Journal of
PERIODONTAL RESEARCH

J Periodont Res 2012; 47: 811–816
All rights reserved

© 2012 John Wiley & Sons A/S

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2012.01501.x

Short communication

Predominant immunoreactivity of *Porphyromonas gingivalis* heat shock protein in autoimmune diseases

E. Jeong[1], J-Y. Lee[2], S-J. Kim[2], J. Choi[2]

[1]Department of Molecular Biology, College of Natural Sciences, Pusan National University, Pusan, South Korea and [2]Department of Periodontology, School of Dentistry, Pusan National University, Yangsan City, South Korea

Jeong E, Lee J-Y, Kim S-J, Choi J. Predominant immunoreactivity of Porphyromonas gingivalis *heat shock protein in autoimmune diseases. J Periodont Res 2012; 47: 811–816. © 2012 John Wiley & Sons A/S*

Background and Objective: Autoimmune diseases, including atherosclerosis, diabetes mellitus and rheumatoid arthritis, can be triggered and aggravated by the pathogen-driven antigenic peptide from *Porphyromonas gingivalis* HSP60. *P. gingivalis* is the major pathogen of chronic periodontitis, which is a global epidemic prevalent in two-thirds of the adult population. The monoclonal antibody raised against peptide 19 (Pep19: TLVVNRLRGSLKICAVKAPG) from *P. gingivalis* HSP60 was polyreactive to the human homolog. The aim of this study was to determine if Pep19 from *P. gingivalis* HSP60 manifests itself as a predominant antigen in infection-triggered autoimmune diseases.

Material and Methods: Pep19 from *P. gingivalis* HSP60, *Mycobacterium tuberculosis* HSP60 and *Chlamydia pneumoniae* HSP60 was synthesized for comparative recognition by the sera from patients with atherosclerosis, type 2 diabetes and rheumatoid arthritis, all with ongoing periodontal disease, and by the sera of a control group of patients with periodontal disease but with no history of atherosclerosis, type 2 diabetes or rheumatoid arthritis.

Results: Of the Pep19 peptides from *P. gingivalis* HSP60, *M. tuberculosis* HSP60 and *C. pneumoniae* HSP60, Pep19 from *P. gingivalis* HSP60 was the peptide epitope predominantly and most consistently recognized by the serum samples of the four disease groups (chronic periodontitis, atherosclerosis, type 2 diabetes mellitus and rheumatoid arthritis).

Conclusion: Seroreactivity to Pep19 of *P. gingivalis* HSP60, an oral pathogen, was predominant in patients with autoimmune disease with ongoing periodontal disease.

Jeomil Choi, DDS, PhD, Department of Periodontology, School of Dentistry, Pusan National University, Beomeo-Ri, Mulgeum-Eop, Yangsan City 626-870, South Korea
Tel: +82 55 360 5200
Fax: +82 55 360 5194
e-mail: jrapa@pusan.ac.kr

Key words: autoimmunity; heat shock protein; periodontitis; *Porphyromonas gingivalis*

Accepted for publication May 19, 2012

The concept of molecular mimicry stems from the tailoring of infectious antigens, in which unique conserved sequences are highly homologous to the human counterparts that might trigger the autoantigen-derived immunopathologic process (1,2). Therefore, identifying the epitope that might operate a double-faced immunoregulatory mechanism is essential for understanding and controlling autoimmune diseases (3,4).

Traditionally, bacterial heat shock proteins (HSPs) of *Mycobacterium tuberculosis* and *Chlamydia pneumoniae* have been proposed as candidate antigenic molecules responsible for triggering autoimmune diseases, including adjuvant arthritis and diabetes (5,6). In contrast, the HSP from *Porphyromonas gingivalis*, a major periodontal pathogen, has attracted considerable interest in the periodontitis–atherosclerosis link (7–9).

The present study proposed that the antigenic load of *P. gingivalis* HSP should be higher than those of M. tuberculosis and *C. pneumoniae* because of the extremely large antigenic burden of *P. gingivalis* derived from the overwhelming number of these bacteria inhabiting the subgingival ecological niche during chronic periodontitis, a global epidemic disease prevailing in two-thirds of the adult population (10).

