J Clin Periodontol 2005; 32 (Suppl. 6): 39–53

Journal of Clinical Periodontology

Immune responses and vaccination against periodontal infections

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Persson RG. Immune responses and vaccination against periodontal infections. J Clin Periodontol 2005; 32 (Suppl. 6): 39–53. © Blackwell Munksgaard, 2005.

Abstract

Background: The infectious aetiology of periodontitis is complex and no curative treatment modality exists. Palliative therapy is available.

Aims: To review the evidence that active or passive immunization against periodontitis provides immune protection.

Material and Methods: PubMed (Medline), the National Institutes of Health, the Food and Drug Administration, and the Center for Disease Control electronic databases were searched to extrapolate information on immune responses to immunization against periodontitis.

Results: Studies in non-human primate models using ligature-induced experimental periodontitis suggest that antibody responses by active immunization against *Porphyromonas gingivalis* can safely be induced, enhanced, and obtained over time. Immune responses to whole bacterial cell and purified protein preparations considered as vaccine candidates have been evaluated in different animal models demonstrating that there are several valid vaccine candidates. Data suggest that immunization reduces the rate and severity of bone loss. It is also, temporarily, possible to alter the composition of the subgingival microflora. Natural active immunization by therapeutic interventions results in antibody titre enhancement and potentially improves treatment outcomes. Passive immunization of humans using *P. gingivalis* monoclonal antibodies temporarily prevents colonization of *P. gingivalis*. Probiotic therapy may be an alternative approach. Regulatory and safety issues for human periodontal vaccine trials must be considered. Shared infectious aetiology between periodontitis and systemic diseases may enhance vaccine effort developments.

Conclusions: Proof of principle that active and passive immunization can induce protective antibody responses is given. The impact of natural immunization and passive immunization in humans should be explored and may, presently, be more feasible than active immunization studies.

Key words: antibody; antigen; immunity; periodontitis; Porphyromonas gingivalis; review; vaccine

Accepted for publication 1 April 2005

The host responses to infectious exposures and from non-infectious substances are physiological functions of the immune system. Specifically, microbial substances including lipids, proteins and polysaccharides trigger immune responses that either eliminate the pathogens or non-self substances, or result in pathological consequences for the host. Prior to the very first infection, from birth, existing innate immunity functions protect the host and provide competent responses to microbes. The innate immune system includes the following: (I) epithelial cell and chemical barriers (defensins), (II) phagocytic and natural killer cells, and (III) serum proteins, complement factors, and cytokines. Virulent microorganisms equipped with sophisticated self-protective and destructive abilities can bypass the innate immune system.

As a second line of host defence, adaptive immunity has explicit capacities to recognize, memorize, and with increasing efficacy, respond to exposures of microorganisms and their by-products (antigens and enzymes). Adaptive immunity consists of two immune competent systems developed by lymphocytes: (I) the cell-mediated immune system, and (II) the humoral immune system. The cell-mediated immune system is mediated by T lymphocytes, and is specifically dedicated to interact with viruses and bacteria that survive and proliferate inside cells (i.e. phagocytes and epithelial cells). Antibodies that explicitly interact with specific external bacteria and their antigens are produced by B lymphocytes. Such antibodies target circulating and non-

invading microorganisms and their toxins via binding through various mechanisms. Thereby, the immune system can neutralize and/or eliminate most pathogens. Interactions between these immune systems occur (for details, see du Pasquier & Flajnik 1999). The B-cell immune interaction has specific interest for periodontal immunization. Current evidence suggests that most pathogens associated with periodontitis are located in the periodontal pockets and are external to host cells. One of the key questions asked is whether a host humoral antibody immune response is protective, destructive, or irrelevant to periodontal infections. Under the assumption that humoral immunity is protective, the perspective of a vaccine to protect against periodontitis would be obvious.

Vaccines

As the successful vaccination against smallpox started in 1796, the importance of prophylactic immunization against infectious diseases is well understood. Effective vaccines have successfully eradicated or significantly reduced the prevalence of several diseases, especially in developed countries. These include the (I) Bacillus–Calmette–Guér in vaccine against tuberculosis and cholera using live attenuated or killed bacteria, (II) polio and rabies vaccines using live attenuated viruses, (III) tetanus and diphtheria vaccines using bacterial cell antigen subunits, (IV) *Haemophilus influenza*, and pneumococcus infections using conjugated vaccines, and (V) synthetic vaccines, i.e. (HBV) against Hepatitis B.

Maybe the most important discovery in recent vaccine development is new knowledge on the gene coding for bacterial protein antigens (attenuated vectors) that can be inserted into cells of other bacteria. Once inserted, these bacteria produce large volumes of the antigen protein used in new vaccines (i.e. hepatitis B surface antigen, lyme disease, pertussis and cholera vaccines). Serial inoculations of vector-based proteins or plasmid DNA vaccines have the potential to induce both strong B- and T-cell responses. Especially in virus research, the development of attenuated vectors and the use of orally administered fruits or vegetables containing vaccine antigens as the route of administration is in progress (Plotkin & Plotkin 1999, Hilleman 2000).

Immunological adjuvants (i.e. lipopeptides, lipopolysaccharides (LPSs), heat-shock proteins (HSP), or zymosan – a mixture of yeast cell wall polysaccharide, proteins, and ash) as substances used in combination with specific antigens in vaccines are used to amplify T-cell immune responses or to absorb complement C3. Freund's adjuvant and other composite adjuvants are also commonly used in vaccines. Syntex adjuvant formulation (SAF) has been used as an alternative to Freund's complete adjuvant. SAF is an oil-in-water emulsion stabilized by Tween 80, and pluronic polyoxyethlene/polyoxypropylene blocks copolymer L121. SAF activates complement by the alternate pathway and is more effective with hydrophobic proteins (Chiron Corporation, Emeryville, CA, USA). Animal studies have demonstrated that adjuvants often interact with Toll-like receptors and activate macrophages, monocytes, and leucocytes, resulting in stimulated secretion of inflammatory products (i.e. TNF-α, IL-8, hydrogen peroxide and arachidonic acid; Young et al. 2001). Thus, adjuvants can have effects on antigen delivery, induction of immune modulatory cytokines, and effects on antigen-presenting cells (Verma et al. 1992). The choice of adjuvant may partly explain differences in vaccine trial outcomes.

Serious concerns regarding the production, purity, content, and safety of vaccines since the early 1900 standards for assessing the safety and efficacy have challenged the development of new vaccines. No vaccine is perfectly safe. The US Food and Drug Administration (FDA) has provided basic regulatory criteria for vaccines. The ''benefit to risk'' consideration must specifically be considered in the review of new vaccines. New vaccines are partly monitored by vaccine-adverse event surveillance methods using computer data mining.

Because of the fact that vaccine development is expensive and time consuming, coordinated support from funding agencies, such as the US National Institutes of Health, and commercial interests are necessary to develop and assess the efficacy, safety, and utility of new vaccines. Between 1990 and 2002, the funding support for vaccine research increased from approximately 200 million US$ to 1.4 billion US$. In the year 2002, the Center for Biologics Evaluation and Research and FDA listed 42 vaccine studies in phase III trials, with more than 300 ongoing vaccine basic research and development projects. Some of these vaccine projects include work using purified proteins, LPS, surface lipoproteins, carbohydrates, and lipid A. Knowledge acquired through such efforts may be indirectly applicable for periodontal vaccine studies. In the development of a new vaccine, it would be important to consider the target population in which the vaccine may have an impact, regulatory factors, outsourcing (basic research, early pre-clinical and clinical work, manufacture, sale), competition, and funding of vaccine projects. These barriers may explain why periodontal vaccine research has been slow to progress.

The FDA requires extensive research and testing before a vaccine can be licenced for general use. Once target antigens have been identified, purified, and prepared for the vaccine, pre-clinical testing in animal models (mice, rats, rabbits, guinea-pigs, or monkeys) is required. Primary safety testing is often specifically studied in guinea-pigs. Before any vaccine can be tested in humans, an investigational new drug application must be approved by the FDA. Specifically, information on how the vaccine works, how it is manufactured, and safety and efficacy data from the animal studied must be provided. Following such approval, phase I safety studies are often performed in less than 20 healthy subjects. Then, phase II efficacy studies with 50–200 subjects are performed, and if successful, are followed by phase III studies of up to several thousand subjects to test the vaccine power in preventing the disease. It might take up to 50 years before a vaccine project can obtain a Biologics Application (BLA) approval. Currently, periodontal vaccine trials that go beyond testing principles in animal models have not been performed.

Periodontitis

The early colonizing bacteria on teeth and on gingival tissues include predominantly *Neisseria*, *Streptococci*, and *Actinomyces* species. Immunization against such bacteria with the objective of preventing colonization of later colonizing pathogens is currently unrealistic. The later phases of bacterial colonization occur in complex biofilm structures.

Thus, different from diseases caused by specific single-type infections and against which vaccines have been successfully developed, the aetiology of periodontitis is a complex mixed infection that includes large numbers of different pathogenic organisms (i.e. Socransky & Haffajee 2003). The bacteria most frequently associated with periodontitis include *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tanerella forsythia (forsythensis)*, *Treponema denticola*, *Actinobacillus actinomycetemcomitans*, and *Fusobacterium* spp. (Genco 1996). Such bacteria and their by-products can elicit strong immune responses (Socransky & Haffajee 1991).

Bacteria in biofilm structures can be protected from host immune responses and are dependent on environmental (passive response) and genetics (active response) factors (Hall-Stoodley & Stoodley 2002, Kjelleberg & Molin 2002). Changes and disconnection from biofilms occur via (I) swarming dispersal in which individual bacteria are released, (II) clumping dispersal in which aggregates of bacteria are released, or (III) surface disposal in which bacterial biofilms move across surfaces. All three models will challenge the host immune system in different ways. For example, *A. actinomycetemcomitans* has a non-motile pathogen that appears to be released passively from biofilm structures by the swarming disposal model and is thereby protected against most host immunity functions (Kaplan et al. 2003).

P. gingivalis has been considered as a key pathogen in periodontitis. In vitro studies have demonstrated that *P. gingivalis* has the ability to invade gingival epithelial cells (Lamont et al. 1995a, b), thereby obtaining protection against humoral immunity factors. *P. gingivalis* interacts with several aspects of the immune system (Lamont & Jenkinson 1998). In fact, *P. gingivalis* can modulate its surface fimbriae dependent on access to them and whether the pathogen resides in an extra- or intracellular position. This might be one of the mechanisms by which *P. gingivalis* is evasive to host immunity (R. P. Darveau, personal communication).

