REVIEW

Relationship between periodontal infections and systemic disease

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ABSTRACT

Oral conditions such as gingivitis and chronic periodontitis are found worldwide and are among the most prevalent microbial diseases of mankind. The cause of these common inflammatory conditions is the complex microbiota found as dental plaque, a complex microbial biofilm. Despite 3000 years of history demonstrating the influence of oral status on general health, it is only in recent decades that the association between periodontal diseases and systemic conditions such as coronary heart disease and stroke, and a higher risk of preterm low birth-weight babies, has been realised. Similarly, recognition of the threats posed by periodontal diseases to individuals with chronic diseases such as diabetes, respiratory diseases and osteoporosis is relatively recent. Despite these epidemiological associations, the mechanisms for the various relationships remain unknown. Nevertheless, a number of hypotheses have been postulated, including common susceptibility, systemic inflammation with increased circulating cytokines and mediators, direct infection and cross-reactivity or molecular mimicry between bacterial antigens and self-antigens. With respect to the latter, cross-reactive antibodies and T-cells between self heat-shock proteins (HSPs) and *Porphyromonas gingivalis* GroEL have been demonstrated in the peripheral blood of patients with atherosclerosis as well as in the atherosclerotic plaques themselves. In addition, *P. gingivalis* infection has been shown to enhance the development and progression of atherosclerosis in apoE-deficient mice. From these data, it is clear that oral infection may represent a significant risk-factor for systemic diseases, and hence the control of oral disease is essential in the prevention and management of these systemic conditions.

Keywords Molecular mimicry, oral disease, systemic disease

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INTRODUCTION

Cardiovascular disease (CVD), including atherosclerosis, myocardial infarction and stroke, is the leading cause of death in many societies (http://www.who.int/cardiovascular_diseases/en/). CVD accounts for an estimated 40% of all deaths worldwide, with atherosclerosis being the underlying aetiology in the vast majority of cases. In many countries, the death rate is often highest among indigenous populations and those from low socio-economic backgrounds. In New Zealand, for example, death rates are highest among Maoris, followed by Pacific people, and lowest among those of neither Maori nor Pacific origin. Fifty-six per cent of Maori males and 34% of Maori females who died from coronary heart disease were under the age of 65 years, compared with 16% of males and 5% of females among those of neither Maori nor Pacific origin [1].

Up to 50% of individuals with CVD may not have any of the traditional risk-factors such as

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smoking, obesity, hypercholesterolaemia, high blood pressure or diabetes. Therefore, it is important to understand all possible risk-factors if morbidity and mortality due to this disease are to be reduced. Risk-factors are the key to prevention, and even a modest change in risk can lead to a significant change in disease burden. The importance of the role of infection and inflammation in the initiation and progression of atherosclerosis is now widely accepted [2–9].

Chronic inflammatory periodontal diseases are found worldwide and are among the most prevalent chronic infections in humans. Advanced forms of the disease can be found in 10–15% of the population and constitute a considerable inflammatory burden (Fig. 1). Individuals with severe chronic periodontitis have been reported to have a significantly increased risk of developing CVD, including atherosclerosis, myocardial infarction and stroke, after adjusting for many of the traditional risk factors [10–16]. Jansson *et al*. [16] found that after adjustment for age, gender, smoking and CVD at baseline, oral health was significantly correlated with fatal coronary events. In fact, these authors made the extraordinary statement that oral health was a risk indicator of death due to CVD.

There have now been over 50 studies investigating the relationship between periodontal disease and CVD, with the majority showing a significant, albeit modest, positive association even after adjusting for confounders. Two recent meta-analyses [17,18] have also concluded that periodontal disease and CVD are significantly related. Despite this, the association between periodontal disease and CVD has been questioned [19] to such a degree that current opinion regarding the relationship is still divided. In this context, longitudinal intervention and pathogenic mechanism studies are urgently required. Several hypotheses, including common susceptibility, direct bacterial damage to the endothelium, systemic inflammation and, finally, cross-reactivity or molecular mimicry between bacterial antigens and self-antigens have, nevertheless, been proposed to explain the relationship [20].

