



# The immunopathology of periodontal disease: links with atherosclerosis?

## Introduction

Chronic inflammatory periodontal disease occurs as a result of an inflammatory response in the periodontium which is elicited by bacteria present in dental plaque. The specific cytokines and chemokines produced by this initial response cause a T cell/macrophage dominated inflammatory infiltrate to develop in the connective tissues. If this cell-mediated immune response does not control the bacterial challenge, progression to a B cell/plasma cell lesion occurs (reviewed in Gemmell *et al.* 2002<sup>1</sup>). A component of the specific T cell and antibody response elicited by these bacteria however, has been implicated in a cross-reactive immune response targeting host antigens on systemic endothelial cells. This cross-reactivity has been proposed to be involved in the pathogenesis of atherosclerosis.

## Periodontal disease and atherosclerosis

Atherosclerotic cardiovascular disease is a leading cause of death worldwide and the importance of the role of infection and inflammation in atherosclerosis is now widely accepted. Chronic inflammatory periodontal disease is a significant oral health problem with *Porphyromonas gingivalis* being one of the major

Table 1. Prevalence of microorganisms detected by real time PCR in atherosclerotic lesions of 25 patients.

Species	% lesions positive
<i>P. gingivalis</i>	100
<i>F. nucleatum</i>	84
<i>T. forsythia</i>	48
<i>C. pneumoniae</i>	28
<i>H. influenzae</i>	4
<i>H. pylori</i>	4

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causative organisms for disease progression. Individuals with severe periodontitis have been reported to have a significantly increased risk of developing cardiovascular diseases including atherosclerosis<sup>2</sup>. Animal models further support this association and murine models of atherosclerosis have demonstrated that repeated inoculation with *P. gingivalis* resulted in atherosclerotic lesions which were more advanced and developed more rapidly than those of control animals<sup>3</sup>.

In atherosclerosis, low density lipoprotein (LDL) from the circulation accumulates and becomes oxidised in the intima. Monocytes and T cells migrate into the arterial wall after adhesion to endothelial cells. The recruited monocytes and smooth muscle cells take up oxidised LDL and eventually die, contributing to the central necrotic core. The macrophages and T cells produce proteins which induce changes in smooth muscle cells which then respond to growth factors from cells in the lesion leading to a generalised immune reaction with the formation of the established plaque.

## Infection and atherosclerosis

Studies have associated many organisms responsible for common chronic infections with atherosclerosis<sup>4</sup>. These include both bacterial and viral infections as well as those associated with periodontal disease. Many of the implicated organisms, such as cytomegalovirus and *Chlamydia pneumoniae* are intracellular pathogens

which can infect cells and remain latent, therefore providing a source of chronic infection and inflammation. We have identified *P. gingivalis*, *Fusobacterium nucleatum*, *Tannerella forsythia*, *C. pneumoniae*, *Helicobacter pylori* and *Haemophilus influenzae* in atherosclerotic plaques, with up to five of these types of organisms detected in the same specimen. Multiple infections were observed to occur more commonly than single infections.

A finding of considerable interest was the presence of *P. gingivalis* in all of the atherosclerotic plaques examined using real time PCR and that the prevalence of periodontopathic bacteria was significantly higher than that of the other bacteria tested (Table 1)<sup>5</sup>. *C. pneumoniae* is a common pathogen of the upper respiratory tract and can cause pneumonia, bronchitis, pharyngitis and sinusitis and an association between infection with this organism and cardiovascular disease has been shown to be very strong<sup>6</sup>.

Until recently however, studies have concentrated on the effect of infection with a single pathogen. Epstein *et al*<sup>7</sup> postulated that multiple pathogens are involved and that 'pathogen burden', or the aggregate pathogen load, is a more significant risk factor than any single infection. Prasad<sup>8</sup> and others have continued this work, demonstrating that aggregate pathogen load is also an independent risk factor for endothelial dysfunction, even in patients with angiographically normal coronary arteries. The authors suggested that the presence of multiple intracellular pathogens in endothelial cells and the resulting endothelial dysfunction may be a mechanism contributing to the initiation of atherosclerosis and increased progression of established disease.



## Heat shock proteins

Infection may initiate and facilitate the progression of atherosclerosis as a result of the immune response to bacterial heat shock proteins (HSPs). All cells, both prokaryotic and eukaryotic, express HSPs on exposure to various forms of stress including temperature, oxidative injury and infection<sup>9</sup>. Factors such as bacterial lipopolysaccharide, cytokines and mechanical stress can induce the expression of protective host (h) HSP60 on endothelial cells (Figure 1). Due to the homologous nature of HSPs among species<sup>10</sup>, cross-reactivity of antibodies to bacterial HSP (GroEL) with hHSP60 on endothelial cells may result in endothelial dysfunction and the subsequent development of atherosclerosis<sup>11</sup>. The presence of risk factors such as high blood cholesterol would enhance the expression of hHSP60 and adhesion molecules by endothelial cells and result in progression from early fatty streak lesions to severe and irreversible atherosclerotic alterations. Aspirin has been shown to protect in part by downregulating adhesion molecule expression on stressed endothelial cells while cyclosporin A induces hHSP60

expression which may explain some of its atherosclerotic promoting effects<sup>12</sup>.

