Infection and Atherosclerosis: Evidence for Possible Associations

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Atherosclerosis and its vascular complications are the leading causes of death in older people in developed countries. There are accumulating, albeit conflicting, data suggesting that infections, particularly Chlamydia pneumoniae, may play a role in atherogenesis and vascular events. Although prospective epidemiological and clinical studies have provided conflicting results, pathological studies have confirmed the association of C. pneumoniae with atherosclerotic disease. Moreover, many in vitro studies on biological mechanisms and studies in animal models have largely supported a plausible role of infections in atherogenesis. These data suggest that infections, especially C. pneumoniae, may be involved in the initiation and acceleration of atherosclerosis and potentially could lead to acute ischemic events by influencing plaque stability and coagulation.

Key words: atherosclerosis, Chlamydia pneumoniae, infections, older people.

Introduction

Atherosclerosis is the underlying pathology responsible for ischemic events and mortality related to cardiovascular disease, stroke and gangrene. It is universally present in older people and results in the leading causes of death in developed countries—myocardial infarction and stroke. The process of atherosclerosis has been recognized for well over a century as a slow, chronic, low grade inflammatory disease.1 Thus, it should not be surprising that infectious agents have been proposed to play a role in atherogenesis since the late 19th century. The mechanisms and possible etiologies of the development of atherosclerosis are complex and multiple. New risk factors for atherosclerotic vascular disease continue to be recognized, and infection—especially Chlamydia pneumoniae—should be considered as a potential new risk factor.

It is possible that up to 40% of patients with myocardial infarction have no known risk factor (such as hypercholesterolemia, hypertension, smoking history, diabetes mellitus or genetic factors). Thus, it is important to explore and investigate new, potentially treatable, risk factors. Infections are the possible link between coronary artery disease and the well recognized risks associated with circulating inflammatory markers, such as C-reactive protein (CRP), fibrinogen, serum amyloid A and interleukin-6 (IL-6).2

Infections Associated with Atherosclerosis

Each year for decades an excess in cardiovascular mortality has been associated with influenza virus outbreaks during the winter season. Recent studies have shown that influenza vaccination reduces these cardiovascular events in older people.3 There are several possible explanations for these observations: the nonspecific stressful effect of influenza may precipitate acute ischemic events in subjects with critical narrowing of the coronary arteries; infection may destabilize a vulnerable plaque or; infection may lead to thrombosis on a plaque by creating a procoagulant environment. In the past decade many studies have assessed the relationship between cardiovascular or cerebrovascular disease and a few infections, notably C. pneumoniae, periodontal disease, Helicobacter pylori and cytomegalovirus. The data is most extensive for C. pneumoniae and less convincing for periodontal disease, although further investigation is warranted. H. pylori data is most controversial and lacks biological plausibility, and a role in atherogenesis is not supported by a study in mice.4 Cytomegalovirus association with cardiac transplant atherosclerosis is well established, the link with coronary artery post-angioplasty restenosis is controversial, and the association with native atherosclerosis is very weak.5 Most of this review will therefore focus on the relationship between C. pneumoniae and atherosclerotic diseases.

Possible Mechanisms in Atherogenesis

Infections could potentially modify the development of atherosclerosis and vascular complications by several mechanisms (Figure). The process of atherogenesis could be initiated by an infectious agent through damage to vascular endothelium, directly by invasion of the vessel intima, or indirectly through release of lipopolysaccharide (LPS, endotoxin) systemically with subsequent activation of the proinflammatory cytokines. Microbes also could lead to acceleration of atherosclerosis at any stage by increasing the recruitment of inflammatory cells (macrophages, T-lymphocytes) to pre-existing lesions. Microbes could be carried to early atherosclerotic lesions by monocytes, macrophages or lymphocytes. These would enhance the generation of adhesion molecules and proinflammatory cytokines, eventually leading to progression of lesions by increasing the uptake in the intima of macrophages, foam cells and proliferation of smooth muscle cell—all critical components in the development of atherosclerosis.

Indirectly, chronic infections could result in alterations in blood lipid profiles through systemic cytokine release, leading to a more pro-atherogenic environment. Infections may result in low-
Infection and Atherosclerosis

Theoretically, infections could potentiate acute precipitation of ischemic events in the later stages of atherosclerotic development. This could occur on mature stable plaques by destabilizing the plaques through activation of metalloproteinases, resulting in a thin fibrous cap that may easily rupture and cause acute thrombosis by release of the lipid-rich thrombogenic contents. Infections also may precipitate acute thrombosis by systemically creating a procoagulant environment.

A representation of a simple scheme for potential mechanisms of infections in influencing development of atherosclerosis. Upregulation of adhesion factors and proinflammatory cytokines can occur locally at the endothelial level, or systemically.

