

Infection or Inflammation: The Link Between Periodontal Disease and Systemic Disease

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ABSTRACT

There is increasing evidence that chronic infections are associated with cardiovascular diseases. A number of hypotheses have been put forward to explain these associations, including common susceptibility, systemic inflammation, direct infection of the blood vessels, and cross-reactivity or molecular mimicry between bacterial and self-antigens. In terms of common susceptibility, a person who is susceptible to progressive periodontal disease is also susceptible to atherosclerosis, but the periodontal disease does not cause the atherosclerosis. In recent years much research has been focused on the role of systemic inflammation and the increase in circulating cytokines and inflammatory mediators. These cytokines and mediators can lead to direct endothelial damage and ultimately to atherosclerosis. A number of studies have shown that periodontal bacteria can directly invade the endothelium and thereby lead to inflammation in the blood vessel wall resulting in atherosclerosis. In terms of molecular mimicry, it is proposed that because of the homology between bacterial GroEL antigens and human heat shock protein (HSP), the local immune response to the periodontopathic bacteria cross-reacts with self-HSP expressed on the endothelium leading to vascular inflammation and hence atherosclerosis. There is increasing evidence in support of this hypothesis; however, none of these possible mechanisms are mutually exclusive, and it is likely that in different people different mechanisms may explain the link between periodontal infection and cardiovascular disease.

There is increasing evidence that chronic infections are associated with cardiovascular diseases (CVDs). These infections include *Helicobacter pylori*, *Chlamydia pneumoniae*, cytomegalovirus, and, more recently, periodontopathic

bacteria such as *Porphyromonas gingivalis*. Although a large number of potential mechanisms have been postulated, the mechanism by which these infections associate with CVDs is still unclear. A number of hypotheses nevertheless exist,

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including common susceptibility, systemic inflammation with increased circulating cytokines and inflammatory mediators, direct infection of the blood vessels, and, finally, cross-reactivity or molecular mimicry between bacterial and self-antigens. This final hypothesis

is gaining support and will be discussed in this review.

COMMON SUSCEPTIBILITY

Common susceptibility involves a genetically determined phenotype, which leads to a greater risk of both atherosclerosis

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 (Figure 1).

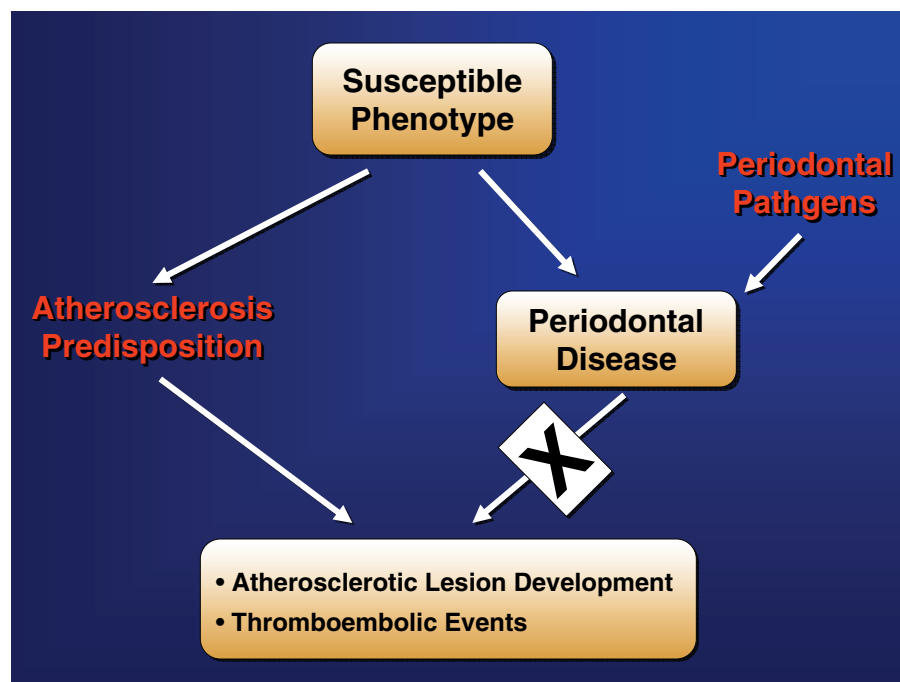


Figure 1 Model of the common susceptibility hypothesis, in which a person would be susceptible to atherosclerosis, but the periodontal disease does not cause the atherosclerosis.