Recently, it was reported that peptide 19 (Pep19: TLVVNRLRGSLKICAVKAPG) from P. gingivalis HSP60 is an immunodominant peptide epitope that might trigger autoimmune responses both in periodontitis and in infection-triggered atherosclerosis; moreover, a monoclonal antibody raised against Pep19 was found to be polyreactive to the human homolog (9). As an extrapolation of this observation, the present study proposed that this antigenic cross-reactive peptide epitope is an immunodominant epitope that might induce a robust autoimmune response in type 2 diabetes mellitus (DM) and rheumatoid arthritis (RA).

The aim of the present investigation was to demonstrate that Pep19 from *P. gingivalis* HSP60 is a predominant antigen recognized by patients with infection-triggered autoimmune diseases.

Material and methods

Study subjects

Twenty patients with atherosclerosis, type 2 DM and RA, suffering from ongoing chronic periodontitis, which is defined as inflammation within the supporting tissues of the teeth with progressive attachment and bone loss Glossary of Periodontal Terms, 4th edition, American Academy of Periodontology, 2001 (11), were enrolled in this study. Atherosclerosis was defined as luminal irregularity or stenosis of the artery by angiography (12). A diagnosis of type 2 DM was based on the following glucose criteria: a fasting plasma glucose level of ≥ 126 mg/dL, or symptoms (such as polyuria, polydipsia and unexplained weight loss) and a casual plasma glucose level of ≥ 200 mg/dL, or a plasma glucose level of ≥ 200 mg/dL 2 h after a 75-g glucose load, or a glycated hemoglobin (HbA1c) level of ≥ 6.5% (13). Patients who fulfilled the American College of Rheumatology 1987 revised classification criteria were diagnosed with RA (14).

Table 1. Profiles of chronic periodontitis manifested by pocket depths and level of attachment*

Variable	Disease			
	Periodontitis	Atherosclerosis	Diabetes mellitus	Rheumatoid arthritis
Number of patients	20	20	20	20
Pocket depth	5.3 ± 1.5	5.5 ± 1.2	5.6 ± 1.4	5.5 ± 1.6
Level of attachment	5.4 ± 1.5	5.5 ± 1.3	5.7 ± 1.6	5.6 ± 1.3

Values are given as mean ± standard deviation, unless indicated otherwise.
*No significant differences in the mean values among disease groups.

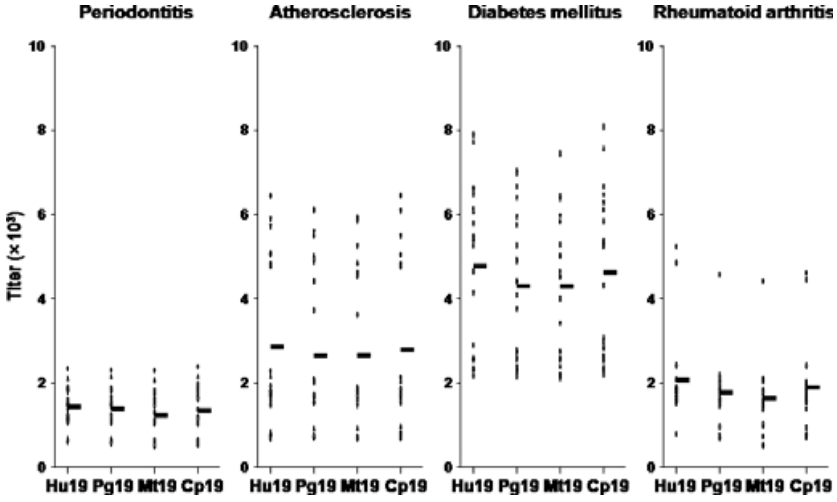

Fig. 1. Scatter diagram of serum immunoglobulin G (IgG) titers, from patients of the four disease groups (periodontitis, atherosclerosis, diabetes mellitus and rheumatoid arthritis), to Pep19 from human HSP60 (Hu19), Pep19 from *Porphyromonas gingivalis* HSP60 (Pg19), Pep19 from *Mycobacterium tuberculosis* HSP60 (Mt19) and Pep19 from *Chlamydia pneumoniae* HSP60 (Cp19). The horizontal bars represent mean values. Each dot represents the serum IgG titer.