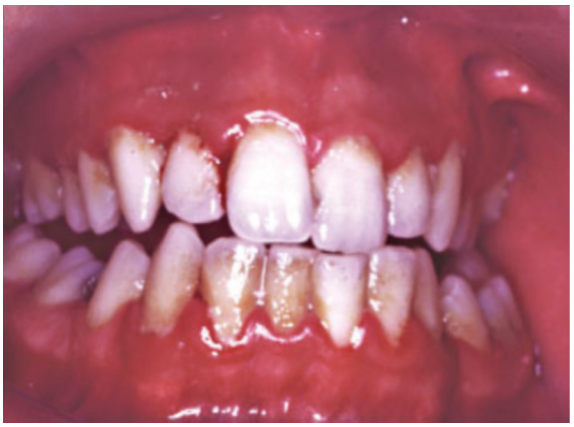

Fig. 1. Patient with chronic inflammatory periodontal disease, illustrating the considerable inflammatory burden.

COMMON SUSCEPTIBILITY MODEL

Common susceptibility involves a genetically determined phenotype, which leads to a greater risk of both artherosclerosis and infection. In this hypothesis, in the presence of periodontal pathogens, a susceptible person develops periodontal disease. This same person would also be susceptible to atherosclerosis, but, in this model the periodontal disease does not cause the atherosclerosis.

SYSTEMIC INFLAMMATION MODEL

A second hypothesis is that of systemic inflammation and increased circulating cytokines and inflammatory mediators. In this hypothesis, inflammation leads to an increase in the levels of circulating cytokines, which in turn damage the vascular endothelium and ultimately result in atherosclerosis. The circulating cytokines of current interest include: C-reactive protein (CRP), interleukin-1, interleukin-6 (IL-6), tumour necrosis factor-α, and prostaglandin E_2. The highest relative risk-factor for myocardial infarction was found to be the levels of CRP together with the ratio of total cholesterol to high-density lipid [21]. A number of studies have shown that chronic periodontitis is associated with increased levels of CRP [22–24]. In our recent study of over 400 patients with defined CVD, the highest levels of CRP were seen in those with the most advanced periodontal disease (Fig. 2).

Measures of endothelial dysfunction

Flow-mediated dilatation.

Flow-mediated dilatation measures the capacity of the arteries to dilate in response to altered flow. It has been shown that flow-mediated dilation is

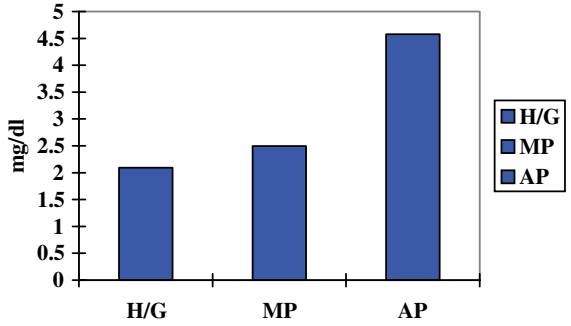

Fig. 2. Levels of C-reactive protein in subjects with diagnosed cardiovascular disease. The highest levels are seen in those with advanced periodontal disease (AP), as compared with those with moderate disease (MP) and those with healthy gingivae or gingivitis (H/G).

decreased in severe periodontal disease and that this is associated with high levels of CRP [22,23]. Recently, Tonetti *et al*. showed that 24 h after intensive periodontal therapy, brachial artery elasticity, as measured by flow-mediated dilatation, was significantly lower than that in the control treatment group [24]. This was associated with increased levels of CRP and IL-6. By 60 and 180 days, however, flow-mediated dilatation was greater in the intensive therapy group and the plasma levels of E-selectin were lower. This degree of improvement in the intensive therapy group was associated with a significant improvement in periodontal health.

Pulse-wave velocity.

Recently, Amanuma *et al*. (85th annual IADR meeting, March 2007, Abstract 2322) used pulse-wave velocity (PWV) to determine the effect of periodontal treatment on arterial elasticity in a Japanese population. PWV is a reflection of arterial stiffness, and an elevated PWV is associated with atherosclerosis, increased cardiovascular events and increased mortality. In addition to PWV, these authors also measured serum levels of high-sensitivity CRP, IL-6 and tumour necrosis factor-α in 39 patients with periodontitis before and after periodontal treatment, and in 22 systemically and periodontally healthy control subjects. They found that the level of PWV in the patient group tended to be higher than in the controls, and that the level decreased after periodontal treatment. They also showed that the levels of high-sensitivity CRP and IL-6, but not tumour necrosis factor-α, decreased after periodontal treatment. These results suggest that chronic inflammatory periodontal disease may affect endothelial cell dysfunction either directly or indirectly via stimulation of CRP synthesis in the liver.