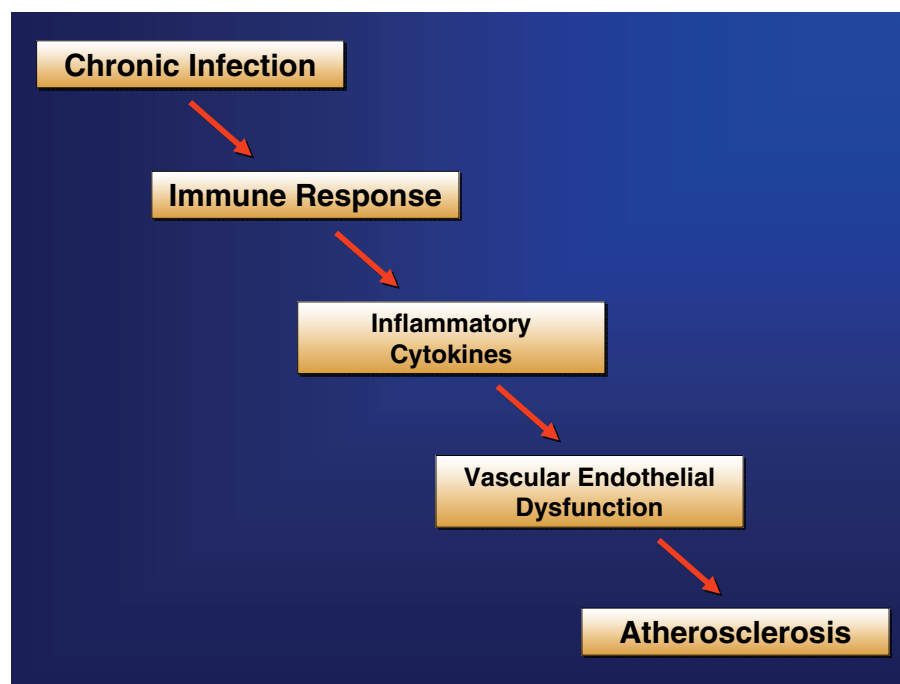


Figure 2 Model of the systemic inflammation hypothesis. Inflammation leads to an increase in the levels of circulating cytokines, which in turn damage the vascular endothelium and ultimately result in atherosclerosis.

SYSTEMIC INFLAMMATION

The 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 (Figure 2). The circulating cytokines of interest include C-reactive protein (CRP), Interleukin-1, Interleukin-6 (IL-6), tumor necrosis factor alpha (TNF- α), and prostaglandin.

The highest relative risk for myocardial infarction was found to be the levels of CRP together with the ratio of total cholesterol to high-density lipid.¹ CRP is a powerful marker of vascular risk and there is some evidence for a direct role in vascular dysfunction and atherogenesis. It is produced by the liver and is stimulated by TNF- α and IL-6, leading to a decrease in nitric oxide availability and an increase in angiotensin 1 receptors. It binds to low density lipids, increasing their uptake by macrophages and hence an increase in foam cell formation. For these reasons, CRP has been postulated as a major mechanism for atherosclerosis.

MEASURES OF ENDOTHELIAL DYSFUNCTION

Endothelium dysfunction can be measured by a number of available techniques. The most common is flow-mediated dilatation. Pulse-wave analysis and pulse pressure have also been used to look at endothelial dysfunction.

Flow-Mediated Dilatation

Flow-mediated dilatation measures the capacity of the arteries to dilate in response to altered flow. Basically, a blood pressure cuff with 300 mm of mercury is applied for 5 minutes. It is then released and the diameter of the artery is measured by ultrasound. The percent increase in diameter after release is a measure of the elasticity of the artery. In healthy subjects, a 7% to 10% increase in the

diameter of the artery is expected. In diabetics, in whom there is a vascular defect, there would only be a 4% to 6% increase. Unfortunately, flow-mediated dilatation is technically demanding, costly, and painful. Nonetheless, it has been shown that flow-mediated dilatation is decreased in severe periodontal disease and that this is associated with high levels of CRP.²

Pulse-Wave Analysis

Pulse-wave analysis measures heart rate, blood pressure (systolic, diastolic, pulse), large artery elasticity, small artery elasticity, and systemic vascular resistance. This technique is clinically feasible, and abnormal results are predictive of CVD. The results are abnormal in hypertension, diabetes, early renal disease, and rheumatoid arthritis. The defects are reversible with angiotensin-converting enzyme inhibitors and statins. Wilson and Jenkins showed that pulse-wave analysis correlates with flow-mediated dilatation.³ Preliminary data indicate that with treatment of periodontal disease, there is not only a decrease in the CRP but also an increase in small artery elasticity.