Table 2. Sequence homology of Pep19 from *Porphyromonas gingivalis* HSP60, *Mycobacterium tuberculosis* HSP60 and *Chlamydia pneumoniae* HSP60 to the human homolog

Pep19 source	Sequence	Homology (%)
P. gingivalis HSP60	TLVVNRLRGSLKICAVKAPG	60
M. tuberculosis HSP60	TLVVNKIRGTFKSVAVKAPG	55
C. pneumoniae HSP60	TLVVNKLQGLLQVTVVTIPQ	50

Twenty periodontitis patients without a history of the aforementioned diseases were included as control subjects. Peripheral blood was drawn from all patients by venipuncture for serum collection. The study was approved by the Institutional Review Board of Pusan National University Hospital. Written, informed consent for the surgical procedure was obtained from the patients. All experiments were performed under the principles of the declaration of Helsinki.

Serum immunoglobulin G titers to Pep19

Pep19 peptides from *P. gingivalis*, *M. tuberculosis* or *C. pneumoniae* HSP60 were synthesized, dissolved in dimethylsulfoxide and diluted with 0.1 M carbonate-bicarbonate buffer. The wells of microtiter plates (Cova-Link plate; NUNC, Roskilde, Denmark) were coated with Pep19 at an antigen concentration of 4 mM/mL using water-soluble 1-ethyl-(3-dimethylaminopropyl) carbodiimide in the presence of N- hydroxysuccinamide. After washing the coated plates with phosphate- buffered saline (PBS)-Tween 20, human serum samples diluted serially with PBS-Tween 20 were added to each well and incubated. After washing the plates, horseradish peroxidase (HRP)-conjugated mouse anti-human immunoglobulin G (IgG) (γ-chain specific; Southern Biotech, Birmingham, AL, USA) was added and incubated. The plates were washed, and tetramethylbenzidine (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA) was added to each well and incubated, followed by the addition of H_2SO_4 to quench the reaction. For ELISA, negative controls were created using the following two protocols: after coating the antigen, the negative control-1 well was incubated in duplicate with dilution buffer only (i.e. without patient serum) followed by a routine procedure for the standard ELISA protocol, whereas the negative control-2 well was incubated in duplicate with patients' serum in the dilution buffer followed by incubation with dilution buffer only, without HRP-conjugated mouse anti-human IgG. The background absorbance (A) values of these control wells were subtracted from the A values of the test samples.

The A values, read at a wavelength of 450 nm, were plotted as a function of the serum-dilution factor for regression analysis. The serum-dilution factor corresponding to an A of 0.5 was designated as the IgG titer for a given sample. The differences in the IgG titers against the Pep19 from different organisms were analyzed using the Student's t-test.

Dot-immunoblot profiles to Pep19

Five micrograms of Pep19 from each bacterial HSP60 was spotted onto each of 40 nitrocellulose membranes (i.e. to allow serum samples from 10 patients in each of the four groups to be tested separately). The membranes were blocked for 30 min with 5% skim milk,