INFECTION MODEL

A third hypothesis is direct infection of the blood vessels by bacteria. In this hypothesis, the bacterial pathogens get into the bloodstream, and subsequently invade the endothelium, leading to endothelial dysfunction, inflammation and atherosclerosis.

Several studies have shown bacteria in the atherosclerotic plaques. In a study by Ford *et al*. [25], real-time PCR was used to show *Porphyromonas gingivalis* in 100% of atherosclerotic plaques. *Fusobacterium nucleatum* was found in approximately 80%, *Tannerella forsythia* in just under 50%, and *Chlamydia pneumoniae* in just under 30%. *Helicobacter pylori* and *Haemophilus influenzae* were both found in approximately 4% of the arteries. These results clearly show that oral organisms can and do invade blood vessel walls, but it is unclear whether they cause the atherosclerosis or simply invade an already damaged artery.

CROSS-REACTIVITY/MOLECULAR MIMICRY MODEL

In this hypothesis [26], the progression of atherosclerosis can be explained in terms of the immune response to bacterial heat-shock proteins (HSPs). All cells express HSPs upon exposure to various forms of stress [27,28], and bacterial HSPs are major antigenic determinants during infection [29]. The immune system may not be able to differentiate between self-HSP and bacterial HSP; thus, during infection, cross-reactive T-cells with specificity for self-HSP may be activated [30], and antibodies produced by the host directed at bacterial HSP could result in an autoimmune response to similar antigenic structures in the host [27,29].

Concerning atherosclerosis, factors such as bacterial lipopolysaccharide, cytokines and mechanical stress may induce the expression of host protective HSP (hHSP60) on endothelial cells. Owing to the homologous nature of HSPs among species [31], cross-reactivity of antibodies

to bacterial HSP (termed GroEL) with hHSP60 on endothelial cells may subsequently result in endothelial dysfunction and induce the development of atherosclerosis [26]. The presence of risk-factors such as high blood cholesterol would enhance the expression of hHSP60 and adhesion molecules by endothelial cells, such that subsequent immune cross-reactivity could result in progression from early fatty streak lesions to severe and irreversible atherosclerosis. A correlation between high anti-HSP60/65 antibody titres and high morbidity and mortality due to atherosclerosis has been demonstrated [32], and these antibodies were shown to be cross-reactive with those of other bacteria and were able to lyse stressed, but not unstressed, endothelial cells [33]. The demonstration of elevated hHSP60 levels in patients with borderline hypertension, and an association between early atherosclerosis and HSP60 levels [34], offer further support for this hypothesis.

GroEL proteins of the HSP60 family have been reported to be major antigens in several pathogenic bacteria [35]. An *Escherichia coli* GroEL homologue has been identified in the periodontopathic bacteria *P. gingivalis*, *F. nucleatum* and *Aggregatibacter actinomycetemcomitans* [36,37], which was immunogenic and recognised by serum antibodies in patients with periodontal disease [37]. GroEL antigens share a high degree of homology with hHSP60 proteins, and antibody to hHSP60 cross-reacts with periodontopathic bacterial GroEL [38]. Patients with periodontal disease were shown to have a higher positive response to *P. gingivalis* GroEL antigens than did healthy controls. Cross-reactivity between *P. gingivalis* GroEL antibodies and hHSP60, and between antibodies to hHSP60 and *P. gingivalis* GroEL, has been demonstrated [39].

Studies in our laboratory [25] have confirmed the expression of hHSP60 on endothelial cells and also on fibroblast/smooth muscle cells in atherosclerotic lesions in humans. We also examined anti-hHSP60 and anti-*P. gingivalis* GroEL antibody levels in serum samples from 130 patients with CVD. Higher levels were detected in patients with moderate/advanced periodontitis. Levels of anti-GroEL and anti-*P. gingivalis* antibodies in plasma samples from atherosclerosis patients were reduced following absorption with hHSP60 in 19/22 patients, thus demonstrating cross-reactivity.