INFECTION

The 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.

A number of studies have shown bacteria in the arteries, but a study by Ford and colleagues⁴ used real-time polymerase chain reaction to show *P gingivalis* in 100% of the arteries. *Fusobacterium nucleatum* was found in approximately 80% of the arteries, *Tannerella forsythia* was found in just under 50%, and *C pneumoniae* was found in just under 30%. *H pylori* and *Haemophilus influenzae* were both found in approximately 4% of the arteries (Figure 3). These results clearly show that oral organisms can and do invade blood vessel walls, but it is unclear as to whether they cause atherosclerosis or simply invade an already damaged artery.

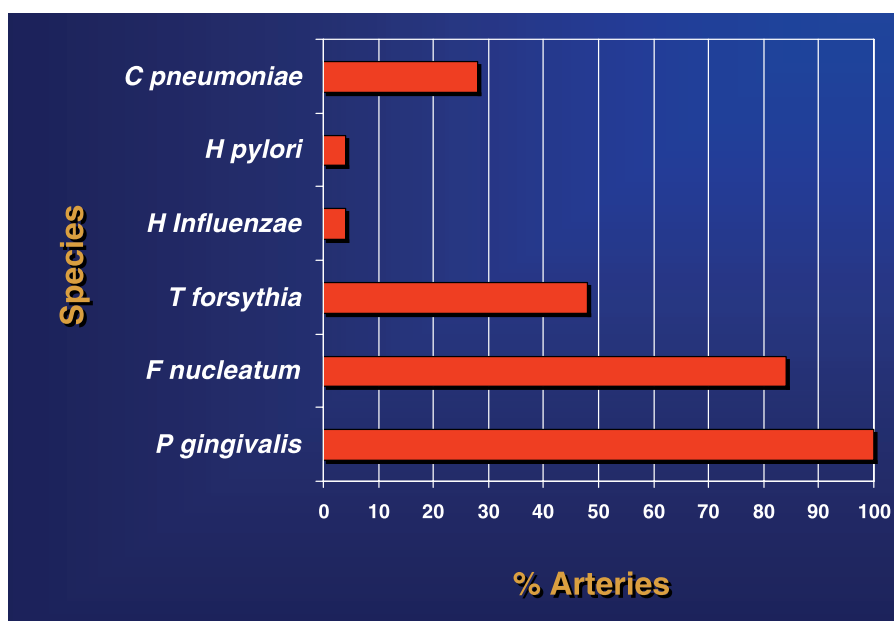


Figure 3 Real-time polymerase chain reaction was used to show that oral organisms can invade blood vessel walls (from Ford et al⁴).

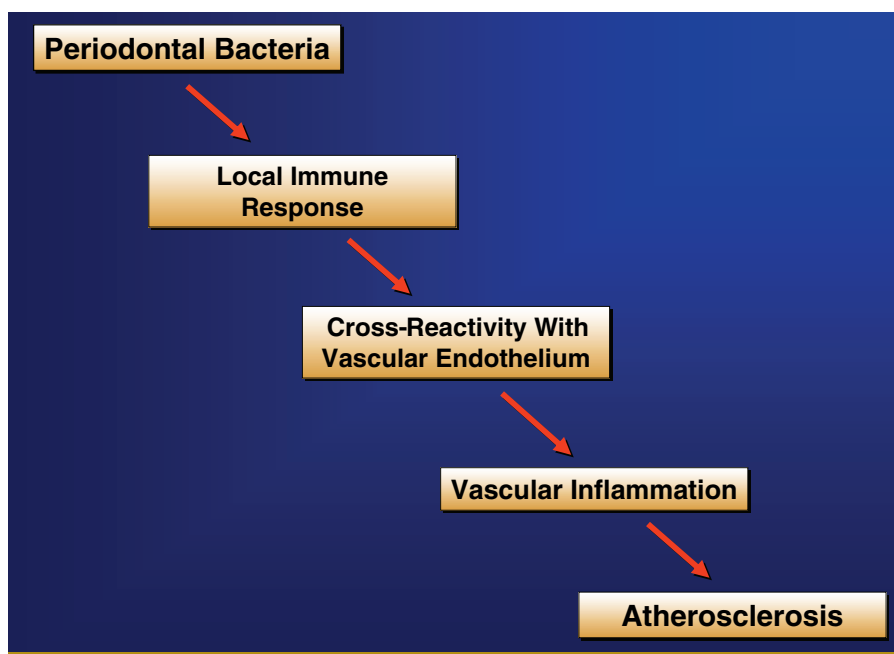


Figure 4 Model of the cross-reactivity hypothesis, in which the periodontal bacteria induce a local immune response and cross-react with self-antigens expressed on the vascular epithelium. This leads to vascular inflammation and atherosclerosis (based on the hypothesis of Wick et al¹¹).