VLDL: very low-density lipoprotein
HDL: high-density lipoprotein
SMC: smooth muscle cell
mille. For instance, through LPS and other inflammatory cytokines can result in increased levels of platelets, fibrinogen and tissue factor, and may down-regulate the fibrinolytic system (thereby decreasing the activation of protein C and thrombomodulin), which may precipitate thrombosis at a critically narrowed blood vessel.

**Evidence for Association Between C. pneumoniae and Atherosclerosis**

*C. pneumoniae* is a common respiratory, intracellular, bacterial pathogen capable of causing pharyngitis, sinusitis, bronchitis and pneumonia in the community. Its geographical distribution is worldwide, and most of the population become infected after five years of age. By age 20 years, 50% of the population would have been exposed and by 65 years up to 80% have antibodies, with predominance in males. The evidences for association with atherosclerotic vascular diseases are derived from seroepidemiological, pathological, in vitro, animal and clinical studies (Table).

**Epidemiological Data**

Numerous retrospective and cross-sectional studies have found an association with *C. pneumoniae* antibodies and cardiovascular disease or stroke, with a mean odds ratio of 2.0. However, the quality of these studies and their adjustment for confounding factors have been variable. Several large prospective studies have confirmed this association whereas others have not. However, meta-analyses of these prospective studies failed to find any strong association. Different diagnostic serological techniques with lack of standardization and varying cut-off points for positivity hamper the interpretation of the meta-analyses.

**Pathological Data**

The strongest body of evidence in favour of *C. pneumoniae* as a risk factor for atherosclerosis comes from over 50 pathological studies. *C. pneumoniae* has been detected in an average of 40–50% of atheromatous plaques from a variety of arteries (aorta, coronary, carotid, iliac, femoral, popliteal) and from aorta-coronary vein graft, but rarely from normal arteries (odds ratio 24.5). A variety of techniques have been used, including immunohistochemical stain, polymerase chain reaction (PCR) for DNA or mRNA, in situ hybridization, electron microscopy and culture. Viable *C. pneumoniae* has been recovered from atheromas by six centres, but this is a rare event in most attempts. This may be due to low numbers or a noncultivatable, persistent state. Chlamydial Heat Shock Protein 60kd (CHSP-60), an inflammatory antigen expressed by persistent chlamydia, has been localized to macrophages in human atherosclerotic lesions. Chronic infection of lesions with persistent forms may result in local CHSP-60 production, a potential stimulus to inflammation and progression of lesion. Although the presence of microbes in plaques does not necessarily mean an active role in atherogenesis as they could be “innocent bystanders”, the finding that *C. pneumoniae* triggers specific cell-mediated immunity within plaques (the detection of chlamydia-specific T-lymphocytes in atherosclerotic lesions) suggests an active process.

**Biological Mechanisms**

*C. pneumoniae* is capable of infecting human monocytes/macrophages and vascular endothelial and smooth muscle cells (key components of atheroma), and can be transferred from monocytes to vascular endothelium. Proliferation of the bacteria intracellularly can result in

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**C. pneumoniae Association with Atherosclerosis: Summary of Evidence**

<table>
<thead>
<tr>
<th>A. Epidemiological Studies</th>
<th>Mean Odds Ratio</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Case-control/Cross-sectional</td>
<td>2.0</td>
<td>11, 13</td>
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<tr>
<td>Prospective/Longitudinal</td>
<td>1.1–1.5</td>
<td>12, 13</td>
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| B. Pathological Studies | 24.5 | 14 |

<table>
<thead>
<tr>
<th>C. Biological Mechanisms</th>
<th>N/A</th>
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<tbody>
<tr>
<td>– can infect human vascular endothelial cells, macrophages, SMC</td>
<td>17</td>
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<tr>
<td>– stimulates adhesion molecules and cytokines in human endothelial cells <em>in vitro</em></td>
<td>18</td>
</tr>
<tr>
<td>– <em>C. pneumoniae</em> LPS stimulates uptake of LDL in macrophages</td>
<td>19</td>
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<tr>
<td>– CHSP-60 enhances intracellular oxidation of LDL</td>
<td>20</td>
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<tr>
<td>– CHSP-60 upregulates TNF-α and matrix metalloproteinase</td>
<td>15</td>
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<tr>
<td>– induces tissue factor in vascular cells</td>
<td>22</td>
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<th>D. Animal Models</th>
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<tr>
<td>– causes endothelial dysfunction in ApoE −/− mice</td>
<td>23</td>
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<tr>
<td>– causes early atherosclerotic lesions (e.g., fatty streaks) in rabbits</td>
<td>24</td>
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<tr>
<td>– causes intimal proliferation in coronary arteries of pigs</td>
<td>26</td>
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<tr>
<td>– accelerates hyperlipidemic lesions in rabbits and mice</td>
<td>27–29</td>
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<th>E. Clinical Trials</th>
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<tr>
<td>– macrolides ↓ cardiovascular events in small trials</td>
<td>30, 31, 33</td>
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<tr>
<td>– macrolides ↓ inflammatory markers but not cardiovascular events</td>
<td>35</td>
</tr>
<tr>
<td>– macrolides failed to reduce vascular events in larger trials</td>
<td>34, 36</td>
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ApoE −/−: apolipoprotein deficient; CHSP-60: Chlamydial Heat Shock Protein 60kd; LDL: low-density lipoprotein; LPS: lipopolysaccharide (endotoxin); N/A: not applicable; SMC: smooth muscle cells; TNF: tumour necrosis factor
stimulation of adhesion molecules (E-selectin, intercellular adhesion molecule 1, vascular cell adhesion molecule 1) and inflammatory cytokines (IL-1, IL-6, tumour necrosis factor-α [TNF-α]) in human endothelial cells in vitro. These augmented expressions of adhesion molecules and cytokines may promote leukocyte adhesion, migration, intimal inflammation and smooth muscle proliferation, and may enhance lesion progression.