A definitive proof of evidence (consistent with the requirement by Koch's postulate) that infection with *P. gingivalis* is associated with the progression of periodontitis defined as alveolar bone loss in *Macaca fascicularis* was demonstrated in studies by Holt et al. (1988). Successful implantation of a rifampin-resistant strain of *P. (Bacteroides) gingivalis* into periodontal pockets of monkeys (*M. fascicularis*) caused an increase in the systemic levels of antibody towards the microorganism. It also resulted in alveolar bone loss. This observation raised questions on whether antibodies against *P. gingivalis* were destructive or protective. Apparently, the natural host immune response failed to provide protection against the infection. If the principles from other vaccines also apply to periodontal infections, active immunization against *P. gingivalis* infection might be feasible and may raise protective immunity against periodontitis.

Vaccine candidate antigens *of P. gingivalis*

P. gingivalis is a potential vaccine candidate because this pathogen carries several high-potent antigens, an LPS capsel, lipids, and outer membrane proteins. Whole-cell formalin-killed *P. gingivalis* has been used as the target antigen. In one study, attempts were made to combine antigens from several bacteria (Ebersole et al. 1991). The results were not conclusive. Shared antigenic determinants (epitopes) exist for many Gram-negative anaerobes. Especially, cell wall surfaces that display polyvalent arrays of protein or carbohydrate antigen determinants in a spatial arrangement may influence antibody binding with cross-over effects. The fact that different bacteria share antigenic determinants would make it feasible to use antigens from a key target pathogen present in the oral biofilm. Different *P. gingivalis* antigens have been studied, and they are presented in Tables 1 and 2. A major virulence factor of *P. gingivalis* is the extracellular noncovalently associated complexes of Arg-X- and Lys-X-specific cysteine proteinases and adhesins designated the RgpA–Kgp complexes. Studies have shown that immunization with RgpA–Pgp induces an immunoglobulin G2a response and with a restricted colonization by *P. gingivalis* and periodontal bone loss in the rat (Rajapakse et al. 2002).

Through sequencing DNA processes, bacterial genome bioinformatics on *P. gingivalis* outer membrane proteins have made it possible to study potential periodontitis vaccine benefits in murine models. This includes the outer membrane protein, porin (PG33) and (PG3), as well as cysteine proteases (PG32) and (PG57) (Ross et al. 2001). Studies with these proteins have shown promise and have shown protection against *P. gingivalis* infection (Ross et al. 2004a, b). Studies have also demonstrated that transcutaneous immunization of mice with a 40 kDa outer membrane protein of *P. gingivalis* induces specific antibodies that inhibit coaggregation by *P. gingivalis* to *Streptococcus gordonii* (Maeba et al. 2005).

Large variations in *P. gingivalis* ribotypes recognized by serum immunoglobulin G (IgG) from subjects with chronic periodontitis also exist. Some outer membrane proteins may be shared by ribotypes (i.e. the 44 and 27 kDa proteins) (Sims et al. 1999). Studies of clinical isolates of *T. forsythia* have also demonstrated a large and subject-defined genotypic variation for *T. forsythia* (Persson et al. 2000). *P. gingivalis* FDC 381 possesses a 53 kDa protein antigen (Ag53) on its outer membrane, which evokes a strong humoral immune response in many patients with periodontal disease, but the humoral immune responses to Ag53 differ greatly among patients and are a result of major B-cell epitopes (Oyaizu et al. 2001).

Animal models

Vaccine trials in animal models are required for safety and efficacy testing of vaccines. The ideal animal research model for vaccine trials against periodontitis with naturally occurring periodontitis based on the same aetiology, pathogenesis, and prevalence in animals as well as in humans does not exist. As substitutes, experimentally induced periodontitis models have been explored. Some of these may not necessarily provide access to clinical conditions that can readily be assessed for clinical efficacy. Page & Schroeder (1982) concluded that because of continuous eruption patterns of teeth and alveolar bone changes, mice, rats, and hamsters might not be suitable for clinical periodontitis vaccine *efficacy* studies. Such animal models might be useful for the *test of principle* in evoking immune responses, and for safety studies (i.e. Burckhardt et al. 1981, Kesavalu et al. 1992, Genco et al. 1999, Mori et al. 2000, O'Brien-Simpson et al. 2000).

Table 1. Examples of non-human primate study models and results from vaccine trials against periodontitis

Study	Antigen	Study type	Results	Conclusion
Moritz et al. (1998)	Purified cysteine protease (porphypain-2) from *P. gingivalis versus* placebo immunization. For experimental protocol, see Ebersole et al. (1991)	Case–control study of experimental periodontitis in *M. fascicularis* Clinical measures, and radiographic analysis (CADIA) ELISA assays	1. Elevated serum IgG titres to whole-cell *P. gingivalis* (36-fold) and Porphypain-2 (194-fold) 2. 25% more Gram-negative bacteria at control sites than in immunized animals 3. Few clinical changes as an effect of immunization 4. No statistically significant changes as defined by CADIA between sham- or test-immunized animals	Immunization with porphypain-2 induces an immune response Immunization may impact microbial colonization Clinical effect remains unclear
Persson et al. (1994)	Vaccine composed of formalin-killed whole-cell *P. gingivalis* (5083, primate strain) and Syntex SAF) adjuvant	Case–control study of 10+10 active/shamimmunized *M. fascicularis* with pre-existing low IgG titres but presence of *P. gingivalis* in pockets over 44 weeks with infectious challenge at week 36. Immunization at baseline weeks 3, 6, and 16 when ligatures were placed. Routine clinical measures, standardized radiographs (CADIA), ELISA assays, and DNA probe analysis	1. Serum IgG titre to *P. gingivalis* elevation through immunization with 50% titre levels remaining at endpoint 2. Trend towards less *P. gingivalis* in immunized animals. 3. Significantly more bone loss in control animals at ligated sites and exaggerated by microbial challenge 4. Variety in antibody response	Immunization with formalin-killed whole-cell *P. gingivalis* with an adjuvant inhibits progression of experimental periodontitis
Persson et al. (1994)	DNA probe analysis of the microflora in relation to clinical features	Cross-section study of the oral microflora in *M. fascicularis* Routine clinical periodontal measures	1. *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *C. rectus*, *T. forsythia* *F. nucleatum* present in majority of sites tested in non-experimental conditions 2. Antibody titre levels to *P. gingivalis* inversely correlated with *P. gingivalis* levels	*M. fascicularis* appears to be a suitable model in which studies of vaccine efficacy for control of periodontitis can be tested
Ebersole et al. (1990)	*A. actinomycetemcomitans* leucotoxin produced in a vector DNA model was used to immunize *M. fascicularis*	Development and testing of a leucotoxin vaccine derived from *A. actinomycetemcomitans* and tested in *M. fascicularis* to assess serum responses. Case–control animal study	1. Most *M. fascicularis* carried antibody titres to *A. actinomycetemcomitans* 2. Primary immunization elicited Ig1 and Ig3 responses 3. Secondary response elicited IgG2 responses and increased antibody avidity	A host response to immunization with *A. actinomycetemcomitans* leucotoxin with potential efficacy possible
Niesengard et al. (1989)	*B. macacae* (non-human primate equivalent to *P. gingivalis* in humans)	Preliminary case–control study in *M. fascicularis* Ligature-induced periodontitis 12-week immunization period Clinical measures, GCF radiographs Serum titres to *B. macacae*	1. Immunization-induced elevated antibody IgG titres to *B. macacae* 2. Ligatures-induced bone loss in all animals 3. Levels *B. macacae* were 2 times higher in non-immunized animals after 6 months	Heightened immune responses and partial prevention of microbial colonization

Table 2. Examples of murine study models and results from vaccine trials

Study	Antigen	Study type	Results	Conclusion
Gemmell et al. (2004)	*F. nucleatum ATCC 25586* *P. gingivalis ATCC 33277* Viable bacteria used in immunization schedule	27 BALB/c female mice. Various immunization schedules and a placebo group (intra-peritoneal injections once per week (4 weeks)	1. ELISA and chemiluminescence assays 2. Production of both IgG1 and IgG2 subclass antibodies and higher levels for the combination vaccine *P. gingivalis/F. nucleatum*	A vaccine candidate may be enhanced by combinations of *P. gingivalis* and *F. nucleatum*
Ross et al. (2004)	*P. gingivalis YH522*, *ATCC 33277*, *ATCC53978* 12 clinical isolates from Seattle, Norway, Romania, Sudan	Genetics research and animal vaccine study with constructed gene vaccine using knockout mice challenged to *P. gingivalis* infection	1. The gene and expressions of PGO695 and PGO0694 are soluble and potentially useful as vaccine candidates 2. Gene expression is highly conserved across *P. gingivalis* species 3. Immunization provides reduction in bone lesions	Additional work needed to improve results to enhance solubility. There is a loss in epitope domains when using truncated versions of proteins as vaccines
DeCarlo et al. (2003)	*P. gingivalis* ATCC 33277 HA2 sequence (Genbank PGU68468.1) cloned with *E. coli*	Fischer CD F(344) rats Test group recombinant HA2 (8 animals) Placebo group (Freunds' adjuvant) (8 animals) 3 immunizations	1. Sham-immunized animals developed no anti-rHA2 IgG2 antibodies, whereas rHA2-immunized animals did develop IgG antibodies measurable over 70 days 2. The extent of protection against bone loss dependent on titre and absence of Freunds' adjuvant in vaccine	Protective effect against bone loss in the absence of adjuvant in the vaccine
Rajapakse et al. (2002)	Whole-cell killed *P. gingivalis* ATCC 33277/ATCC53978+adjuvant Rgp (ArgX and LysX proteinase)+adjuvant	4 groups (2 of them (sham immunized)) of Sprague–Dawley rats 2 immunizations with 3-week intervals After antibiotics treatment challenged with *P. gingivalis* infection	1. Immunization with the RgpA–Kgp *P. gingivalis* W50-induced high IgG titres 2. Immunization restricted colonization with *P. gingivalis* ATCC 33277 3. Cross-reactivity between ATCC 33277 and W50 (ATCC53978) 4. Immunization prevents bone loss	Immunization with Kgp39 and Rgp44 may prevent or reduce periodontitis in humans
Gibson III et al. (2001)	*P. gingivalis* A7A1–A28	BALB/c mice Gingipain PgpA and RgpB Whole-cell heat-killed *P. gingivalis* 3 immunizations	1. Immunizations with gingipains PrgA and PrgB stimulate *P. gingivalis* serum IgG antibodies 2. Purified denaturated PgpA and RgpB identified 5 polypeptide bands identified as 45, 44, 17, 15, 27 kDa 3. Immunization with RgpA but not RgpB protects against *P. gingivalis*-induced bone loss	Whole-cell heat-killed *P. gingivalis* and gingipain RgpA (haemagglutinin domain) provide protection against bone loss

Table 2. (Contd.)