An animal model<sup>13</sup> has demonstrated that rabbits immunised with human or rabbit atherosclerotic lesion proteins induced atherosclerosis. Complete Freund's adjuvant (CFA) was used in the immunisations and this was found to result in atherosclerosis when used alone. A major component of the heat-killed mycobacteria contained in CFA was HSP65 and immunisation with recombinant mycobacterial HSP65 again induced atherosclerotic lesions. Xu *et al.*<sup>14</sup> subsequently reported increased levels of anti-HSP antibodies in clinically healthy humans with sonographically demonstrable atherosclerotic lesions in their carotid arteries compared with individuals with no lesions. There was a correlation between high anti-HSP60/65 antibody titres and high mortality. *in vitro*, these antibodies were cross-reactive with those of other bacteria and were able to lyse stressed but not unstressed endothelial cells.

Chronic infections, including those caused by periodontopathic bacteria such as *P. gingivalis*, have been associated with

atherosclerosis possibly due to cross-reactivity of the immune response to bacterial GroEL with hHSP60. This cross-reactivity would occur as a result of the structural similarity or 'molecular mimicry' of these antigens. We have recently demonstrated the presence and cross-reactivity of hHSP60-, GroEL- and *P. gingivalis*-specific T cells in human atherosclerotic lesions<sup>15</sup> and have also shown that anti-*P. gingivalis* antibodies from the plasma of atherosclerosis patients cross-reacted with hHSP60. As well, we have demonstrated that periodontopathic bacteria, particularly *P. gingivalis*, are present in human atherosclerosis lesions.

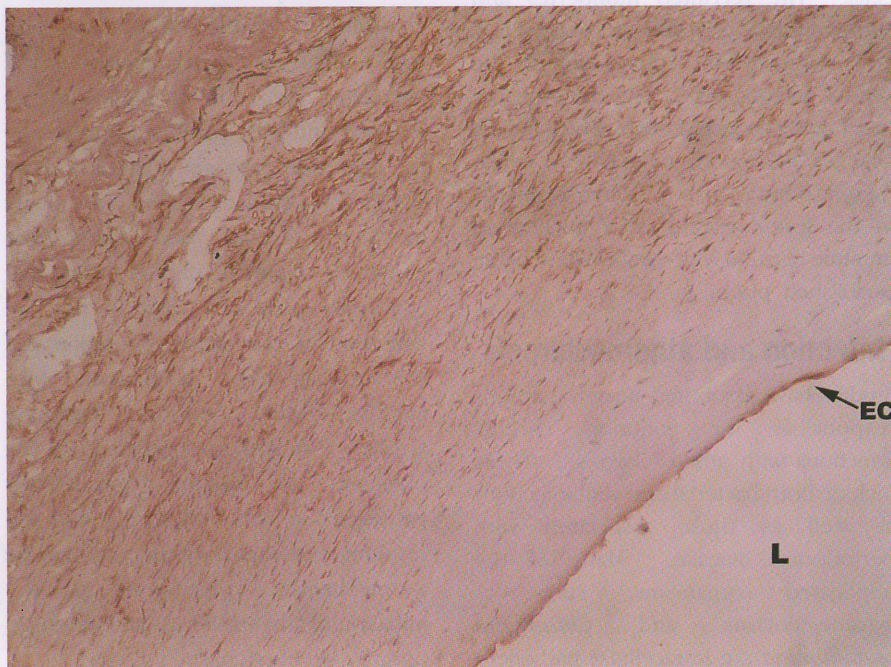
## Conclusions

Much evidence therefore exists that immune responses are central to atherogenesis. Our recent studies support the hypothesis of molecular mimicry of GroEL and hHSP60 and the importance of *P. gingivalis* infection and therefore of periodontitis, in atherosclerosis. Should infection be established as a risk factor for atherosclerosis as many studies suggest, then effective treatment of chronic infections such as periodontitis would have an important preventive role in atherosclerosis. It is likely, however, that many factors are involved in this disease process and that the infectious component may be more significant for some patients than for others.

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Figure 1. Expression of hHSP60 in a human atherosclerotic carotid artery. Positively staining cells include the endothelial cells (EC) adjacent to the lumen (L) and other cells with the appearance of inflammatory leukocytes and smooth muscle cells within the lesion. Original magnification x 100.





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