Furthermore, intact C. pneumoniae or its LPS can increase the uptake of LDL in human macrophages to become cholesterol ester laden foam cells in vitro, a key component in early atherogenesis. Moreover, CHSP-60 can enhance intracellular oxidation of LDL, the toxic form of LDL that is believed to be the primary mediator of atherogenesis.

Destabilization of plaques could be induced by C. pneumoniae as CHSP-60 regulates matrix metalloproteinase expression, and in vitro infected macrophages induce secretion of the 92-kDa gelatinase that could weaken plaques and predispose to rupture. In addition, C. pneumoniae could predispose to acute thrombosis on plaques, as human vascular endothelial cells and smooth muscle cells infected with these bacteria can induce procoagulant protein and proinflammatory cytokine expression, such as tissue factor, plasminogen activator inhibitor-1 and IL-6, along with activation of nuclear factor-kappa B.

Animal Models

The accepted paradigm in the genesis of atherosclerosis is the initial occurrence of endothelial dysfunction. In hyperlipidemic, apolipoprotein E (ApoE)-deficient mice, infection with C. pneumoniae impairs arterial relaxation by causing endothelial dysfunction. The subsequent steps in atherogenesis include migration of macrophages and lymphocytes to the intima, and accumulation of cholesterol ester laden macrophages (foam cells) to form fatty streaks. We have previously demonstrated that normo-cholesterolmic rabbits infected with C. pneumoniae can develop early atherosclerotic lesions of the aorta equivalent to fatty streaks, and others have shown inflammatory changes in the rabbit intima. Moreover, in the pig model C. pneumoniae induces moderate intimal proliferation in coronary arteries. The typical mature atheroma seen in humans with a lipid core and fibrous cap is absent in the infected normocholesterolmic rabbits and likely requires hyperlipidemia to occur. Hypercholesterolemic rabbits and ApoE- or LDL receptor-deficient mice have demonstrated enhanced or accelerated atherosclerotic lesions with C. pneumoniae infection, which has been partly reversed in rabbits with early treatment with azithromycin.

Acceleration of atherosclerosis is not seen in hyperlipidemic mice with C. trachomatis or H. pylori, and Mycoplasma pneumoniae fails to induce early lesions in the rabbit aorta.

There is no animal model to demonstrate acute precipitation of an ischemic event or induction of an unstable plaque with infection.

Clinical Trials

A few pilot prospective clinical trials stimulated worldwide interest in the field of infections and atherosclerosis by showing early decrease in secondary cardiac events in patients treated with new macrolides after recent acute myocardial infarction or unstable angina. However, in one of these trials the initial beneficial effect was not sustained after six months. In another recent trial of 324 patients with acute myocardial infarction or unstable angina, subjects receiving either the macrolide azithromycin (effective against chlamydia) or amoxicillin, metronidazole and omeprazole for a week (the regimen used for H. pylori) had reduced secondary cardiac events even at one year compared to the placebo arm. However, a larger trial of 1,439 patients with acute coronary syndrome did not show any benefit with five days of azithromycin treatment in preventing recurrent ischemic events.

Other clinical trials have examined the value of antibiotics in patients with stable coronary artery disease (CAD). In a trial of 302 patients (not powered to detect even a 30% difference), no reduction in cardiovascular events was observed after two years following a three-month course of azithromycin. However, there was a reduction of global rank score of four serum inflammatory markers (CRP, IL-1, IL-6 and TNF-α) in the treated group. In a recent international randomized study of 7,724 patients with previous myocardial infarction and stable CAD, azithromycin given once weekly for three months did not reduce secondary vascular events over placebo after two to three years in patients with C. pneumoniae Ig titer ≥ 1:16.

Summary

Infection, particularly C. pneumoniae, remains a potential atherosclerotic risk factor. Current trials will not prove or disprove a causal role in humans. These studies are addressing the issue of acute precipitation of ischemic events in subjects with established and advanced atherosclerotic disease. Studies in the future should assess the role of infections in accelerating atherosclerosis in younger, asymptomatic subjects by novel imaging techniques.

No competing financial interests declared.

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