Study	Antigen	Study type	Results	Conclusion
O'Brien-Simpson et al. (2000)	*P. gingivalis* ATCC 33277 *P. gingivalis* W50	BALB/c mice immunized with Rgp–Kgp proteinase adhesion complex of ATCC 33277 or W50 with or without adjuvant (IFA) Abdominal injections	1. When challenged with W50 post-immunization, those immunized against W50 showed significantly smaller abdominal lesions 2. When challenged with ATCC 33277, no lesions were found post-immunization 3. Protection against *P. gingivalis* infection may be mediated via Fc receptor-dependent phagocytosis	RgpA–Kgp protein–adhesin complex protects against *P. gingivalis* challenge in the murine model
Katz et al. (1999)	*P. gingivalis* ATCC 33277, 381, A7A1–A28 Hagb gene from 381 was cloned in a pET vector and expressed in *E. coli*	Fischer CD F 344 rats were immunized with recombinant Hagb plus Freunds' adjuvant subcutaneously and then orally exposed to fresh *P. gingivalis* at days 13 and 14 post-immunization	1. No elevation in salivary IgA as a result of immunization 2. Serum IgG elevated in immunized+infected and in infected-only animals 3. Supernatants from rHagb immunized stimulated lymphoid cell cultures with high levels of interferon, followed by IL2, IL-1, and then IL-4 consistent with T-helper type 1 (Th1) and Th2 responses rHagb reacted with *P. gingivalis* ATCC 33277, 381, A7A1–A28, and W50 with a 50 kDa protein band representing rHagb	Immunization with purified rHag B induces protective immunity against *P. gingivalis* infection and provides a potential vaccine candidate against chronic periodontitis in humans
Genco et al. (1998)	*P. gingivalis A7436 and HG66-extracted cysteine protease (GingipainR)*	148 BALB/c female mice: non-immunized Peptide A Peptide D Whole-cell Gingipain R1 (95 kDa) Gingipain R2 (50 kDa)	1. When challenged with *P. gingivalis*, non-immunized and peptide A immunized develop ulcerated lesions, weight loss 2. Gingipain R immunized were protected from abscess formation 3. Reduction in viable *P. gingivalis* counts 4. Antisera from *P. gingivalis* HG66 recognize gingipains from many different *P. gingivalis* strains	Immunization with gingipain R provides protection against *P. gingivalis* infection in mice
Evans et al. (1992)	*P. gingivalis* 381, 2561 and *P. gingivalis* ATCC 33277 fimbriae	Germ-free Sprague–Dawley rats (6 groups of 8 rats per group) Sham-immunized non-infected, Sham-immunized infected Whole-cell heat-killed *P. gingivalis* Purified 43 kDa protein Purified 75 kDa protein Combined 43 and 75 kDa vaccine	1. 43 kDa immunized were protected from alveolar bone loss 2. 75 kDa immunized had no protection against bone loss 3. The combination 43, and 75 kDa provided protection 4. Gingival fluid collagenase activity reduced in 43 kDa immunized animals	43 kDa fimbrial protein may be useful as a vaccine candidate against periodontitis

Dogs have not been considered for periodontal vaccine studies. It is of interest that sheep (ovine) appear to develop naturally occurring periodontitis. At least for *P. gingivalis* in sheep, there is homology to human strains. There is also a similarity in humoral immune responses and periodontitis responses in sheep and humans (Duncan et al. 2003).

Non-human primates, including *M. fascicularis*, *M. nemestrina*, Marmosets, Baboons, and Chimpanzees, have been considered for periodontal vaccine trials. Naturally occurring periodontitis in *M. fascicularis* (wild caught or domestically bred) is less than 5% (Friskopp & Blomlöf 1988). Studies have demonstrated that key pathogens associated with periodontitis can be identified in samples taken from adult *M. fascicularis* and *M. nemestrina* and identified by DNA probes aimed for studies of strains found in humans (Moncla et al. 1994, Persson et al. 1994a). Thus, the patterns of bacterial presence in older *versus* young *M. nemestrina* are consistent with what is known from bacterial patterns in humans. Higher serum IgG titres against key pathogens associated with periodontitis, suggesting an impact over time from exposure, can also be found in older *M. nemestrina* (Persson et al. 1993). In order to predictably induce periodontitis in non-human primates, the experimentally induced periodontitis model has been used. In most periodontal vaccine studies in non-human primates, the design presented by Kornman et al. (1981) has been used.

Active immunization

Active immunity is induced by exposure to a foreign antigen. This activates lymphocytes to produce antibodies against the antigen. The immune system of the host plays an active role in responding to the antigen.

Vaccine studies in animal models against periodontitis

Non-human primate vaccine studies

In the guinea-pig model, the safety of a vaccine against periodontitis composed of formalin-killed whole-cell *P. gingivalis* and the SAF (Syntex, Palo Alto, CA, USA) has been tested. Unpublished data from the vaccine trials at the University of Washington (R. C. Page, R. P. Darveau, G. R. Persson, personal communication) necropsy revealed no organ abnormalities or residual organ effects (kidney, liver, eye balls, lymph nodes). However, Western blot analysis demonstrated that specifically protein and carbohydrate antigen residues were recognized in immunized animals (Houston et al. 1995).

The ligature-induced model using *M. fascicularis* for studies of experimental periodontitis has the advantage that it allows for clinical assessments of oral and periodontal conditions consistent with human examinations (Kornman et al. 1981, Nalbandian et al. 1985). The model also allows for the use of standardized intra-oral dental radiographs (Ebersole et al. 1991, Persson et al. 1994a, b). The oral microflora in *M. fascicularis* has been documented (Holt et al. 1988, Ebersole et al. 1991, Moncla et al. 1994, Persson et al. 1994a, b). The distribution of different bacteria associated with periodontitis in non-human primates is quite similar to that of human periodontitis (Moncla et al. 1994).

Vaccine strategies that only prevent colonization of one pathogen may have limited value if they are only effective against one bacterial species. Studies of sera from immunized *M. fascicularis* have, however, demonstrated that there exist shared antigens between, i.e. *P. gingivalis* and *T. forsythia* (Vasel et al. 1996). Thus, shared epitopes in lipid A and core carbohydrate of LPS between these two pathogens exist, which makes it feasible to induce immunity to more than one pathogen, although only antigen from a specific bacterial species is used as target antigen in the vaccine.

Results from active immunization studies using an experimental periodontitis model in *M. fascicularis* are presented (Table 1). Detailed assessments of immunization impact with regard to immunological, microbiological, and clinical outcomes are summarized. The results from these studies demonstrate that the relationship between antibody titres and killing abilities, and protection against challenge with *P. gingivalis* infection in the non-human primate model is complex.

A wide variability in functional capacity of sera from individual *M. fascicularis* has been reported. Thus, Anderson et al. (1995), studying available sera (Ebersole et al. 1991), demonstrated that antibody- and complement-mediated killing of *P. gingivalis* was enhanced by immunization. Unique differences, however, in immunogenic potential by different pathogens associated with periodontitis have also been demonstrated in non-human primates (Ebersole et al. 1997). This suggests that the ability of the immune system is highly dependent on the characteristics of antigens produced by oral bacteria. The data emerging from the studies by the research group at San Antonio (Holt et al. 1988, Ebersole et al. 1991, 1997, Anderson et al. 1995) suggest that the complexity of the subgingival microbiota dictates the functional protection from periodontal tissue destruction induced by oral microorganisms that already colonize or infect the host (Holt et al. 1995). Additional studies in the *M. fascicularis* model using porphypain-2 as a purified *P. gingivalis* vaccine has demonstrated promising elevation of enduring antibody titres in *M. fascicularis*, and with an impact on the oral microflora (Moritz et al. 1998).

The vaccine trials group in Seattle used the same experimental model as used by Ebersole et al. (1991). In a blinded, randomized-controlled case–control study over 44 weeks, immunization of *M. fascicularis* with formalin-killed whole-cell *P. gingivalis* (strain 5083) vaccine with SAF adjuvant or placebo adjuvant only resulted in significant differences in alveolar bone loss. This was assessed by both computer-assisted density image analysis and by standard bone height measurements, suggesting protection in experimentally induced periodontitis (Persson et al. 1994c). In a subset of animals, the immunization effects were further documented in bacterial challenging studies (Persson et al. 1994c).

The studies demonstrated that, in control monkeys, no significant IgG, IgA, or IgM titres were elevated. In contrast, serum IgG and IgA titres to *P. gingivalis* appeared early and persisted throughout the 36-week observation period. In immunized *M. fascicularis*, IgM titres were elevated until 6–12 weeks and then decreased through week 36. Significant opsonic capacity was seen by 6–12 weeks and persisted throughout the study in immunized animals, whereas sera from control animals showed only low opsonization capacity (Houston et al. 1999). Moreover, no correlation was seen between peak IgG titres against *P. gingivalis* and protec-

tion against bone loss, whereas a significant association was found between protection against bone loss and pre-immunization IgM titres to *P. gingivalis*.

Evidence of a protective mechanism from a formalin-killed whole-cell *P. gingivalis* vaccine with SAF adjuvant can be found in the reported effect that vaccine-induced serum antibody titres to *P. gingivalis* resulted in a blockage of prostaglandin E_2 (PGE_2) response to LPS challenge (Bainbridge et al. 1997). Furthermore, the whole-cell formalin-killed *P. gingivalis* (monkey strain 5083) in combination with an adjuvant results in a lower levels of PGE_2 levels in gingival fluid in immunized *M. fascicularis* than in placebo-immunized animals (adjuvant only). The extent of alveolar bone loss was directly correlated to the levels of PGE_2 (Roberts et al. 2004). Decreased (PGE_2) levels in GCF in immunized animals suggest a positive effect of the vaccine, as PGE_2 is a major inflammatory mediator and is associated with bone loss.

Analysis of data from additional studies using purified vaccines (cysteine proteases) with adjuvant by the Seattle vaccine study group is in progress (G. R. Persson, R. C. Page, personal communication). In these series of studies, no adverse effects as a consequence from immunization were found during study progression or from post-mortem analysis of pertinent organs, consistent with the conclusions from findings after the whole-cell formalin-killed *P. gingivalis* vaccine plus adjuvant immunization.