CROSS-REACTIVITY/ MOLECULAR MIMICRY

The fourth hypothesis is that of cross-reactivity or molecular mimicry. In this hypothesis, the periodontal bacteria induce a local immune response, which subsequently cross-reacts with self-antigens expressed on the vascular epithelium. This in turn leads to vascular inflammation and atherosclerosis (Figure 4).

Recently, there has been increasing awareness that immune responses are central to atherogenesis,⁵ and a mechanism by which infection may initiate and facilitate the progression of atherosclerosis can be explained in terms of the immune response to bacterial heat shock genes and heat shock proteins (HSPs). All cells express HSPs on exposure to various forms of stress, including temperature,

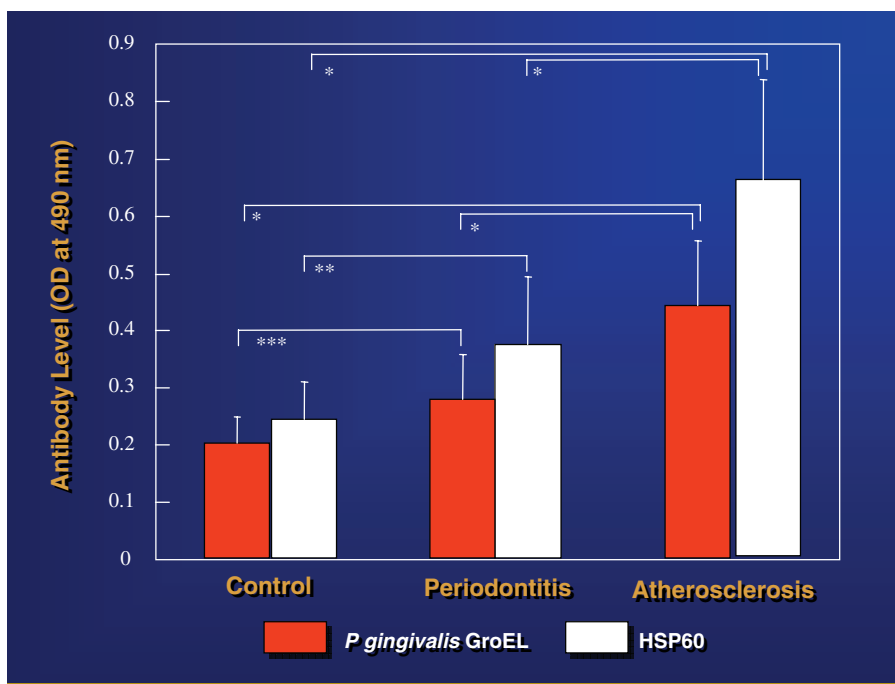


Figure 5 Higher levels of antibodies HSP60, *P. gingivalis*, and GroEL were found in patients with atherosclerosis compared with periodontitis and controls (from Tabeta et al¹⁹).

