Editorial

Chlamydia pneumoniae–Atherosclerosis Link
A Sound Concept in Search for Clinical Relevance

Franz-Josef Neumann, MD

The potential role of Chlamydia pneumoniae (Cpn) in atherosclerosis has attracted considerable attention. On the basis of early encouraging results, it was anticipated that infection with Cpn might prove a novel treatable risk factor for atherosclerosis, with a relevance comparable to that of Helicobacter pylori in peptic ulcer disease. The effect of antibiotic treatment on clinical outcome became widely accepted as benchmark for the Cpn–atherosclerosis link. Several studies investigated macrolide antibiotics for secondary prevention in vascular disease. Due to limited sample sizes, however, most of these trials need to be interpreted cautiously. At the 51st Annual Scientific Sessions of American College of Cardiology, 2002, two adequately sized antibiotic trials, WIZARD (Weekly Intervention with Zithromax for Atherosclerosis and its Related Disorders) and AZACS (AZithromycin in Acute Coronary Syndromes), were reported. The negative results of these trials deadened the enthusiasm for the role of Cpn in atherosclerosis. WIZARD included 7747 patients with previous myocardial infarction (MI) and elevated Cpn titers who were randomly assigned to placebo or azithromycin for 11 weeks. During 2-year follow-up, the composite of all-cause mortality, recurrent MI, revascularization, and hospitalization for angina was not significantly affected by antibiotic treatment. Likewise, AZACS did not show a significant effect of short-term treatment with azithromycin on 6-month rate of all-cause mortality, nonfatal MI, or recurrent ischemia requiring revascularization in 1400 patients with unstable angina.

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Chain of Evidence Linking Cpn to Atherosclerosis

Since Saikku and co-workers reported the potential link between coronary disease and previous infection with Cpn in 1988, the association between Cpn and incident atherosclerotic disease has been repeatedly confirmed. Cpn has been demonstrated frequently in atherosclerotic plaques by diverse techniques, including immunostaining of Cpn antigen, detection of Cpn DNA by polymerase chain reaction, and even culturing of viable Cpn from plaque material. Cpn in atherosclerotic plaques is not a mere stowaway, as evidenced by the retrieval of Cpn-reactive T lymphocytes from human atherosclerotic plaques. In cell culture, monocytes, endothelial cells, and smooth muscle cells can be infected. Cellular infection with Cpn causes transcriptional activation of various prothrombotic, proliferative, and proinflammatory genes. This involves genes encoding tissue factor and adhesion molecules such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin, as well as cytokines and growth factors, including intercellular (IL)-6, IL-1, tumor necrosis factor-α, basic fibroblast growth factor, and platelet-derived growth factor. Cpn induces preferential arterial attachment of monocytes, as shown by intravital microscopy in mice. In cholesterol-fed rabbits or mice with apolipoprotein E or low-density lipoprotein receptor-deficiency, repeated infection with Cpn but not Chlamydia trachomatis accelerates the progression of atherosclerosis. This effect can be prevented by azithromycin treatment.

The chain of evidence for the Cpn–atherosclerosis link is completed by mechanistic studies in patients. Corresponding to the cell culture studies showing endothelial activation by Cpn infection, clinical studies demonstrated infection-related endothelial dysfunction that was responsive to antibiotic treatment. With respect to the relation between progression of early atherosclerosis and previous infection with Cpn, the current issue of Circulation contains the report of an elegant study on patients with cerebrovascular disease. In this study, Sander et al included 272 consecutive patients with a first-ever cerebral ischemia. In the first part of their study, which was reported earlier, they prospectively investigated the progression of intimal medial thickness (IMT) of the common carotid artery during 3-year follow-up using duplex ultrasonography. They found that patients with seropositivity for Cpn had a significantly higher increase in intimal medial thickness than seronegative patients. In addition, the rate of fatal and nonfatal cardiovascular and cerebrovascular events was by 47% lower in seronegative patients as compared with seropositive patients. This part of the study demonstrated a relation of previous infection with Cpn to the progression of the early stages of atherosclerosis. In the current issue of Circulation, Sander et al report an important continuation of their study. After the initial 3-year follow-up, the cohort, stratified according to serostatus for Cpn, was randomly assigned to treatment with roxithromycin (150 mg twice daily for 30 days) or placebo, and IMT was re-evaluated after additional 2-year follow-up. In both the seronegative and the seropositive patients on placebo, IMT progressed at virtually the same rate as during the pretreatment phase. Roxithromycin, however, significantly reduced the progression rate of...
IMT in seropositive patients to the level of progression found in seronegative patients. There was no effect of roxithromycin in seronegative patients. The study strongly supports the concept that antibiotic treatment directed against Cpn reduces the progression of early atherosclerosis in seropositive patients with cerebrovascular disease.

**Discrepancy Between Mechanistic Studies and Large-Scale Clinical Endpoint Trials**

Hence, the mechanistic clinical and experimental studies support the Cpn–atherosclerosis link and demonstrate a beneficial effect of antibiotic treatment. On the other hand, the large randomized clinical endpoint trials WIZARD and AZACS do not reveal any significant advantage from antibiotic treatment. To reconcile these discrepant findings, several aspects need to be considered.