Murine vaccine studies

Murine models are extensively used in studies assessing humoral immune factors and the effects of immunizations. For example, studies of an outer membrane protein derived from *P. gingivalis* ATCC 33277 with at least four specific antigen bands have demonstrated that immunized BALB/c mice after immunization acquire immune protection against *P. gingivalis* infection (Bird et al. 1995). Immunization with whole-cell heat-killed *P. gingivalis* provides protection both in subcutaneous chamber models and to oral challenge models, and with an elevation in serum IgG-specific titres to *P. gingivalis* (Gibson & Genco 2001, Gibson et al. 2004).

Other studies have been performed using different strains of *P. gingivalis* being able to identify shared bacterial antigenic epitopes with immune-inducing capacity (see Table 2). In one study, a combination vaccine based on *P. gingivalis* and *F. nucleatum* enhanced humoral immune responses and protection against consequences of infections (Gemmell et al. 2004). The murine model for studies of humoral immunity and periodontal infection focusing on various strains of *P. gingivalis* has lately been used to specifically address whether specific purified antigens derived from genetic vectors use bioinformatics to identify genes predictive of proteins with surface localization and with immunogenic potential (Ross et al. 2001). In further studies, the characterization of proteins from *P. gingivalis* and the conservation of such genes across different strains of *P. gingivalis* using genetic information from the Institute of Genome Research (TIGR) have identified two proteins (PG0695), and (PGO694), which are potentially most effective as vaccine candidates against periodontitis (Ross et al. 2004a, b).

Fimbriae appear to be a significant adherence-mediating determinant of *P. gingivalis*. *P. gingivalis* possesses two distinct different types of fimbriae (FimA, 41 kDa protein) and (Fai1, 67 kDa protein). Studies have demonstrated that both types of fimbriae can induce a strong cytokine response (IL-1 β, IL-6, TNF-α). Murine peritoneal macrophages with the ability to induce osteoclast activity can be blocked by an anti-Toll-like receptor antibody (Hiramine et al. 2003).

P. gingivalis has an obligate iron requirement for growth. *P. gingivalis* also secretes proteases to provide enhanced access to iron and peptides that are also involved in tissue destruction and evasion and modulation of host immune defences. Therefore, efforts have been made to identify such proteases that may have immunogenic potential and may serve as vaccine candidates. Several murine studies have been used to assess such potential vaccine candidates. *P. gingivalis* cysteine proteases (gingipains) are involved in hem and haemoglobin binding. The pathogenicity of *P. gingivalis* has specifically, been attributed to fimbriae, haemagglutinins LPS and the Arg-X- and Lys-X-specific cysteine proteases and their adhesions (Lamont & Jenkinson 1998, O'Brien-Simpson et al. 2000). It is known that cysteine proteases, especially from *P. gingivalis* W50, deregulates host defences and up-regulates cytokine responses (Okamoto et al. 1998). Immunization studies with the cysteine protease complex of RgpA–Kgp have shown that immunization results in high serum IgG2 titres and protection against *P. gingivalis* and bone loss in a rat model (Rajapakse et al. 2002). Specifically, the 44 kDa adhesion/haemagglutinin (RgpA44), the 27 kDa adhesion (RgpA27) of RgpA, and the 39 and 42 kDa adhesion (Kgp39) of KgP are recognized by anti-RgpA antisera and provide protection against *P. gingivalis* infection in mice (Genco et al. 1998).

P. gingivalis fimbrillin (FimA) is involved both in bacterial adhesion and the induction of an inflammatory response. Biologically active domains of *P. gingivalis* fimbrillin can be expressed on the surface of *S. gordonii*. Immunization with recombinants expressing terminal epitopes of FimA using *S. gordonii* vectors result in the induction of FimA-specific serum IgG and salivary IgA antibody responses. The efficacy of such mucosal vaccination against *P. gingivalis* has been demonstrated in a germ-free rat model (Sharma et al. 2001).

Thus, studies using murine models have independently proven that purified proteases from *P. gingivalis* evoke strong humoral immune responses with potential periodontitis-protective functions. Similar conclusions have been pointed out in the studies using non-human primates (Moritz et al. 1998).

Stimulation of humoral immunity by treatment

Stimulation of self-antibody production as a consequence of treatment of infected tissues causing bactaeremia can induce an elevation of antibodies against a target antigen. Several studies have assessed the effects of such ''uncontrolled'' immunization against pathogens associated with periodontitis (Table 3).

Studies of serum IgG antibodies to whole-cell sonicate, protein, and purified LPS fractions of *A. actinomycetemcomitans* from patients diagnosed with aggressive periodontitis (AgP), but untreated, and from healthy controls have demonstrated large variation in titres (Ou et al. 1997). In a case–control

Table 3. Summary of findings from human studies on serum titre responses to therapy or passive immunization in subjects with aggressive periodontitis (AgP) or chronic periodontitis (CP)

Study	Antigen	Study type	Results	Conclusion
Aukhil et al. (1988)	*P. gingivalis* *A. viscosus* *P. intermedia* *T. vincentii* *T. denticola*	Longitudinal case series of 23 subjects with CP over 12 months Serum IgG ELISA assay	Reduction in serum IgG titres to: *P. gingivalis*, *A. viscosus*, *F. nucleatum*, *P. intermedia*, *T. vincentii*, *T. denticola* Unclear for *Capnocytophaga* spp.	No immunity effect resulting in sustained titres at one year
Kohyama (1989) (only abstract review, publ. in Japanese)	*P. gingivalis* *A. viscosus* *A. actinomycetemcomitans* (serotype a) *P. intermedia*	Longitudinal case series of subjects with CP ($n = 52$) before and after ICRT Serum IgG ELISA assay	1. Subjects with CP had higher serum IgG titres to *P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans* than healthy controls 2. Titres increased after therapy, whereas the presence of pathogens decreased	Subjects with CP experience elevated serum IgG titres to pathogens associated with periodontitis. Potential passive immune response
Chen et al. (1995)	*P. gingivalis* (ATCC 33277)	Longitudinal case–control study. 36 subjects with AP and 20 healthy controls Whole-cell-purified LPS, and protein fractions from *P. gingivalis* Serum IgG ELISA titre chemiluminescence assays	1. IgG titres to LPS and protein factions in subjects with high baseline titres decreased after ICRT, but increased in those with low baseline titre 2. Avidities increased for all subjects after treatment	Subjects with untreated AgP may not produce functional antibodies against *P. gingivalis*. infection. Treatment results in elevation of titres against *P. gingivalis*
Johnson et al. (1993)	*P. gingivalis* (ATCC 33277)	Longitudinal case series 28 subjects with AP (12-month study) Serum IgG ELISA titre assay to *P. gingivalis*	1. Serum IgG titre decrease from 3rd to 12th months after ICRT after concurrent with clinical improvement	Elevated serum titres were not observed as effect of treatment Serum IgG titres' decrease consistent with clinical improvements
Sjöström et al. (1994)	*A. actinomycetemcomitans* (ATCC 43718 serotype b, strain Y4)	Case intervention longitudinal study (12 months). Subjects (22 with AgP, 20 healthy controls) Serum IgG ELISA antibody titre assay to *A. actinomycetemcomitans* Chemiluminescence (CL) assay (PMN cell-killing capacity)	1. Sero-conversion one year after ICRT 2. Elevated IgG serum antibodies in sero-negative subjects to whole-cell and LPS from *A. actinomycetemcomitans* antigen 3. Increased CL capacity	ICRT results in a humoral immune response in sero-negative subjects consistent with beneficial treatment effects
Mooney et al. (1995)	*P. gingivalis* (NCTC 11834) *A. actinomycetemcomitans* (ATCC 29523)	Longitudinal case–control intervention study of 18 subjects with CP and 23 healthy controls. Samples prior to and 3 months after completion of ICRT Serum IgG ELISA assay, avidity assay (ammonium thiocyanate)	1. Enhanced avidity and serum IgG titres to *P. gingivalis* and *A. actinomycetemcomitans* in sero-positive CP cases 2. Elevated serum IgG ELISA titres in sero-negative CP subjects 3. No differences in treatment effect explained by IgG titre or avidity changes	Unique humoral immune responses Previous exposure to pathogens of significance
Horibe et al. (1995)	*P. gingivalis* (ATCC FDC 381) *P. intermedia* (ATCC 25611) *P. Loescheii* (ATCC 15930) *F. nucleatum* (ATCC 25586) *A. actinomycetemcomitans* (ATCC Y 4) *E. corrodens* (FDC 1073) *C. ochraea* (M1)	Longitudinal case series with 20 AP subjects Serum IgG ELISA assay	1. Decrease in serum IgG titres *to P. gingivalis*, *P. intermedia* 2. Decrease in serum IgG titres consistent with suppression of pathogens in subgingival plaque	Study *failed to demonstrate* passive immunization effect Study suggested that serum tires linked to presence of bacteria in plaque
Smith et al. (1996)	*A. actinomycetemcomitans (ATCC 45718 serotype b, strain Y4* *P. gingivalis (ATCC 33277)* *T. forsythensis (clinical)*	Case intervention study over 2 months in 18 subjects with IDDM type 1 and CP Serum IgG ELISA antibody titre assay	1. Treatment did not eliminate the bacteria studied 2. Limited treatment effects 3. No effect on serum IgG titres	*No evidence* of passive immunization in subjects with IDDM

Table 3. (Contd.)

Study	Antigen	Study type	Results	Conclusion
Booth et al. (1996)	Radioimmunoassay for serum IgG, Iga, IgM	Longitudinal case–control study of subjects with periodontitis Monoclonal antibody to *P. gingivalis* (MAb 61GB 1.3) or placebo in subgingival applications	1. No impact on difference in decrease of periodontal measures other than for *P. gingivalis*	Passive immunization can selectively prevent colonization of *P. gingivalis* for up to 9 months
Papapanou et al. (2004)	*Antigens from 19 different bacteria including* *P. gingivalis (FDC 381)* *P. intermedia (ATCC 25611* *P. nigrescens (ATCC 33563)* *T. forsythensis (ATCC 43037)*	Longitudinal intervention case–control study 89 CP patients and healthy control subjects Checkerboard immunoblotting	1. No impact on serum IgG titres as an effect of therapy 2. Higher titres in CP subjects 3. Different patterns of titres between CP and healthy subjects	Titres linked to the presence and challenge from microbiota *No effect* on titers suggesting passive immunization

ICRT, initial cause related therapy.

study of subjects with AgP, Chen et al. (1995) demonstrated that non-surgical treatment-induced antibody avidity and elevation of serum antibody titres to both purified LPS and to protein fractions from whole-cell *P. gingivalis*. This is consistent with a concept that patients with AgG and with initially low functions and levels of antibodies to *A. actinomycetemcomitans* can be stimulated to produce biologically functional antibodies during the course of non-surgical periodontal therapy. Studies on the one-stage non-surgical debridement suggesting a Schwartzman reaction after treatment also suggest that such a treatment causes bacteraemia, which turns on a host immune response (Quirynen et al. 1999).