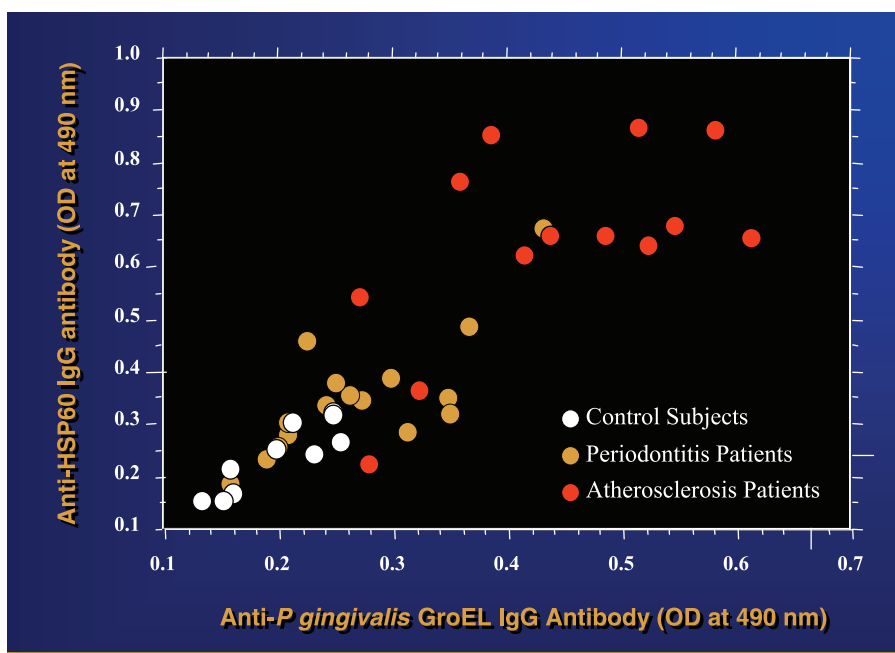


Figure 6 A high correlation was found between the anti-GroEL and anti-HSP60 antibodies (from Tabeta et al¹⁹).

oxidative injury, and infection.^{6,7} There is a remarkable conservation in the structure of HSPs across species, and many pathogens bear antigens that are homologous to human HSPs. During infection, bacterial HSPs constitute major antigenic determinants,⁸ which have been studied extensively to determine their role in the induction of protective or nonprotective

immune responses. The immune system may not be able to differentiate between self-HSP and bacterial-HSP. Cross-reactive epitopes of T cells with specificity for self-HSP can be activated during infection⁹ and antibodies generated by the host directed at pathogenic HSP could result in an autoimmune response to similar sequences in the host.⁶ In this

context, human heat shock protein 60 (hHSP60) shows a remarkable similarity with a very large number of auto-antigens. In terms of atherosclerosis, factors such as bacterial lipopolysaccharide, cytokines, and mechanical stress may induce the expression of host protective hHSP60 on endothelial cells. Because of the homologous nature of HSPs among species,¹⁰ cross-reactivity of antibodies to bacterial HSP (termed GroEL) with hHSP60 on endothelial cells may subsequently result in endothelial dysfunction and the development of atherosclerosis.¹¹ 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. A correlation between high anti-HSP60/65 antibody titers and high morbidity and mortality because of atherosclerosis has been demonstrated.¹² These antibodies were shown to be cross-reactive with those of other bacteria and were able to lyse stressed but not unstressed endothelial cells.¹³ The demonstration of elevated hHSP60 levels in patients with borderline hypertension and an association between early atherosclerosis and HSP60 levels offers further support for this hypothesis.¹⁴

GroEL proteins, belonging to the HSP60 family, have been reported to be major antigens in several pathogenic bacteria.¹⁵ An *Escherichia coli* GroEL homologue has been identified in the periodontopathic bacteria *P. gingivalis*, *F. nucleatum*, and *Actinobacillus actinomycetemcomitans*,^{16,17} which was immunogenic and was recognized by serum antibodies in patients with periodontal disease.¹⁷ GroEL antigens share a high degree of homology with hHSP60 proteins and antibody to hHSP60 cross-reacts with this periodontopathic bacterial GroEL.¹⁸ Patients with periodontal disease were shown to have a higher positive response to *P. gingivalis* GroEL than healthy controls and cross-reactivity between anti-*P. gingivalis* GroEL antibodies in the serum of these periodontitis patients with hHSP60 and between antibodies to hHSP60 with *P. gingivalis* GroEL has been demonstrated.¹⁹

Studies conducted in our laboratory⁴ have confirmed the expression of hHSP60 on endothelial cells and also on fibroblast/smooth muscle cells in atherosclerotic lesions in humans. Tabeta and colleagues¹⁹ were able to show that there are higher levels of antibodies HSP60, *P. gingivalis*, and GroEL in patients with atherosclerosis compared with periodontitis and controls (Figure 5). They were also able to show that there was a high correlation between the anti-GroEL and anti-HSP60 antibodies (Figure 6). Ford and colleagues⁴ were able to show the same in their CVD patients. In this case, again, the highest levels of antibody to HSP60 and GroEL were in those subjects with advanced periodontitis (Figure 7). They were also able to show that if those antibodies were absorbed with HSP60, the reactivity could be absorbed out, indicating a high degree of cross-reactivity between the antibody to the HSP60 and GroEL (Figure 8).