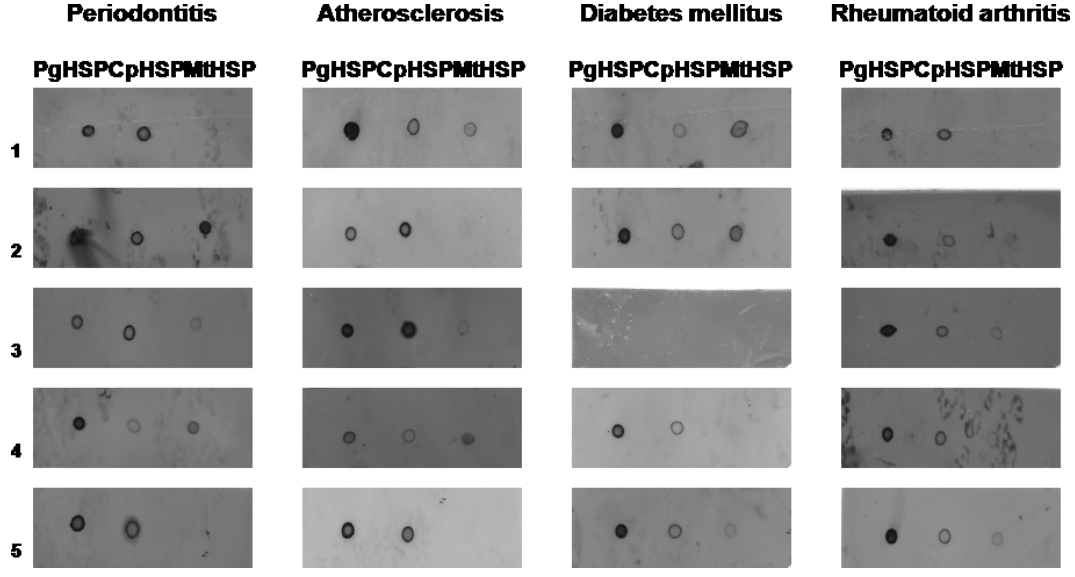

Fig. 2. Dot-immunoblot profiles of reactivity of serum from patients of the four disease groups (periodontitis, atherosclerosis, diabetes mellitus and rheumatoid arthritis) to whole HSP60 protein from *Porphyromonas gingivalis* (PgHSP), whole HSP60 protein from *Mycobacterium tuberculosis* (MtHSP) and whole HSP60 protein from *Chlamydia pneumoniae* (CpHSP). The black margins are artifacts produced during the blotting procedure.

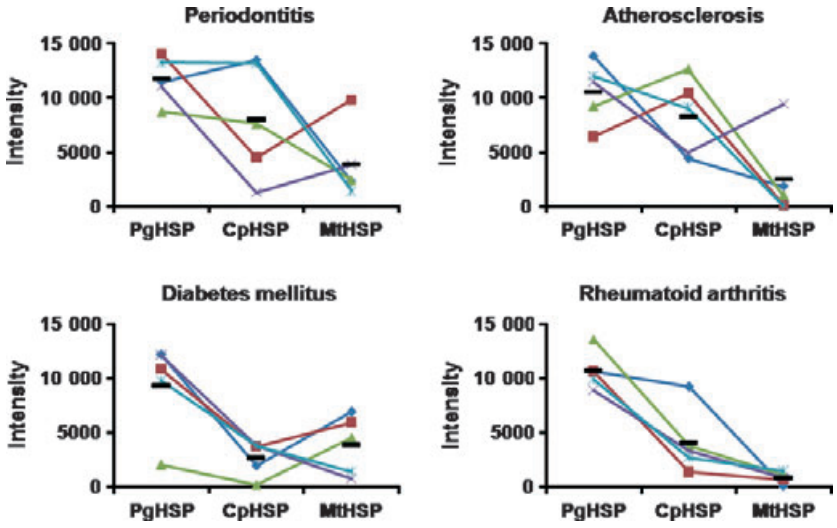

Fig. 3. Densitometric image analysis of the dot-immunoblot profiles of reactivity of serum from patients of the four disease groups (periodontitis, atherosclerosis, diabetes mellitus and rheumatoid arthritis) to whole HSP60 protein from *Porphyromonas gingivalis* (PgHSP), whole HSP60 protein from *Mycobacterium tuberculosis* (MtHSP) and whole HSP60 protein from *Chlamydia pneumoniae* (CpHSP).

