosclerosis and the potent inflammatory potential of endotoxin, it is surprising that the contribution of endotoxin to atherogenesis has received little attention to date. In this issue of JACC, Wiedermann et al. (11) report on a carefully conducted and executed prospective study in which they assessed the patient’s risk of developing atherosclerosis as a function of baseline endotoxemia. For the study sample of 516 patients, these investigators found a median plasma endotoxin value of 14 pg/ml. The authors hasten to caution that the absolute plasma concentrations may be somewhat misleading in that there may be method- and laboratory-dependent variability in values. Indeed, a similar study (12) reported a median of 6 pg/ml with only 2.5% of specimens falling above 17 pg/ml. To extend the applicability of their findings to other laboratories, these investigators collapsed the data into deciles for analysis. Employing this method, they observed a clear association between endotoxin levels >50 pg/ml (90th percentile) and newly developing carotid atherosclerosis among patients available for follow-up (n = 466). Their findings were similar for coronary artery atherosclerosis.

*Editorials published in the Journal of the American College of Cardiology reflect the views of the authors and do not necessarily represent the views of JACC or the American College of Cardiology.

From the Division of Cardiology, Department of Medicine, and Molecular Pathology Laboratory, University of Utah School of Medicine and LDS Hospital, Salt Lake City, Utah.
Although the data presented in Wiedermann et al.'s report clearly identify that the risk for atherosclerosis is associated with elevated (highest decile) endotoxin, it is very difficult to firmly establish what place endotoxin assumes in the etiology of this disease. This uncertainty arises from the inability to discern any trend toward risk at blood levels of endotoxin below 50 pg/ml and from the apparent lack of risk (odds ratio 0.96) with elevated endotoxin in the absence of other risk factors (smoking and infection). Although the actual number of subjects in this last group is not given, it is likely that the inability to identify risk among these subjects may have resulted from a small sample size. Also in this study, 37% of end point events occurred among subjects with endotoxin levels <50 pg/ml. Thus, one could conclude from the totality of findings that endotoxin is neither necessary nor sufficient in the pathogenic process. It is beyond dispute, however, that endotoxin greatly augments the risk associated with smoking and underlying infection.

What part does endotoxin play in the etiology of atherosclerosis? The present study does not identify this function, but it does provide clues. The study design measures only baseline endotoxin levels and, therefore, cannot distinguish between transiently elevated and persistently elevated levels. One may speculate that only chronically elevated endotoxin is associated with risk for disease, whereas transient elevations are not. Within this scheme, transient elevation would account for those individuals who were in the highest decile at entry into the study but did not go on to develop disease. When viewed in this perspective, the study results could be interpreted to suggest that some chronic conditions (e.g., infection or smoking) maintain the level of endotoxin sufficiently high to promote disease. Alternatively, persistently elevated endotoxin levels may be an indicator of chronic conditions of sufficient inflammatory potential to promote disease. These similar models differ only in the relative contribution of endotoxin itself to the disease process, and either situation would provide a possible explanation for the strong effect modification seen between endotoxin and smoking and/or infection.

With respect to the investigators' hypothesis, some of the findings of this study appear paradoxical: some individuals with elevated endotoxin remained disease-free, whereas decreased plasma endotoxin was not necessarily protective. There was also a surprising absence of trend toward risk among subjects with baseline endotoxin levels <50 pg/ml. Thus, the relation between endotoxin and vascular disease appears more complex than expected. The ability for endotoxin to promote disease may depend on its ability to initiate an inflammatory response. This effect may be controlled by additional regulatory factors. Under certain conditions, modifying factors could minimize the deleterious effects of high endotoxin but, conversely, could also augment the pathogenic effects of lower levels. Factors that negatively modify the effects of endotoxin are known. For example, the inflammatory effects of endotoxin are blocked by the cytokine IL-10 (13) and antioxidants (14). In contrast, the inflammatory potential of endotoxin present in small quantities is augmented by lipopolysaccharide binding protein (15,16). Modifying factors also regulate the availability and action of downstream effector molecules (e.g., TNF-alpha, IL-1, IL-6) in the inflammatory cascade. Future studies will need to address not only the presence and quantity of endotoxin, but also its potential to produce inflammation and, ultimately, vascular disease.

It is of interest to compare the findings of Wiedermann et al. (11) with those of Ridker et al. (17) in a widely cited study measuring the risk for acute ischemic events associated with CRP. Similarities between the studies might be expected in that CRP production by the liver is stimulated by the proinflammatory cytokines that are upregulated in response to endotoxin. In the Ridker et al. study, risk was limited to the upper quartile of CRP values, and there was a significant trend across all quartiles. In the present study, risk was also limited to a restricted, albeit smaller subset (upper 10%) of subjects, but there was no overall trend toward risk among the remainder of the sample. These small differences might be explained by differences in study design (e.g., period of follow-up [five vs. eight years]) or in the measured end point (incident atherosclerosis vs. ischemic event), but may also be biologic in origin. Proinflammatory cytokines and hence CRP production are downstream events after stimulation of target cells by endotoxin. There may be additional upstream initiating events independent of endotoxin that nonetheless increase CRP levels (e.g., immune-mediated inflammation, gram-positive infection, bacterial nucleic acids and HSP). Within this model, endotoxin-associated atherosclerosis would be a subset of all cases of CRP-associated atherosclerosis. As seen by Wiedermann et al. (11), endotoxin was associated with a subset of newly arising cases of disease that was smaller than the CRP-related subset found in the study by Ridker et al. (17). Likewise, within this model, cases of endotoxin-associated atherosclerosis would predictably be associated with elevated CRP. In the present study, elevated CRP did correlate with elevated endotoxin. Interestingly, an association between CRP and smoking has been previously noted (18). The precise interrelation of all of these variables will require further investigation.

Given the biologic significance of endotoxin and the heightened awareness of the contribution of inflammation to atherosclerosis, a study such as that by Wiedermann et al. (11) has been long overdue. The stage was set for a very profound revelation. Unhappily, an unambiguous role for endotoxin in the pathogenesis of carotid or coronary atherosclerosis was not forthcoming, as is frequently the case in biology. The authors convincingly demonstrate risk for disease associated with endotoxin; however, the independent contributions to disease and pathogenic mechanism(s) of this substance remain elusive. This study has provided a needed point of departure for future investigations into this relation; further investigation will need to address the long-term contribution of small quantities of endotoxin to
disease etiology, the correlation between endotoxin and inflammatory mediators, the effects of modifying factors on the effects of endotoxin and possibly the ability to therapeutically modify its deleterious effects. Based on this and other studies, it is clear that endotoxin will take its place among other significant risk factors for atherosclerosis.

It is unlikely that the human population will be able to easily rid itself of its microbial burden. In contrast, the list of agents that chronically infect humans continues to grow, as does the spectrum of complications that arise from such infections. The challenge to medical science in the coming years will be to identify the factors that give rise to the array of effects that chronic infections impart. There are many pieces in this complex puzzle—some seen but many, as yet, unseen. Hopefully in the near future we will bring to light all of the presently hidden contributors to atherosclerosis. A complete understanding of all of the etiologic processes will provide avenues to prevention and cure. Or, stated more simply: what ceases to be a phantom, ceases to be a menace.

Reprint requests and correspondence: Dr. John F. Carlquist, Division of Cardiology, Department of Medicine, and Molecular Pathology Laboratory, University of Utah School of Medicine, and LDS Hospital, 8th Avenue and C Street, Salt Lake City, Utah. E-mail: ldjcarlq@ihc.com.

REFERENCES