Contradictory results have been reported, in that some studies suggest that enhanced immunity occurs as an effect of therapy (Sjöström et al. 1994, Chen et al. 1995, Mooney et al. 1995). Other studies have failed to demonstrate such an effect (Aukhil et al. 1988, Johnson et al. 1993, Horibe et al. 1995, Smith et al. 1996, Darby et al. 2001). Mooney et al. (1995) demonstrated that periodontal therapy affects the magnitude and quality of the humoral immune response. Mooney et al. (1995) also demonstrated that the immune response to treatment is linked to pre-treatment antibody titre levels and that sero-positive subjects responded clinically better to treatment than sero-negative subjects. No significant post-therapy effects on the humoral immune response other than reduced antibody avidity to *P. gingivalis* and *P. intermedia* have also been demonstrated (Darby et al. 2001). The observations by Papapanou et al. (2001, 2004) also suggested a correlation between serum titre status and the antibody responses to periodontal microbiota but in relation to the severity of periodontitis.

Apatzidou & Kinane (2004) failed to demonstrate that non-surgical periodontal therapy performed as either full-mouth or sequential debridement resulted in increased antibody titres or avidities to pathogens associated with periodontitis. A trend towards reduction in titres was noticed, and with a marked inter-subject variability. Similar results have been demonstrated for antibody titres to HSP (human HSP60 and *P. gingivalis* GroEL, a bacterial homologue of human HSP60) (Yamazaki et al. 2004b).

Thus, whether therapy can trigger effective immune responses enhancing resistance to disease and elimination of pathogens remains unclear.

Differences in antigen/antibody complexes studied, differences in serum assays used, differences in periodontitis disease severity classification and existing subgingival microflora, and differences in therapy efficacy are some of the factors explaining the disparate results.

Passive immunization

Protective immunity can be obtained through passive immunization. This can be obtained by transfer of specific antibodies against the target bacteria (antigen). A passive immune response can be achieved by transfer of antibodies via serum, lymphocytes from immunized individuals, or monoclonal antibodies against specific pathogens. Transfer of maternal antibodies to the foetus is another example of passive immunization. The advantages of using antibody molecules to treat infectious diseases include their specificity and versatility (i.e. neutralizing toxins and viruses, activating complement, and opsonization) have been demonstrated (Casadevall et al. 2004). Passive immunization is short-lived and remains effective only as long as the injected antibody persists. The host will not respond to the immunization.

Animal studies

The murine monoclonal antibody Guy's 13 recognizing *S. mutans* and *S. sobrinus* has successfully been used to prevent colonization of *S. mutans* in non-human primates (Lehner et al. 1985). Based on the murine monoclonal antibody Guy's 13, it has been possible to develop a human derivate that has demonstrated binding to the surface adhesion of *S. mutans* in vitro. It has therefore been suggested that this vaccine candidate may be a useful passive immunization candidate against caries (Kuepper et al. 2005). In vitro testing of a recombinant (r)40 kDa outer membrane protein derived from immunizing mice with purified r40 kDa OMP has demonstrated that the IgG1 monoclonal antibody (Pg-ompA2) is bactericidal against *P. gingivalis* strain W381 (Katoh et al. 2000). Thus, Pg-ompA2 may contribute to the development of a local immunotherapy that can be applied in the gingival crevice of a patient with *P. gingivalis*-related perio-

dontitis (Teshirogi et al. 2003). Other monoclonal antibodies have been used by oral or intra-nasal immunization of mice with *P. gingivalis*-coated monoclonal antibodies, demonstrating differences in antigenic specificity of anti- *P. gingivalis* serum IgG (Van Tilburg et al. 2001). Similar results in mice have also been obtained from passive immunization with monoclonal antibodies against the *A. actinomycetemcomitans* Y4 strain (Herminajeng et al. 2001).

Promising results have been reported using monoclonal antibodies (MAb 61BG 1, 3) that are specifically recognized by at least 22 laboratory strains and 105 clinical isolates of *P. gingivalis*. Passive immunization with MBb 61BG 1, 3 resulted in the suppression of *P. gingivalis* for at least 6 months (Booth et al. 1996, Kelly et al. 1997). Recent construction of a human monoclonal antibody (HuMAb-HMGD1) that is capable of recognizing the 43 and 49 kDa proteins from *P. gingivalis* and inhibiting the haemagglutinating ability of *P. gingivalis* may prove useful in passive immunization against periodontitis (pending safety and efficacy studies) (Kaizuka et al. 2003). If selective inhibition of pathogens associated with periodontitis can be induced by topical application of a preparation containing monoclonal antibodies or attenuated vectors in combination with proteins or DNA vaccines it may elicit strong B- and T-cell responses. Such efforts are currently being pursued in other fields (tuberculosis research) and may also become applicable in periodontal research and clinical prevention.

Probiotics

Probiotics are live microorganisms administered in adequate amounts with beneficial health effects on the host. The Food and Agriculture Organization of the United Nations has defined Probiotics as ''live microorganisms administered in adequate amounts conferring beneficial health effect on the host''.

Most probiotic products contain bacteria from the genera *Lactobacillus* or *Bifidobacterium*, although other genera, including *Escherichia*, *Enterococcus*, *Bacillus*, and *Saccharomyces* (a yeast), have been suggested as probiotics. Such bacteria are often identified from the human gastrointestinal system. These bacteria are purified, grown to large numbers, concentrated to high doses, and preserved. They are provided in products as (1) a culture concentrate added to a food (usually a dairy product) with a low or no opportunity for culture growth, (2) inoculants into a milk-based food (or dietary supplement) as fermented food, or (3) dietary supplements such as powders, capsules, or tablets. There are currently no published reports in the English language on the use of probiotics in the treatment of periodontitis. Mucosal immune responses may be invoked by probiotic immunization. Studies of adhesion molecules have shown that superficial cell layers of the gingiva can be affected and can be stimulated to enhance the presence of immune potent cells (Lappin et al. 2003).

A recent (03.15.05) PubMed search identified three studies on the topic in the Russian language. Regulation of microflora composition (e.g. by probiotics and prebiotics) may offer the possibility to influence the development of mucosal and systemic immunity, but it can also play a role in the prevention and treatment of diseases such as periodontitis and heart disease (Tlaskalova-Hogenova et al. 2004).

Non-conclusive short-term beneficial effects of a probiotics in the form of cheese containing *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG) in reducing *S. mutans* and yeast counts have been demonstrated in humans (Ahola et al. 2002). However, others have shown increases of *lactobacilli* and no impact on *S. mutans* in subjects who received administration of probiotics, both in capsule and in liquid form. The increased salivary counts of *lactobacilli* may indicate the need to closely monitor the dental health of patients undergoing long-term probiotics treatment (Montalto et al. 2004).

A link between periodontitis and systemic disease with impending shared vaccine benefits

A potential association between stress and periodontitis in humans has been suggested (Pistorius et al. 2002, Merchant et al. 2003). It is therefore of interest to notice that lower serum IgG1/IgG2 ratios against *P. gingivalis* were found both in immunized and sham-immunized mice as compared with non-stressed animals (Houri-Haddad et al. 2003). This observation may also have implications for the interpretation of data from vaccine studies in animals in general.

There is evidence that microbial HSP are immunodominant antigens of many microorganisms. HSP (i.e. HSP60, GROEL) have been associated with atherosclerosis and *Chlamydia pneumoniae* infection (i.e. Chiu et al. 1997). Elevated antibody levels against *C. pneumoniae*, human HSP60, and mycobacterium HSP65 have been identified in subjects with myocardial infarction and ischaemic heart disease (Heltai et al. 2004). The 60 kDa HSP has been associated with delayed-type hypersensitivity responses, and the isolation and sequence analysis of HSP60 from bacteria are important (Kikuta et al. 1991).

Elevated serum antibody titres against human HSP60 and *P. gingivalis* HSP60 in subjects with cardiovascular disease and periodontitis and with a T-cell clonality between these HSP60 proteins have been shown (Yamazaki et al. 2004a). In a pilot project, Yamazaki et al. (2004b) also demonstrated significant variation in antibody titres to HSP60 after periodontal treatment. Other studies have shown that subjects with high anti-HSP (HSP90, DnaK, and GroEL) antibody concentrations tended to have significantly healthier periodontal tissues (Lopatin et al. 1999). Thus, it might be feasible to develop a vaccine against periodontitis based on *P. gingivalis*-specific HSP or HSP epitopes.

Other studies have demonstrated that *Campylobacter rectus* and *Heliobacter pylori* share common antigens belonging to the HSP60 family of antigens. *H. pylori* has been linked to several chronic diseases including cardiovascular disease, gastritis, and gastro-duodenal ulcers (Tanabe et al. 2003). There is also a symbiotic relationship between *C. rectus* and *P. gingivalis*, which may provide additional interest in vaccine trials with potentially shared protective outcomes. A relationship between serum titres to HSP70, HSP90, GAD65 (an autoimmune factor in type 1 diabetes mellitus) and serum titres to *P. gingivalis* has been demonstrated, suggesting that periodontal infection with *P. gingivalis* may be related to severity of IDDM (Sims et al. 2003).

In immunization studies with DNA fragments of human 60 kDa HSP using the rat model effects, it has been demonstrated that boosting the HSP60 regulatory response may provide an approach to manage human rheumatoid arthritis

(Quintana et al. 2003). Studies have identified several T-cell epitopes of *P. gingivalis* HSP60 (Choi et al. 2004). Further studies identifying cross-reactivity between pathogens associated with periodontitis and other diseases with a potential infectious aetiology may provide avenues for the development of a peptide vaccine strategy not only effective against periodontitis but also against other infectious diseases. Stress protein-derived peptides (HSP60) might provide a significant advantage if they do not induce auto-immune disease while providing immunity to infections of HSP60-carrying bacteria (Amir-Kroll et al. 2003).