We have established GroEL-, hHSP60- and *P. gingivalis*-specific T-cell lines from peripheral blood and from human atherosclerotic plaques.²⁰ Of particular note was the cross-reactivity observed by a number of GroEL-specific T-cell lines to hHSP60 and hHSP60-specific lines to GroEL, again suggesting molecular mimicry of GroEL and hHSP60. These results demonstrate the presence of T cells specific for GroEL in the peripheral blood as well as in lesions of atherosclerosis and their cross-reactivity with hHSP60. The artery T-cell lines specific for GroEL, hHSP60, and *P. gingivalis* demonstrate a predominant Th2 phenotype in the CD4 subset and a Tc0 predominance in the CD8 subset with a high proportion of CD8 cells expressing the chemokines IP-10, RANTES, MCP-1, and MIP- α . Finally, there was an over-expression of the T-cell receptor V β 5.2 family in all lines suggesting clonality within the cell lines. The cytokine, chemokine, and V β results are similar to those demonstrated previously for *P. gingivalis*-specific lines from patients with periodontal disease.²¹ Yamazaki and colleagues²² demonstrated that HSP60-stimulated peripheral blood mononuclear cells (PBMC) and *P. gingivalis* GroEL-stimulated PBMC had identical nucleotide sequences in the CDR3 of

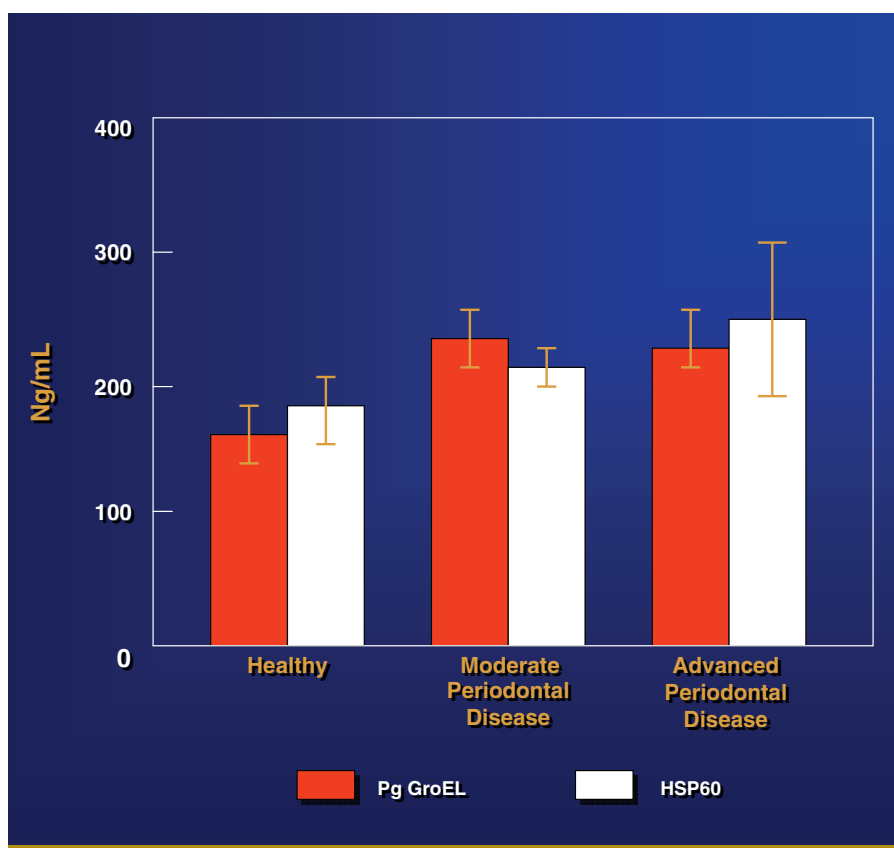


Figure 7 In CVD patients, the highest levels of antibody to HSP60 and GroEL were in those subjects with advanced periodontitis (from Ford et al²⁷).

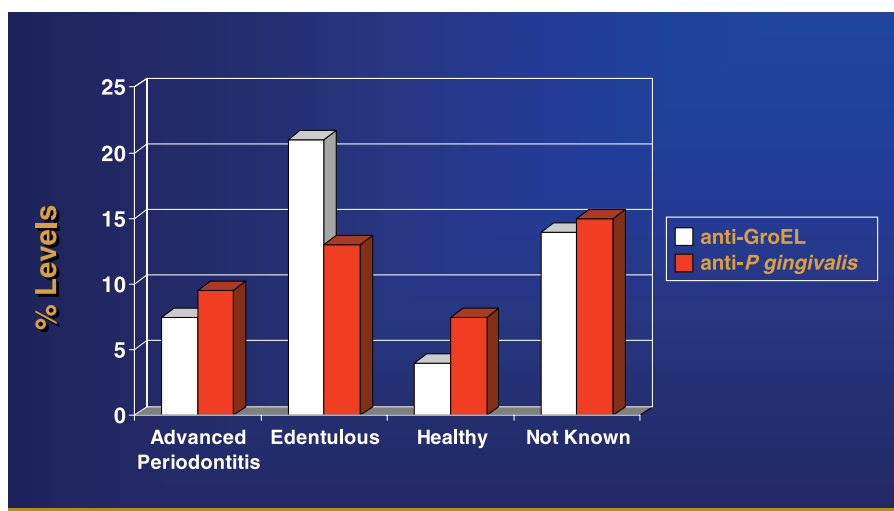


Figure 8 The percent levels of anti-GroEL and anti-*P. gingivalis* antibodies in plasma of atherosclerosis patients after absorption with hHSP60 (from Ford et al²⁷).