In addition, we have examined anti-hHSP60 and anti-*P. gingivalis* GroEL antibody levels in serum from patients with diagnosed CVD, as well as from patients with both low and high cardiovascular risk. Levels of anti-hHSP60 antibodies were significantly greater in patients with high cardiovascular risk than in patients with low risk and diagnosed CVD. Levels of antibodies to *P. gingivalis* GroEL were also significantly greater in patients with high cardiovascular risk than in low-risk patients. There was no significant difference in antibody levels between those with diagnosed CVD and those with low risk. However, patients with diagnosed CVD were receiving therapy, which may explain the reduction to an antibody level comparable to that of low-risk patients. We also examined the cross-reactivity of anti-hHSP60 antibodies with *P. gingivalis* antigens. After absorption with *P. gingivalis*, there was a significant reduction in the level of anti-hHSP60 antibodies in patients with diagnosed CVD, as compared with low-risk and high-risk patients. That is, patients with diagnosed CVD had a greater amount of cross-reactivity between anti-hHSP60 antibodies and *P. gingivalis* antigens. While cardiovascular therapy may have lowered the levels of anti-hHSP60 and anti-GroEL antibodies, the amount of cross-reactivity was unchanged (unpublished data).

We have established GroEL-, hHSP60- and *P. gingivalis*-specific T-cell lines from peripheral blood and from human atherosclerotic plaques [40]. Of particular note was the cross-reactivity of a number of GroEL-specific T-cell lines with hHSP60 and of hHSP60-specific lines with GroEL, again suggesting molecular mimicry of GroEL and hHSP60. These results demonstrated the presence of T-cells specific for GroEL in the peripheral blood, as well as in atherosclerotic lesions, and their cross-reactivity with hHSP60 [40]. The cytokine, chemokine and V Beta profiles of these cells are similar to those demonstrated previously for *P. gingivalis* – specific T cell lines from patients with periodontal disease [41–43]. Furthermore, Yamazaki *et al*. [44] have demonstrated that HSP60-stimulated peripheral blood mononuclear cells and *P. gingivalis* GroEL-stimulated peripheral blood mononuclear cells had identical nucleotide sequences in the CDR3 of the T-cell receptor (TCR) β-chain and that T-cells with the same nucleotide sequences were present in

the gingival tissues as well as in atherosclerotic aneurysmal tissues.

We now have preliminary data showing that peripheral blood T-cells from young adults (aged <35 years) without periodontal disease or clinically evident atherosclerosis do not respond to either HSP60 or *P. gingivalis* outer-membrane preparation containing GroEL (as confirmed by western blot), thus adding further weight to the proposed hypothesis that molecular mimicry may be a mechanism involved in atherosclerosis, at least in some patients [45, 46].

The development of murine models of atherosclerosis has provided a valuable tool for the study of factors involved in atherogenesis. Immunisation of C57BL/6 mice (on a cholesterol-rich diet) with recombinant HSP has been shown to significantly increase the development of atherosclerosis [47], suggesting that hHSP60 may play a major role in inducing atherosclerosis. Using the apoE-deficient mouse model, studies have demonstrated that repeated inoculation with *P. gingivalis* results in atherosclerotic lesions that are more advanced and develop more rapidly than those in control mice [48,49]. We have also shown that, in this model, weekly intraperitoneal injections of either *P. gingivalis* or *C. pneumoniae* resulted in marked atherosclerotic lesions in the proximal aorta, while control mice showed no lesions (Fig. 3). Furthermore, while lesions developed later with *P. gingivalis*, as compared with *C. pneumoniae*, an increase in the pathogen burden of *P. gingivalis*, but not of *C. pneumoniae*, enhanced atherosclerosis [50]. An increased pathogen load also resulted in increased titres of specific antibody in the case of *P. gingivalis* but not in the case of *C. pneumoniae*, suggesting that the peak atherosclerotic response to *P. gingivalis* coincided with the peak antibody response. There was a significant correlation between anti-GroEL antibody levels and lesion size ($p < 0.05$) ($r = 0.77$), which further suggests that immunological cross-reactivity could be involved.

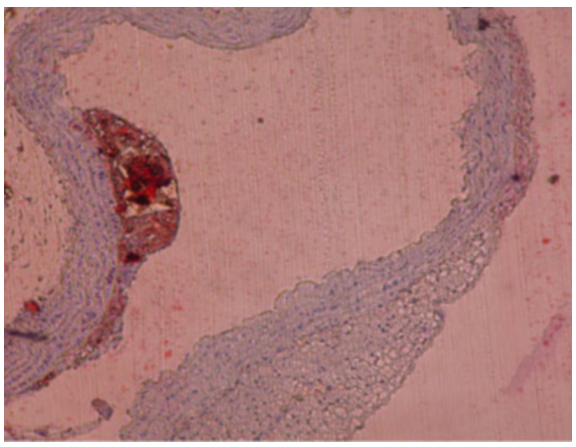

Fig. 3. After 11 weekly injections, immunised mice demonstrate marked atherosclerotic lesions in the proximal aorta, while control mice show no lesions. The dark staining area in the wall of the aorta is a lipid-filled atherosclerotic lesion from an immunised mouse.