Summary

Major efforts have been dedicated to the development of vaccines against serious diseases, highly prevalent diseases, and diseases without effective treatments. With regard to periodontitis, there are well-established treatment modalities of chronic periodontitis, the most common type of periodontitis. The prevalence of AgP in most societies is by all estimates rather low. Whether there is merit in developing a periodontal vaccine for the prevention of periodontitis in general, and for special risk populations, must be considered before major efforts can be pursued in periodontal vaccine development. Recent findings of associations between periodontitis and other systemic diseases may provide a rationale for the development of a vaccine against periodontitis, especially a vaccine that could have additional benefits and reduce risks for other diseases. Current knowledge of these associations does not possess sufficient merit to pursue such vaccine developments. The opposite might be proven more effective, that a vaccine developed for treatment of infection linked to pre-term birth or cardiovascular disease caused by infections may have cross-over benefits for periodontal health.

The research efforts associated with host immune responses and potential vaccine candidates against *P. gingivalis* infection have enriched the understanding of immune functions in periodontitis. The information on serum IgG antibody titres to bacteria associated with periodontitis may soon become useful as diagnostic methods, serving as predictors of treatment outcomes, and may guide clinicians in therapeutic decisions.

The current evidence collected from a large series of diverse and independent studies has clearly demonstrated that active immunization using vaccines against *P. gingivalis* will induce a significant humoral response across animal study models. If passive immunization studies are included, such evidence can also be gathered from human observational studies. Further studies are needed to assess the potential of passive immunization by either clinical treatment inducing bacteraemia and a host immune response, or from exposure to monoclonal antibodies and vector antigens developed against periodontal bacteria, which can be administered via the mucosal route.

Further studies are needed to develop scientific models for studies of naturally occurring periodontitis. The impact of trauma induced by ligature placement alone will, in itself, cause an inflammatory response with bone loss. Thus, the induced mechanical trauma from ligatures will mask any positive impact of infection prevention in a vaccine project. There are few suitable animal models. The ovine model discussed in this review appears to be promising. Additional studies are, however, necessary before this animal model can be recommended.

There is a need for a better understanding of the infections in periodontitis. The majority of studies that can be linked to vaccine trials have been focused on *P. gingivalis*. Although it appears clear that this pathogen is involved in periodontitis, other pathogens may be more critical in the early development of the biofilm resulting in periodontitis. Knowledge from caries vaccine efforts may be useful in the development of a vaccine against periodontitis. In fact, animal studies have demonstrated that it is possible to induce protection against caries in rodents with protein antigens derived from *S. mutans* or *S. sobrinus* against oral colonization by mutans streptococci and the development of dental caries. The protection has been focused on salivary IgA antibodies inhibiting different sucrose mechanisms of streptococcal accumulation on tooth surfaces (Russell et al. 2004). Recent advances in mucosal immunology and the introduction of novel strategies for inducing mucosal immune responses now raise the possibility that effective and safe vaccines can be developed (Abiko 2000, Kruger et al. 2004).

Whereas most vaccine efforts are directed against infections caused by single viral or bacterial infectious agents, periodontitis is believed to have a complicated infectious pattern. Few vaccines based on a composition of antigens from different bacteria/viruses and linked to different infectious diseases have been tested or developed. One example is the classical measles, mumps, and rubella virus vaccine combination, against three different diseases (Weibel et al. 1980). Vaccines studied in mice using conjugate multiple-antigen peptides against highly variable pathogens have been shown to elicit antibodies to a huge number of clinical isolates (Iglesias et al. 2005). Thus, similar methods might be applicable to combined periodontal and caries vaccine trials.

The current evidence collected from a large series of diverse and independent studies have clearly demonstrated that active immunization using vaccines against *P. gingivalis* will induce a significant humoral response across animal study models. If passive immunization studies are included, such evidence can also be gathered from human observational studies.

Conclusions

(I) There is sufficient concurring evidence that serum antibodies against *P. gingivalis* antigens are induced by either infection or immunization.

(II) There are non-human primate and murine study results with evidence of specific methods to induce an enduring antibody titre without recognizable systemic side-effects. The ambiguity in some study results may depend more on the study model (ligature-induced disease) than vaccine efficacy. High antibody titres appear to provide protection.

(III) Immunization against *P. gingivalis* results in a reduction of the quantity of the target organism in animal models. *P. gingivalis* levels at infected periodontal sites are inversely correlated with antibody titres against the pathogen.

(IV) Collaborative efforts are needed to ensure successful vaccine development against periodontitis.

References

Abiko, Y. (2000) Passive immunization against dental caries and periodontal disease: development of recombinant and human monoclonal antibodies. *Critical Reviews in Oral Biology and Medicine* **11**, 140–158.

Ahola, A. J., Yli-Knuuttila, H., Suomalainen, T., Poussa, T., Ahlstrom, A., Meurman, J. H. & Korpela, R. (2002) Short-term consumption of probiotic-containing cheese and its effect on dental caries risk factors. *Archives Oral Biology* **47**, 799–804.

Amir-Kroll, H., Nussbaum, G. & Cohen, I. R. (2003) Proteins and their derived peptides as carriers in a conjugate vaccine for *Streptococcus pneumoniae*: self-heat shock protein 60 and tetanus toxoid. *Journal of Immunology* **170**, 6165–6171.

Anderson, D. M., Ebersole, J. L. & Novak, M. J. (1995) Functional properties of nonhuman primate antibody to *Porphyromonas gingivalis*. *Infection and Immunity* **63**, 3245–3252.

Apatzidou, D.A & Kinane, D. F. (2004) Quadrant root planing *versus* same-day full-mouth root planing. *Journal of Clinical Periodontology* **31**, 152–159.

Aukhil, I., Lopatin, D. E., Syed, S. A., Morrison, E. C. & Kowalski, C. J. (1988) The effects of periodontal therapy on serum antibody (IgG) in plaque microorganisms. *Journal of Clinical Periodontology* **15**, 544–550.

Bainbridge, B. W., Page, R. C. & Darveau, R. P. (1997) Serum antibodies to *Porphyromonas gingivalis* block the prostaglandin E2 response to lipopolysaccharide by mononuclear cells. *Infection and Immunity* **65**, 4801–4805.

Bird, P. S., Gemmell, E., Polak, B., Paton, R. G., Sosroseno, W. & Seymour, G. J. (1995) Protective immunity to *Porphyromonas gingivalis* infection in a murine model. *Journal of Periodontology* **66**, 351–362.

Booth, V., Ashley, F. P. & Lehner, T. (1996) Passive immunization with monoclonal antibodies against *Porphyromonas gingivalis* in patients with periodontitis. *Infection and Immunity* **64**, 422–427.

Burckhardt, J. J., Gaegauf-Zollinger, R., Schmid, R. & Guggenheim, B. (1981) Alveolar bone loss in rats after immunization with *Actinomyces viscosus*. *Infection and Immunity* **31**, 971–977.

Casadevall, A., Dadachova, E. & Pirofski, L.-A. (2004) Passive antibody therapy for infectious diseases. *Nature Reviews Microbiology* **2**, 695–703.

Chen, H. A., Weinberg, A., Darveau, R. P., Engel, D. & Page, R. C. (1995) Immunodominant antigens of *Porphyromonas gingivalis* in patients with rapidly progressive periodontitis. *Oral Microbiology and Immunology* **10**, 193–201.

Chiu, B., Viira, E., Tucker, W. & Fong, I. W. (1997) *Chlamydia pneumoniae*, cytomegalovirus, and herpes simplex virus in atherosclerosis of the carotid artery. *Circulation* **96**, 2144–2148.

Choi, J.-L., Kang, H.-S., Park, Y.-M., Kim, S.-J. & Kim, U.-S. (2004) Identification of T-cell epitopes of *Porphyromonas gingivalis* heat-schock protein 60 in periodontitis. *Oral Microbiology and Immunology* **19**, 1–5.

Darby, I. B., Mooney, J. & Kinane, D. F. (2001) Changes in subgingival microflora and humoral immune response following periodontal therapy. *Journal of Clinical Periodontology* **28**, 796–805.

DeCarlo, A. A., Huang, Y., Collyer, C. A., Langley, D. B. & Katz, J. (2003) Feasibility of an HA2 domain-based periodontitis vaccine. *Infection and Immunity* **71**, 562–566.

Du Pasquier, L. & Flajnik, M. (1999) Origin and evolution of the vertebrate immune system. In : Paul, W. E. (ed). *Fundamental Immunology*, 4th edition, pp. 605–650. Philadelphia: Lippincott-Raven Publishers.

Duncan, W. J., Persson, G. R., Sims, T. J., Braham, P., Pack, A. R. C. & Page, R. C. (2003) Ovine periodontitis as a potential model for vaccination studies. Cross-sectional analysis of clinical, microbiological and serum immunological parameters. *Journal of Clinical Periodontology* **30**, 63–72.

Ebersole, J. L., Bauman, G. R., Cox O'Dell, D. S. E. & Giardino, A. (1997) Evidence for serum immunoglobulin G (IgG) antibody response in *Macaca fascicularis* identified by monoclonal antibodies to human IgG subclasses. *Oral Microbiology and Immunology* **12**, 193–203.

Ebersole, J. L., Brunsvold, M., Steffensen, B., Wood, R. & Holt, S. (1991) Effect of immunization with *Porphyromonas gingivalis* and *Prevotella intermedia* on progression of ligature induced periodontitis in the non-human primate *Macaca fascicularis*. *Infection and Immunity* **59**, 3351–3359.

Ebersole, J. L., Kraig, E., Bauman, G., Spitznagel, J. K. & Kolodrubetz, D. (1990) Molecular approaches to leukotoxin as a virulence component to *Actinobacillus actinomycetemcomitans*. *Archives Oral Biology* **35** (Suppl.), 69S–78S.

Evans, R. T., Klausen, B., Sojar, H. T., Bedi, G. S., Sfintescu, C., Ramamurthy, N. S., Golub, L. M. & Genco, R. J. (1992) Immunization with *Porphyromonas gingivalis* fimbriae protects against periodontal destruction. *Infection and Immunity* **60**, 2926–2933.

Friskopp, J. & Blomlöf, L. (1988) Spontaneous periodontitis in a sample group of the monkey *Macaca fascicularis*. *Journal of Periodontal Research* **23**, 265–267.