the T-cell receptor β chain and that T cells with the same nucleotide sequences were present in the gingival tissues as well as in atherosclerotic aneurysmal tissues (Figure 9). 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. This provides strong support to 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.²³

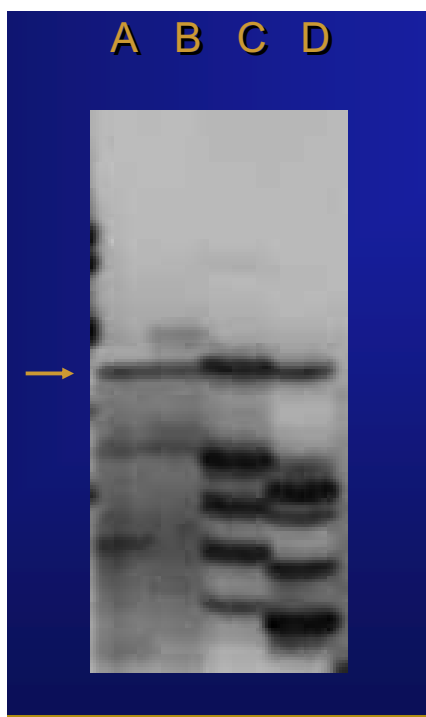
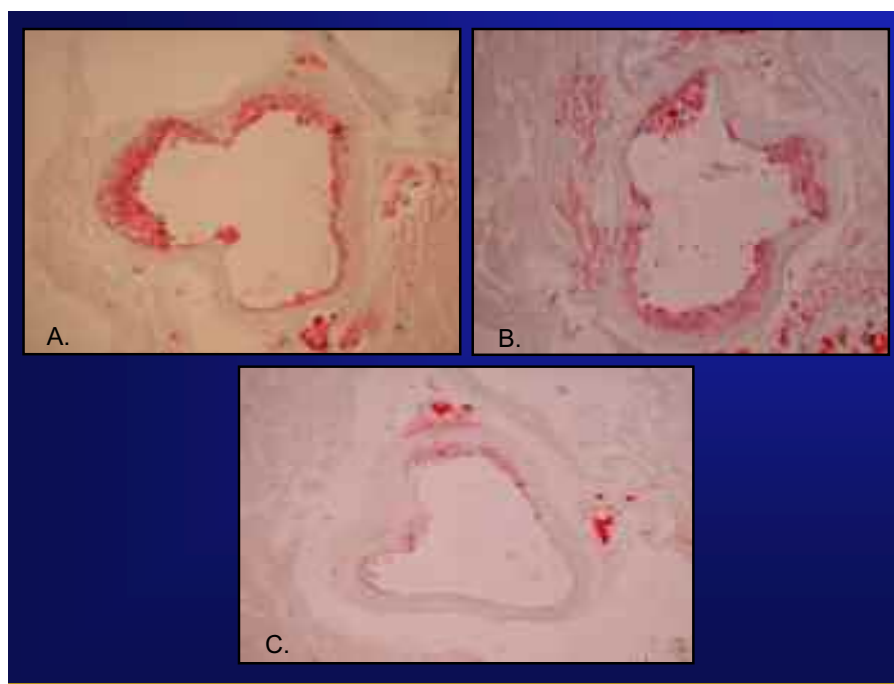


Figure 9 (A) HSP60-stimulated PBMC. (B) *P gingivalis*-stimulated PBMC. (C) Atherosclerotic aneurismal-derived T-cell clones. (D) Gingival tissue-derived T-cell clones. Arrow indicates identical TCR sequence in all four clones (from Yamazaki et al²²).

Ang and colleagues²⁴ 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.