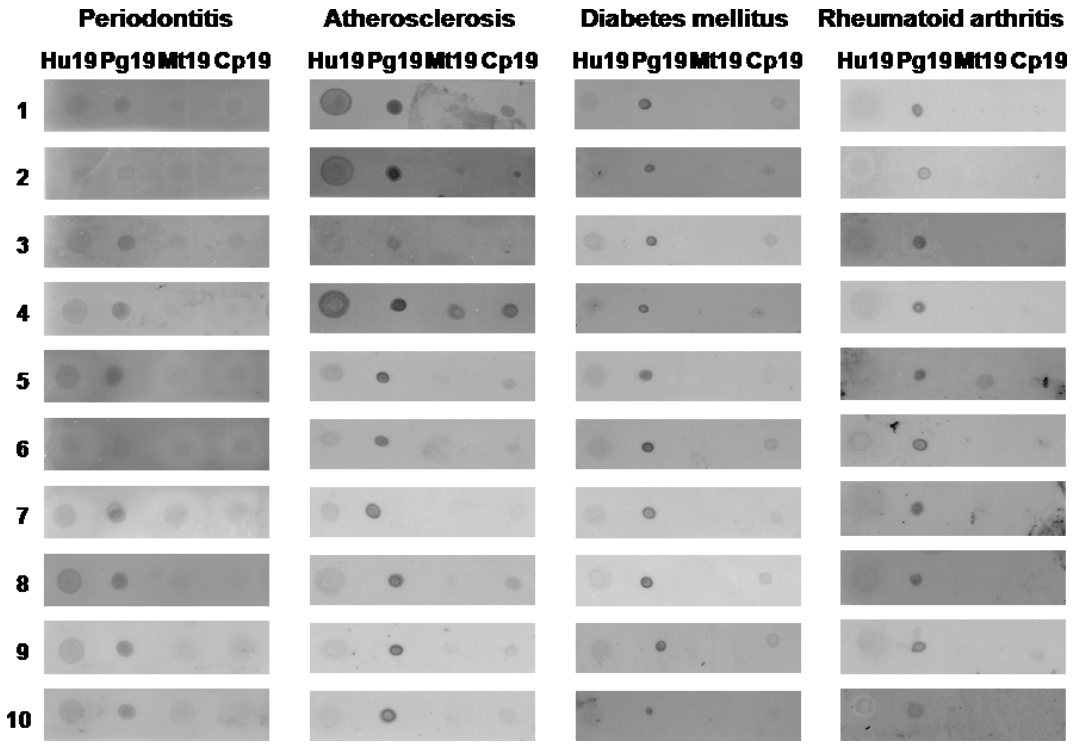

Fig. 4. Dot-immunoblot profiles of reactivity of serum from patients of the four disease groups (periodontitis, atherosclerosis, diabetes mellitus and rheumatoid arthritis) to Pep19 from human HSP60 (Pg19), Pep19 from *Porphyromonas gingivalis* HSP60, Pep19 from *Mycobacterium tuberculosis* HSP60 (Mt19) and Pep19 from *Chlamydia pneumoniae* HSP60 (Cp19). The black margins are artifacts produced during the blotting procedure.

serum samples from 10 representative patients in each group were added to separate membranes and the membranes were incubated for a further 2 h at room temperature. After washing the membranes with PBS-Tween 20 for 30 min at room temperature, HRP-conjugated mouse anti-human IgG (γ-chain specific; Jackson Immuno Research Laboratories, West Grove, PA, USA) was added, and the membranes were incubated for a further 1 h. The membranes were washed with PBS-Tween 20, tetramethylbenzidine was added and any color development

was recorded. An identical procedure was performed using five representative serum samples from each disease group for the whole HSP60 proteins of *P. gingivalis*, *M. tuberculosis* or *C. pneumoniae* (kindly provided by Dr. Grant Pierce). Each dot-blot image was converted for densitometric image analysis using imaging software (IMAGE J software version 1.45; National Institutes of Health, Bethesda, MD, USA).

Results and Discussion

Table 1 lists the mean pocket depths and attachment levels of patients in each of the four disease groups. Both probing pocket depth and attachment level were similar in each of the disease groups. The serum IgG titer seemed highest to Pep19 from *C. pneumoniae* HSP60 among the three microorganisms tested, which was comparable to the IgG titer to Pep19 from *P. gingivalis* HSP60, in the patients of the four disease groups; however, the intermicrobial differences failed to reach statistical significance (Fig. 1). The magnitudes of the antibody titers were enhanced greatly in the groups with systemic disease plus chronic periodontitis (particularly in the groups with atherosclerosis and type 2 DM). The antibody titers to Pep19 of human HSP60, *P. gingivalis* HSP60, *C. pneumoniae* HSP60 and *M. tuberculosis* HSP60 were similar. Although the antibody to Pep19 of *P. gingivalis* HSP60/GroEL was measured against purified Pep19 of *P. gingivalis* HSP60/GroEL, it is likely to represent the antibody to the GroEL homologues of other microorganisms. In a previous study (9), *P. gingivalis* HSP60/GroEL was shown to cross-react with the HSP60 homologues from other bacteria.

Despite the similar degree of sequence homology of the Pep19 from the three different bacterial species to the human homolog (Table 2), the highest serum immunoblot intensity was observed consistently with Pep19 from *P. gingivalis* HSP60 or with the whole *P. gingivalis* HSP60 in patients from all four disease groups compared with those from the other two microorganisms (Figs 2–5).

The severity of periodontal disease was similar among the four test groups, which demonstrated a unique serum-response pattern to Pep19 or to whole protein from *P. gingivalis* HSP60. In accordance with the emerging concept that oral pathogens might contribute to the initiation and perpetuation of systemic autoimmune diseases (15), it was a primary concern as to whether *P. gingivalis* HSP would play a key role in infection-triggered autoimmune responses operating in the immunopathogenic mechanisms of atherosclerosis, type 2 DM or RA.