**Potential Differential Effect on Proliferative Versus Prothrombotic Mechanisms**

The results of the mechanistic clinical studies suggest that Cpn promotes the earliest inflammatory stages of atherosclerosis, that is, endothelial dysfunction, and the proliferative responses thereafter. On the other hand, the large clinical endpoint studies assess events that are driven by the late thrombotic complications. A common mechanism for these late complications is plaque rupture or plaque erosion. These events reflect a change in the equilibrium between stabilizing mechanisms, such as smooth muscle cell proliferation and extracellular matrix production, and destabilizing mechanisms, such as apoptosis and matrix digestion by metalloproteinas. Hence, the catastrophic late complications are rather characterized by lack of proliferation. The discrepancy between the results of the mechanistic studies on the early stages of atherosclerosis and the clinical endpoint trials looking at late complications may thus reflect a predominant effect of Cpn on proliferative responses.

**Limited Contribution of Cpn to the Pathogenesis of Atherosclerosis**

Atherosclerosis is an inflammatory disease of the vessel wall. Although Cpn has the potential to boost vascular inflammation, it is only one potential player among many others. The well-established cardiovascular risk factors hypercholesterolemia, smoking, hypertension, and diabetes mellitus all exert potent proinflammatory vascular effects. In particular, modified low-density lipoprotein fractions are known as powerful potent proinflammatory vascular effects. In particular, modified low-density lipoprotein fractions are known as powerful proinflammatory vascular effects. In particular, modified low-density lipoprotein fractions are known as powerful proinflammatory vascular effects. In particular, modified low-density lipoprotein fractions are known as powerful proinflammatory vascular effects. In particular, modified low-density lipoprotein fractions are known as powerful proinflammatory vascular effects. In particular, modified low-density lipoprotein fractions are known as powerful proinflammatory vascular effects. In particular, modified low-density lipoprotein fractions are known as powerful proinflammatory vascular effects. In particular, modified low-density lipoprotein fractions are known as powerful proinflammatory vascular effects.

Consistent with the concept that infection with Cpn is only one atherogenic mechanisms among many others, the associations between Cpn seropositivity and atherosclerosis that have been found are weak; in a recent study, the odds ratio was 1.25 (95% confidence interval: 1.03 to 1.53). Likewise, it is not surprising that any treatment specifically directed against Cpn has limited efficacy, as it can only eliminate one of many vascular proinflammatory stimuli. Consistently, in the study of Sander et al, treatment with roxithromycin only partially reduced the progression of carotid IMT.

**Role of Host Conditions**

The impact of Cpn infection on atherosclerosis and, thus, the effect of antibiotic treatment, depends on the extent of innate and/or acquired immune responses to the pathogen. The extent of these responses is subject to individual host conditions. It may not be the infection as such but the variable host responses to infection that impact atherosclerosis. Consistent with this concept, the ISAR-3 (Intracoronary Stenting and Antibiotic Regimen) trial found a graded effect of roxithromycin treatment on restenosis after stenting that was dependent on immunoglobulin G titers against Cpn. In this randomized, double blind, placebo-controlled trial including 1010 patients, roxithromycin had no effect in the entire population, whereas in patients with high titers (1/512), roxithromycin reduced the rate of restenosis significantly. Likewise, Sander et al found a strong relation between intimal medial thickness progression and seropositivity for Cpn only in patients with elevated C-reactive protein. These findings support the concept that an individual propensity to inflammatory responses is a prerequisite for the atherogenic effect of Cpn.

Another potential mechanism for the Cpn–atherosclerosis link determined by host conditions is autoimmunity by molecular mimicry. Depending on antigen presentation by the individual host-specific major histocompatibility complex molecules, microbial proteins that show strong homology to self proteins may induce antibodies that cross-react with self protein. As suggested by the work of Xu et al, an attractive candidate for such molecular mimicry is Chlamydial heat shock protein (hsp) 60, which is highly conserved across species, including bacteria. Human antibodies against Chlamydial hsp60 have been shown to cross-react with self hsp60 that is expressed in activated endothelium and atheroma. As shown by experimental and clinical studies, antibodies against hsp60 exert atherogenic effects. In a recent study from Huittinen et al, seropositivity for Cpn only had a substantial impact on coronary risk if there were concomitant antibodies against hsps60. These findings lend strong support to the concept that immunity against hsps60 represents an important mechanism that links Cpn infection to atherosclerosis.

Host-dependent variations in innate and acquired immune responses to Cpn might limit the global effect of antibiotic treatment in studies with non-selected patient populations.

**Clinical Consequences?**

As Sander et al correctly state, surrogate parameters such as IMT are highly useful in gaining further insights into the Cpn–atherosclerosis link. For clinical decision-making, however, true endpoint studies are needed. At the current stage, the use of antibiotics cannot be recommended for treatment or prevention of atherosclerotic diseases, because the adequately powered studies currently available do not reveal a reduction in major clinical events. Other large trials assessing this issue.
are still under way (for example, ACES [Azithromycin and Coronary Events Study] and PROVE-IT [Pravastatin OratorVastatin Evaluation and Infection Therapy]). The results of WIZARD and AZACS have dampened the expectations that these trials will show a benefit large enough to justify the widespread use of antibiotics. If there is marginal advantage of antibiotic treatment in upcoming trials, there will be a strong need to identify those patient subsets that benefit substantially. Mechanistic studies using surrogate parameters, such as the one by Sander et al.\(^{15}\) may then help guide the direction of future clinical research.

At present, there is evidence that Cpn plays a role in atherosclerosis, but this role appears to be limited, and clinical relevance in risk stratification, prevention, and therapy of atherosclerosis is still doubtful.

References

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