There is clear evidence of an epidemiological association between periodontal disease and CVD. The results of our studies in Brisbane and Niigata (references 4, 19, 20, 21, 22, and 27 reviewed in this article) provide evidence for criteria two and three. Together 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



Figures 10A through 10C In the apoE mouse model, weekly injections of either *P gingivalis* or *C pneumoniae* intraperitoneally resulted in marked atherosclerotic lesions in the proximal aorta. Control mice showed no lesions. (A) *P gingivalis*. (B) *C pneumoniae*. (C) Control. (From Ford et al, unpublished data.)

tissues; and we have shown that these cells are in the peripheral blood.

Li and colleagues²⁵ showed that repeated intravenous injection with *P gingivalis* accelerates the progression of atherosclerosis in apoE-deficient mice. Lalla and coworkers²⁶ showed that oral infection with *P gingivalis* also accelerates the progression of atherosclerosis in apoE-deficient mice. We have also shown that in the apoE mouse model, weekly injections of either *P gingivalis* or *C pneumoniae* intraperitoneally resulted in marked atherosclerotic lesions in the proximal aorta, while control mice showed no lesions compared with controls (Figures 10A through 10C). We have further shown that, although lesions developed later with *P gingivalis* compared with *C pneumoniae*, increasing the pathogen burden of *P gingivalis* but not *C pneumoniae* enhanced atherosclerosis.²⁷ Increased pathogen load resulted in increased titers of specific antibody in the case of *P gingivalis* but not *C pneumoniae*, suggesting that the peak atherosclerotic response to *P gingivalis* coincided with the peak immune (antibody) response. This further suggests that immunological cross-reactivity could be involved.

CONCLUSION

The results from these studies add to the weight of evidence implicating infection, including periodontal infection, with atherosclerosis. Taken together, these results provide evidence for all four molecular mimicry criteria 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. None of the hypotheses is mutually exclusive and it is clear that in some people one or either of the proposed mechanism may be more important. In this context, it is clear that infection can contribute to atherosclerosis via molecular mimicry. This infection could be respiratory (eg, *C pneumoniae*), gastrointestinal (eg, *H pylori*), or oral (eg, *P gingivalis*). Together these all contribute to the total burden of infection; and in some people 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 dental professionals to ensure that all oral infection is kept to a minimum. In addition, all of these infections lead to inflammation in the respective tissue and in this context contribute

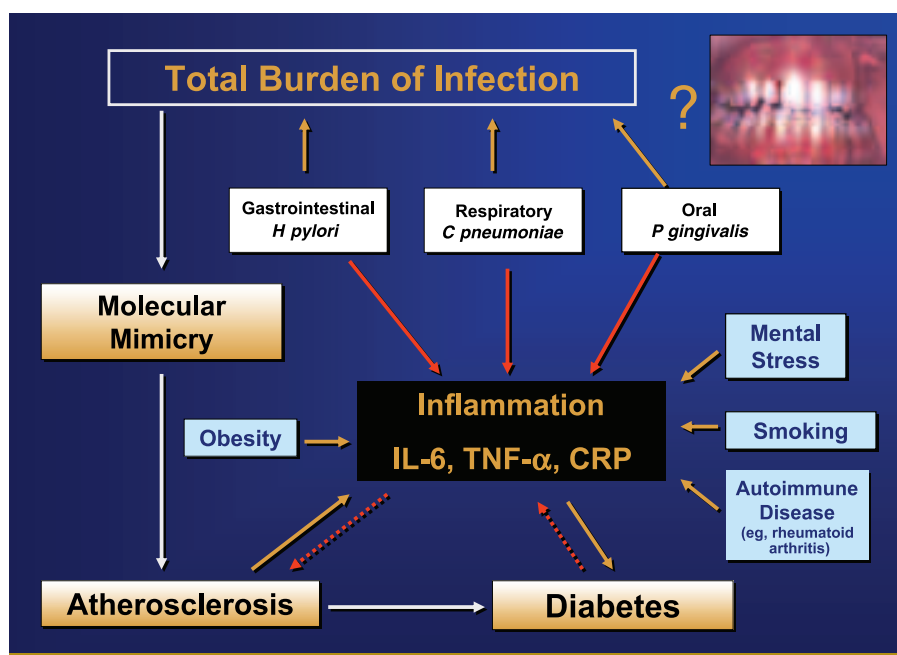


Figure 11 Model of the complex interactions of the observed associations between periodontal disease, atherosclerosis, diabetes, rheumatoid arthritis, smoking, mental stress, etc.

to the total burden of inflammation, which in turn can contribute to atherosclerosis. Obesity, diabetes, and other auto-immune diseases can also contribute to this total burden of inflammation and atherosclerosis. Atherosclerosis itself is an inflammatory condition which contributes to the total burden of inflammation. All of these interactions can be seen in Figure 11, 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.

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