These results are in accordance with the recent observation that anti-GroEL and anti-*P. gingivalis* IgG antibodies cross-react with human HSP60 (16). Ford *et al.* (17) reported that increasing the pathogenic burden of *P. gingivalis* enhanced the size of the atherosclerotic lesion, which also correlated with the anti-GroEL IgG antibody levels, supporting the concept of molecular mimicry.

Pep19 from *P. gingivalis* HSP60 has been reported to lack the ability to mobilize regulatory T cells in patients with chronic periodontitis (9). Furthermore, Pep19 from *P. gingivalis* HSP60 aggravated both periodontitis and atherosclerosis (18). Whether the epitope enhances or suppresses the infection-induced autoimmune responses by mobilizing the effector or regulatory T cells (19, 20) needs to be determined in a sophisticated manner for molecular mimicry-based immunotherapy through antigen tailoring by comparing the sequence alignment (1). In an attempt to circumvent this inherent problem, the polyreactive monoclonal antibody that recognized Pep19 from both *P. gingivalis* HSP60

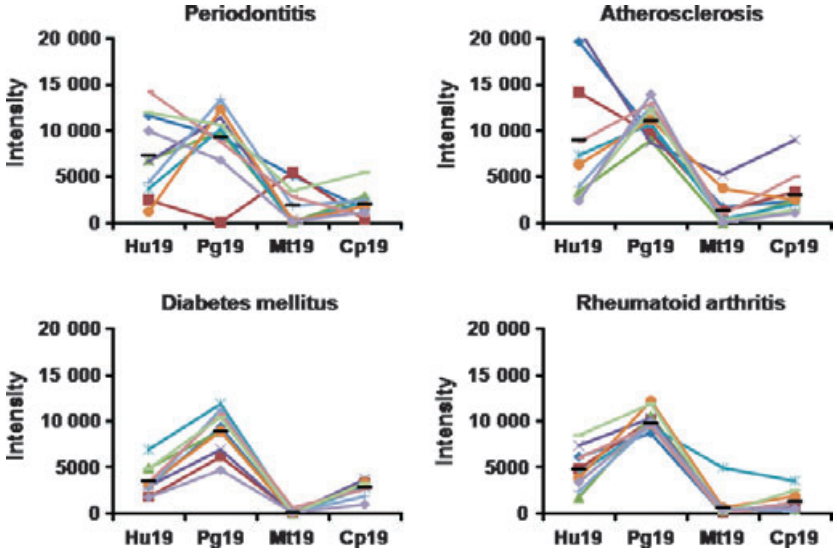

Fig. 5. Densitometric image analysis of the dot-immunoblot profiles of reactivity of serum from patients of the four disease groups (periodontitis, atherosclerosis, diabetes mellitus and rheumatoid arthritis) to Pep19 from human HSP60 (Hu19), Pep19 from *Porphyromonas gingivalis* HSP60 (Pg19), Pep19 from *Mycobacterium tuberculosis* HSP60 (Mt19) and Pep19 from *Chlamydia pneumoniae* HSP60 (Cp19).

and human HSP60 was used as a tool to examine an immunodominant peptide from an infectious pathogen that might trigger autoimmune disease (9). In addition, the other peptide recognized only by a monoreactive monoclonal antibody is being tested for its potential to suppress autoimmunity.

No attempt was made to quantify the severity of disease in each patient with atherosclerosis, type 2 DM or RA. Instead, an attempt was made to adhere to the physician's clinical diagnosis made according to their routine diagnostic guideline. A future study will examine the correlation between the severity of autoimmune diseases and the reactivity to Pep19 of HSP60.

Within the limits of this study, it is tempting to hypothesize that Pep19 might be a pan biomarker for pathogen-derived autoimmunity prevailing in atherosclerosis, type 2 DM and RA. Further mechanistic studies will be needed to delineate this (14,20,21).

Acknowledgements

This work was supported by a Clinical Research Grant from Pusan National University Hospital (2010), a Medical Research Institute Grant (2008–25), Pusan National University, KOSEF RO1-2008-000-20044-0, the National Research Foundation of Korea (NRF) grant funded by the Korean Government (MEST) (No. 2012-0005540) and the Korea Healthcare Technology R&D Project, Ministry of Health & Welfare, Republic of Korea A080391. The support of Professors Jun-Hong Kim and Seok-Man Son, and of Sung-Il Kim, in patient recruitment is appreciated.