CONCLUSION

Taken together, these results show that HSPs are expressed in atherosclerotic plaques, and that cross-reactive T-cells exist in periodontal disease tissue, in peripheral blood and in atherosclerosis lesions, thus strongly supporting the hypothesis that cross-reactivity of the immune response to bacterial HSPs with arterial endothelial cells expressing hHSP60 may be a mechanism involved in the disease process of atherosclerosis, at least in some patients.

Ang *et al*. [51] have put forward four criteria for diseases mediated by molecular mimicry.

1. Establishment of an epidemiological association between the infectious agent and the immune-mediated disease.
2. Identification of T-cells or antibodies directed against host target antigens.
3. Identification of the microbial mimic of the target antigen.
4. Reproduction of the disease in an animal model.

As indicated above, there is clear evidence of an epidemiological association between periodontal disease and CVD. The results of our studies in Brisbane and Niigata provide data that fulfil criteria 2 and 3. We have identified the microbial target; we have shown that there are antibodies and T-cells that react with this target; we have identified the human antigens with which these cells and antibodies cross-react; we have shown that these cells are in the arteries as well as in the gingival tissues; and we have shown that these cells are in the peripheral blood of patients with atherosclerosis. Furthermore, the studies of Li *et al*. [48] and Lalla *et al*. [49], together with our own studies [50], have shown that infection with *P. gingivalis* also accelerates the progression of atherosclerosis in apoE-deficient mice. Taken

together, these results provide data that fulfil all four criteria for diseases mediated by molecular mimicry and, therefore, support for the concept that cross-reactivity between GroEL and HSP60 represents, at least in part, the link between infection, including periodontal disease, and CVD.

It is clear, therefore, that infection can contribute to atherosclerosis via molecular mimicry. This infection could be respiratory, e.g., *C. pneumoniae*, gastrointestinal, e.g., *H. pylori*, or oral, e.g., *P. gingivalis*. These all contribute to the total burden of infection; in some individuals, oral infection may make a significant contribution to the total burden of infection, while in others it may be only a minor contributor. Nevertheless, it is the responsibility of all health professionals to ensure that all oral infection is kept to a minimum. All such infections lead to inflammation in the respective tissue and contribute to the total burden of inflammation, which in turn can contribute to atherosclerosis. Obesity, diabetes and other autoimmune diseases such as rheumatoid arthritis can also contribute to the total burden of inflammation and hence to atherosclerosis. Atherosclerosis itself is an inflammatory condition that contributes to the total burden of inflammation. All these interactions are shown in Fig. 4, which begins to explain the observed associations between periodontal disease, atherosclerosis, diabetes, rheumatoid arthritis, smoking, mental stress, etc. Overall, it is an extremely complex interaction, and no one mechanism is likely to provide the complete explanation.

Nevertheless, the importance of preventing and treating infections, especially chronic infections such as periodontitis, would become paramount in advising and treating patients with coronary heart disease. Effective health policy needs to focus on risk-factors, as even modest changes in risk can have significant changes in disease burden. If periodontal disease proves to be a risk-factor for CVD, this will clearly establish the need for adult oral health programmes, together with diet, exercise and smoking control, in the treatment and prevention of CVD. In terms of the 'health payback model', understanding the relationship between oral infection and systemic diseases has the potential to change health policy with ensuing economic benefits.

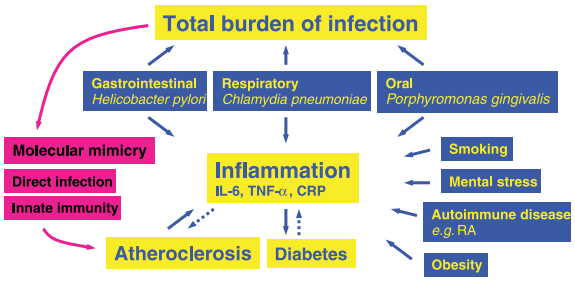

Fig. 4. Model for the interaction of infection and inflammation in the pathogenesis of atherosclerosis and diabetes. The contribution of oral disease to the total burden of infection and the total burden of inflammation will vary from one patient to the next, but could still represent a significant risk-factor.

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