Gemmell, E., Bird, P. S., Ford, P. J., Ashman, R. B., Gosling, O., Hu, Y. & Seymour, G. J. (2004) Modulation of the antibody response by *Porphyromonas gingivalis* and *Fusobacterium nucleatum* in a mouse model. *Oral Microbiology and Immunology* **19**, 247–251.

Genco, C. A., Odusanya, B., Potempa, J., Mikolajczyk-Pawlinska, J. & Travis, J. (1998) A peptide domain on gingipain R which confers immunity against *Porphyromonas gingivalis* infection in mice. *Infection and Immunity* **66**, 4108–4114.

Genco, C. A., Potempa, J., Mikolajczyk-Pawlinska, J. & Travis, J. (1999) Role of gingipains R in the pathogenesis of *Porphyromonas gingivalis* in periodontal disease. *Clinical Infectious Diseases* **28**, 456–465.

Genco, R. J. (1996) Current view of risk factors for periodontal diseases. *Journal of Periodontology* **67** (Suppl. 10), 1041–1049.

Gibson, F. C. III. & Genco, R. A. (2001) Prevention of *Porphyromonas gingivalis* induced oral bone loss following immunization wit GingipainR1. *Infection and Immunity* **69**, 7959–7963.

Gibson, F. S. III., Gonzales, D. A., Wong, J. & Genco, C. A. (2004) *Porphyromonas gingivalis*-specific immunoglobulin G prevents *P. gingivalis* elicited oral bone loss in a murine model. *Infection and Immunity* **72**, 2408–2411.

Hall-Stoodley, L. & Stoodley, P. (2002) Development regulation of microbial biofilms. *Current Opinion in Biotechnology* **13**, 228–233.

Heltai, K., Kis, Z., Burian, K., Endresz, V., Veres, A., Ludwig, E., Gonczol, E. & Valyi-Nagy, I. (2004) Elevated antibody levels against *Chlamydia pneumoniae,* human HSP60 and mycobacterial HSP65 are independent risk factors in myocardial infarction and ischaemic heart disease. *Atherosclerosis* **173**, 339–346.

Herminajeng, E., Asmara, W., Yuswanto, A., Barid, I. & Sosroseno, W. (2001) Protective humoral immunity induced by surface-associated material from *Actinobacillus actinomycetemcomitans* in mice. *Microbes Infection* **3**, 997–1003.

Hilleman, M. R. (2000) Vaccines in isotric evolution and perspective: a narrative of vaccine discoveries. *Vaccine* **18**, 1436–1447.

Hiramine, H., Watanabe, K., Hamada, N. & Umemoto, T. (2003) *Porphyromonas gingivalis* 67 kDa fimbriae induced cytokine production and osteoclast differentiation utilizing TLR2. *FEMS Microbiology Letters* **229**, 49–55.

Holt, S. C., Brunsvold, M., Jones, A., Wood, R. & Ebersole, J. L. (1995) Cell envelope and cell wall immunization of *Macaca fascicularis* and effect on the progression of ligature-induced periodontitis. *Oral Microbiology and Immunology* **10**, 321–333.

Holt, S. C., Ebersole, J., Felton, J., Brunsvold, M. & Kornman, K. S. (1988) Implantation of *Bacteroides gingivalis* in nonhuman primates initiates progression of periodontitis. *Science* **239**, 55–57.

Horibe, M., Watanabe, H. & Ishikawa, I. (1995) Effect of periodontal treatments on serum IgG antibody titer to periodontal bacteria. *Journal of Clinical Periodontology* **22**, 510–515.

Houri-Haddad, Y., Itzchaki, O., Ben-Nathan, D. & Shapiro, L. (2003) The effect of chronic emotional stress on the humoral immune response to *Porphyromonas gingivalis* in mice. *Journal of Periodontal Research* **38**, 204–209.

Houston, L., Moncla, B. J. & Page, R. C. (1995) Response of guinea pigs to a vaccine containing a new adjuvant (SAF) and Gram-negative bacteria. *Laboratory Animal Science* **45**, 59–66.

Houston, L. S., Lukehart, S. A., Persson, G. R. & Page, R. C. (1999) Function of anti-*Por-*

phyromonas gingivalis immunoglobulin classes in immunized *Macaca fascicularis*. *Oral Microbiology and Immunology* **14**, 86–91.

Iglesias, E., Aguilar, J. C., Cruz, L. J. & Reyes, O. (2005) Broader cross-reactivity after conjugation of V3 based multiple antigen peptides to HBsAg. *Molecular Immunology* **42**, 99–104.

Johnson, V., Johnson, B. D., Sims, T. J., Whitney, C. W., Moncla, B. J. Engel, L. D. & Page, R. C. (1993) Effects of treatment on antibody titer to Porphyromonas gingivalis in gingival crevicular fluid of patients with rapidly progressive periodontitis. *Journal of Periodontology* **64**, 559–565.

Kaizuka, K., Hosogi, Y., Hayakawa, M., Shibata, Y. & Abiko, Y. (2003) Human monoclonal antibody inhibits *Porphyromonas gingivalis* hemagglutinin activity. *Journal of Periodontology* **74**, 38–43.

Kaplan, J. B., Meyenhofer, M. F. & Fine, D. H. (2003) Biofilm growth and detachment of *Actinobacillus actinomycetemcomitans*. *Journal of Bacteriology* **185**, 1399–1404.

Katoh, M., Saito, S., Takiguchi, H. & Abiko, Y. (2000) Bactericidal activity of a monoclonal antibody against a recombinant 40-kDa outer membrane protein of *Porphyromonas gingivalis*. *Journal of Periodontology* **71**, 368–375.

Katz, J., Black, K. P. & Michalek, S. M. (1999) Host responses to recombinant hemagglutinin B of *Porphyromonas gingivalis* in an experimental rat model. *Infection & Immunity* **67**, 4352–4359.

Kelly, C. G., Booth, V., Kendal, H., Slaney, J. M., Curtis, M. A. & Lehner, T. (1997) The relationship between colonization and haemagglutination inhibiting and B cell epitopes of *Porphyromonas gingivalis*. *Clinical Experimental Immunology* **110**, 285–291.

Kesavalu, J. J., Ebersole, J. L., Machen, R. L. & Holt, S. C. (1992) *Porphyromonas gingivalis* virulence in mice: induction of immunity to bacterial components. *Infection and Immunity* **60**, 1455–1464.

Kikuta, L. C., Puolakkainen, M., Kuo, C.-C. & Campbell, L. A. (1991) Isolation and sequence analysis of the *Chlamydia pneumoniae* GrE Operon. *Infection and Immunity* **59**, 4664–4669.

Kjelleberg, S. & Molin, S. (2002) is there a role for quorum sensing signals in bacterial biofilms. *Current Opinion in Microbiology* **5**, 254–258.

Kohyama, A. (1989) Microbiological and serological investigation of periodontal disease activity. *Nippon Shishubyo Gakkai Kaishi*, **31**, 360–379.

Kornman, K. S., Holt, S. C. & Robertson, P. B. (1981) The microbiology of ligature-induced periodontitis in the cynomolgus monkey. *Journal of Periodontal Research* **16**, 363–371.

Kruger, C., Pearson, S. K., Kodama, Y., Vacca Smith, A., Bowen, W. H. & Hammarström, L. (2004) The effects of egg-derived antibodies to glucosyltransferases on dental caries in rats. *Caries Research* **38**, 9–14.

Kuepper, M. B., Huhn, M., Spiegel, H., Ma, J. K., Barth, S., Fischer, R. & Finnern, R. (2005) Generation of human antibody fragments against *Streptococcus mutans* using a phage display chain shuffling approach. *BioMed Central Biotechnology* **25**, http://www.biomedcentral.com/1472-6750/5-4.

Lamont, R. J. D., Chan, C. M., Belton, K. T., Izutsu, T., Vasel, D. & Weinberg, A. (1995a) *Porphyromonas gingivalis* invasion of gingival epithelial cells. *Infection and Immunity* **63**, 3878–3885.

Lamont, R. J. D. & Jenkinson, H. F. (1998) Life below the gum line: pathogenic mechanisms of *Porphyromonas gingivalis*. *Microbiology and Molecular Biology Reviews* **62**, 1244–1263.

Lamont, R. J. D., Oda, D., Persson, R. E. & Persson, G. R. (1995b) Interaction of *Porphyromonas gingivalis* with gingival epithelial cells in culture. *Oral Microbiology and Immunology* **7**, 364–367.

Lappin, D. F., McGregor, A. M. & Kinane, D. F. (2003) The systemic immune response is more prominent than the mucosal immune response in the pathogenesis of periodontal disease. *Journal of Clinical Periodontology* **30**, 778–786.

Lehner, T., Caldwell, J. & Smith, R. (1985) Local passive immunization by monoclonal antibodies against streptococcal antigen I/II in the prevention of dental caries. *Infection and Immunity* **50**, 796–799.

Lopatin, D. E., Shelburne, C. E., Van Poperin, N., Kowalski, C. J. & Bagramian, R. A. (1999) Humoral immunity to stress proteins and periodontal disease. *Journal of Periodontology* **70**, 1185–1193.

Maeba, S., Otake, S., Namikoshi, J., Shibata, Y., Hayakawa, M., Abiko, Y. & Yamamoto, M. (2005) Transcutaneous immunization with a 40-kDa outer membrane protein of *Porphyromonas gingivalis* induces specific antibodies which inhibit coaggregation by *P. gingivalis*. *Vaccine* **31**, 2513–2521.

Merchant, A. T., Pitiphat, W., Ahmed, B., Kawachi, I. & Joshipura, K. (2003) A prospective study of social support, anger expression and risk of periodontitis in men. *Journal of American Dental Association* **134**, 1591–1596.

Moncla, B. J., Braham, P., Persson, G. R., Page, R. C. & Weinberg, A. (1994) Direct detection of *Porphyromonas gingivalis* in *Macaca fascicularis* in dental plaque samples using an oligonucleotide probe. *Journal of Periodontology* **65**, 398–403.

Montalto, M., Vastola, M., Marigo, L., Covino, M., Graziosetto, R., Curigliano, V., Santoro, L., Cuoco, L., Manna, R. & Gasbarrini, G. (2004) Probiotic treatment increases salivary counts of lactobacilli: a double-blind, randomized, controlled study. *Digestion* **69**, 53–56.

Mooney, J., Adonogianaki, E., Riggio, M. P., Takahashi, K., Haerian, A. & Kinane, D. F. (1995) Initial serum antibody titer to *Porphyromonas gingivalis* influences development of antibody avidity and success of therapy for chronic periodontitis. *Infection and Immunity* **63**, 3411–3416.