References

1. Wucherpfennig KW. Structural basis of molecular mimicry. *J Autoimmun* 2001; **16:**293–302.
2. Wucherpfennig KW. Mechanisms for the induction of autoimmunity by infectious agents. *J Clin Invest* 2001;**108:**1097–1104.
3. Fourneau JM, Bach JM, Endert PM, Bach JF. The elusive case for a role of mimicry in autoimmune diseases. *Mol Immunol* 2004;**40:**1095–1102.
4. Hansson KG, Libby P. The immune response in atherosclerosis: a double-edged sword. *Nat Rev Immunol* 2006;**6:**508–519.
5. Harats D, Yacov N, Gilburd B, Shoenfeld Y, George J. Oral tolerance with heat shock protein 65 attenuates *Mycobacterium Tuberculosis*-induced and high-fat-diet-driven atherosclerotic lesions. *J Am Coll Cardiol* 2002;**40:**1333–1338.
6. Anderton SM, van der Zee R, Prakken B, Noordzij A, van Eden W. Activation of T-cells recognizing self 60-kD heat shock protein can protect against experimental arthritis. *J Exp Med* 1995;**181:**943–952.
7. Choi JI, Chung SW, Kang HS, Rhim BY, Kim SJ, Kim SJ. Establishment of *Porphyromonas gingivalis* heat-shock-protein specific T-cell lines from atherosclerosis patients. *J Dent Res* 2002;**81:**344–348.
8. Choi JI, Chung SW, Kang HS *et al*. Epitope mapping of *Porphyromonas gingivalis* heat-shock protein and human heat-shock protein in human atherosclerosis. *J Dent Res* 2004;**83:**936–940.
9. Choi J, Lee SY, Kim K, Choi BK. Identification of immunoreactive epitope of *Porphyromonas gingivalis* heat shock protein peptide in periodontitis and atherosclerosis. *J Periodont Res* 2011;**46:**240–245.
10. Haffajee AD, Socransky SS. Introduction to microbial aspects of periodontal biofilm communities, development and treatment. *Periodontol 2000* 2006;**42:**7–12.
11. Glossary of Periodontal Terms, 4th edition. American Academy of Periodontology, 2001.
12. Ryan TJ, Bauman WB, Kennedy JW *et al*. Guidelines for percutaneous transluminal coronary angioplasty. A report of the American Heart Association/American College of Cardiology Task Force on assessment of diagnostic and therapeutic cardiovascular procedures. *Circulation* 1993;**88:**2987–3007.
13. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 2012;**35:**S64–S71.
14. Arnett FC, Edworthy SM, Bloch DA *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;**31:**315–324.
15. Kebschull M, Demmer RT, Papapanou PN. Gum bug, leave my heart alone! – epidemiologic and mechanistic evidence linking periodontal infections and atherosclerosis. *J Dent Res* 2010;**89:**879–902.
16. Ford PJ, Gemmell E, Hamlet SM *et al*. Cross-reactivity of GroEL antibodies with human heat shock protein 60 and quantification of pathogens in atherosclerosis. *Oral Microbiol Immunol* 2005;**20:**296–302.
17. Ford PJ, Gemmell E, Timms P, Chan A, Preston FM, Seymour GJ. Anti-*P. gingivalis* response correlates with atherosclerosis. *J Dent Res* 2007;**86:**35–40.
18. Choi J, Kim K. Vaccination of Pep19 from *Porphyromonas gingivalis* HSP60 aggravates both periodontitis and atherosclerosis. Keystone Symposium abstract. 142, Seattle, 2010.
19. van Eden W, Wick G, Albani S, Cohen I. Stress, heat shock proteins, and autoimmunity. *Ann N Y Acad Sci* 2007;**1113:**217–237.
20. Sherer Y, Shoenfeld Y. Mechanisms of disease: atherosclerosis in autoimmune diseases. *Nat Clin Pract Rheumatol* 2006; **2:**99–106.
21. Choi JI, Seymour GJ. Vaccines against periodontitis: a forward-looking review. *J Periodontal Implant Sci* 2010;**40:**153–163.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.