Mori, Y., Kitamaru, H., Song, Q. H., Kobayashi, T., Umemura, S. & Cyong, J. C. (2000) A new murine model for atherosclerosis with inflammation in the periodontal tissue induced by immunization with heat shock protein 60. *Hypertensive Research* **23**, 475–481.

Moritz, A. J., Capelli, D., lantz, M. S., Holt, S. C. & Ebersole, J. L. (1998) Immunization with *Porphyromonas gingivalis* cysteine protease effects on experimental gingivitis and ligature induced periodontitis in *Macaca fascicularis*. *Journal of Periodontology* **69**, 686–697.

Nalbandian, J., Brecx, M. C., Ooya, K., Kornman, K. S. & Robertson, P. B. (1985) Morphological studies on periodontal disease in the cynomolgus monkey. I. Light microscopic observations on gingivitis. *Journal of Periodontal Research* **20**, 154–164.

Niesengard, R., Blann, D., Zelonis, L., Mchenry, K., Reynolds, H. & Zambon, J. J. (1989) Effects of immunization with *B. macace* on induced periodontitis. Preliminary findings. *Immunological Investigations* **18**, 225–237.

O'Brien-Simpson, N. M., Paolini, R. A. & Reynolds, E. C. (2000) RgpA–Kgp peptide based immunogens probide protection against *Porphyromonas gingivalis* challenge in a murine lesion model. *Infection and Immunity* **68**, 4055–4063.

Okamoto, K. K., Nakayama, T., Kadowaki, N., Abe, D. B., Ratnayake, D. B. & Yamamoto, K. (1998) Involvement of a lysine-specific cysteine protease in haemoglobin absorption and hem accumulation by *Porphyromonas gingivalis*. *Journal of Biology and Chemistry* **273**, 21225–21231.

Ou, J. G., Bainbridge, B., Gu, K., Sims, T. J., Whitney, C. W., Darveau, R. P., Houston, L. S. & Page, R. C. (1997) Recognition of antigenic epitopes in lipopolysaccharide and protein from *Actinobacillus actinomycetemcomitans* by serum antibody in untreated rapidly progressive periodontitis patients. *Oral Microbiology and Immunology* **12**, 11–19.

Oyaizu, K., Ohyama, H., Nishimura, F., Kurihara, H., Matsushita, S., Maeda, H., Kokeguchi, S., Hongyo, H., Takashiba, S. & Murayama, Y. (2001) Identification and characterization of B-cell epitopes of a 53-kDa outer membrane protein from *Porphyromonas gingivalis*. *Oral Microbiology and Immunology* **16**, 73–78.

Page, R. C. & Schroeder, H. (1982) *Periodontal Disease in Man and Other Animals. A Comparative Review*, pp. 1–325. Basel: Karger.

Papapanou, P., Neiderud, A.-M., Disick, E., Lalla, E., Miller, G. C. & Dahlén, G. (2004) Longitudinal stability of serum immunoglobulin G responses to periodontal bacteria. *Journal of Clinical Periodontology* **31**, 989–990.

Papapanou, P. N., Neiderud, A.-M., Sandros, J. & Dahlén, G. (2001) Interleukin-1 gene polymorphism and periodontal status. A case–control study. *Journal of Clinical Periodontology* **28**, 389–396.

Persson, G. R., Engel, D., Moncla, B. J. & Page, R. C. (1993) *Macaca nemestrina*: a non-human primate model for studies of periodontal disease. *Journal of Periodontal Research* **28**, 294–300.

Persson, G. R., Engel, D., Whitney, C., Darveau, R., Weinberg, A., Brunsvold, M. & Page, R. C. (1994a) Immunization against *Porphyromonas gingivalis* inhibits progression of experimental periodontitis in nonhuman primates. *Infection and Immunity* **62**, 1026–1031.

Persson, G. R., Engel, L. D., Whitney, C. W., Weinberg, A., Moncla, B., Darveau, R., Houston, L., Braham, P. H. & Page, R. C. (1994b) *Macaca fascicularis* as a model in which to assess the safety and efficacy of a vaccine for periodontitis. *Oral Microbiology and Immunology* **9**, 104–111.

Persson, G. R., Engel, D., Whitney, C. W., Weinberg, A., Moncla, B. J., Darveau, R., Houston, L., Braham, P. & Page, R. C. (1994c) *Macaca fascicularis* as a model in which to assess the safety and efficacy of a vaccine for periodontitis. *Oral Microbiology and Immunology* **9**, 104–111.

Persson, G. R., Schlegel-Bregenzer, B., Chung, W. O., Houston, L., Oswald, T. & Roberts, M. C. (2000) Serum antibody titers to *Bacteroides forsythus* in elderly subjects with gingivitis or periodontitis. *Journal of Clinical Periodontology* **27**, 839–845.

Pistorius, A., Krahwinkel, T., Willershausen, B. & Boekstegen, C. (2002) Relationship between stress factors and periodontal disease. *European Journal of Medical Research* **30**, 393–398.

Plotkin, S. L. & Plotkin, S. A. (1999) A short history of vaccination. In: Plotkin, S. L. & Orenstein, W. A. (eds). *Vaccines*, 3rd edition, pp. 1–27. Philadelphia: W.B. Saunders.

Quintana, F. J., Carmi, P., Mor, F. & Cohen, I. R. (2003) DNA fragments of the human 60-kDa heat shock protein (HSP60) vaccinate against adjuvant arthritis: identification of a regulatory HSP60 peptide. *Journal of Immunology* **171**, 3533–3541.

Quirynen, M., Mongardini, C., Pauwels, M., Bollen, C. M., Van Eldere, J. & van Steenberghe, D. (1999) One stage full- *versus* partial-mouth disinfection in the treatment of chronic adult or generalized early-onset periodontitis. II. Long-term impact on microbial load. *Journal of Periodontology* **70**, 646–656.

Rajapakse, P. S., O'Brien-Simpson, N. M., Slakeski, N., Hoffmann, B. & Reynolds, E. C. (2002) Immunization with the RgpA–Kgp proteinase–adhesin complexes of *Porphyromonas gingivalis* protects against periodontal bone loss in the rat periodontitis model. *Infection and Immunity* **70**, 2480–2486.

Roberts, F., Houston, L. S., Lukehart, S. A., Mancl, L. A., Persson, G. R. & Page, R. C. (2004) Periodontitis vaccine decreases local prostaglandin E2 levels in a primate model. *Infection and Immunity* **72**, 1166–1168.

Ross, B. C., Czajkowski, L., Vandenberg, K. L., Camuglia, S., Woods, J., Agius, C., Paolini, R., Reynolds, E. & Barr, I. G. (2004a) Characterization of two outer membrane protein antigens of *Porphyromonas gingivalis* that are protective in a murine lesion model. *Oral Microbiology and Immunology* **19**, 6–15.

Ross, C. R., Czajkowski, L., Hocking, D., Margetts, M., Webb, E., Rothel, L., Patterson, M., Agius, C., Camuglia, S., Reynolds, E., Littlejohn, T., Gaeta, B., Ng, A., Kuczek, E. S., Mattlick, J., Gearing, D. & Barr, I. G. (2001) Identification of vaccine candidate antigens from a genomic analysis of *Porphyromonas gingivalis*. *Vaccine* **19**, 4135–4142.

Ross, C. R., Czajkowski, L., Vandenberg, K. L., Camuglia, S., Woods, J., Agius, C., Paolini, R., Reynolds, E. & Barr, I. G. (2004b) Characterization of two outer membrane protein antigens of *Porphyromonas gingivalis* that are protective in a murine lesion model. *Oral Microbiology and Immunology* **19**, 6–15.

Russell, M. W., Childers, N. K., Michalek, S. M., Smith, D. J. & Taubman, M. A. (2004) A caries vaccine? The state of the science of immunization against dental caries. *Caries Research* **38**, 230–235.

Sharma, A., Honma, K., Evans, R. T., Hruby, D. E. & Genco, R. J. (2001) Oral immunization with recombinant *Streptococcus gordonii* expressing *Porphyromonas gingivalis* FimA domains. *Infection and Immunity* **69**, 2928–2934.

Sims, T., Ali, R. W., Brockman, E. S., Skaug, N. & Page, R. C. (1999) Antigenic variation in *Porphyromonas gingivalis* ribotypes recognized by serum immunoglobulin G of adult periodontitis patients. *Oral Microbiology and Immunology* **14**, 73–85.

Sims, T., Lernmark, Å., Mancl, L. A., Schifferle, R. E., Page, R. C. & Persson, G. R. (2003) Serum IgG to heat shock proteins and *Porphyromonas gingivalis* antigens in diabetic patients with periodontitis. *Journal of Clinical Periodontology* **29**, 551–562.

Sjöström, K., Ou, J., Whitney, C., Johnson, B., Darveau, R., Engel, D. & Page, R. C. (1994) Effect of treatment on titer, function, and antigen recognition of serum antibodies to *Actinobacillus actinomycetemcomitans* in patients with rapidly progressive periodontitis. *Infection and Immunity* **62**, 145–151.

Smith, G. T., Greenbaum, C. J., Johnson, B. D. & Persson, G. R. (1996) Short-term responses to periodontal therapy in insulin-dependent diabetic patients. *Journal of Periodontology* **67**, 794–802.

Socransky, S. S. & Haffajee, A. D. (1991) Microbial mechanisms in the pathogenesis of destructive periodontal diseases: a critical assessment. *Journal of Periodontal Research* **26**, 195–212.

Socransky, S. S. & Haffajee, A. D. (2003) Microbiology of periodontal disease. In: Lindhe, J., Karring, T. & Lang, N. P. (eds). *Clinical Periodontology and Implant Dentistry*, 4th edition. Oxford: Blackwell Munksgaard.

Tanabe, S., Hinode, D., Yokoyama, M., Fukui, M., Nakamuru, R., Yoshioka, M., Grenier, D. & Mayrand, D. (2003) *Heliobacter pylori* and *Campylobacter rectus* share a common antigen. *Journal of Oral Microbiology and Immunology* **18**, 79–87.

Teshirogi, K., Hayakawa, M., Ikemi, T. & Abiko, Y. (2003) Production of monoclonal antibody inhibiting dipeptidylaminopeptidase IV activity of *Porphyromonas gingivalis*. *Hybrid Hybridomics* **22**, 147–151.

Tlaskalova-Hogenova, H., Stepankova, R., Hudcovic, T., Tuckova, L., Cukrowska, B., Lodinova-Zadnikova, R., Kozakova, H., Rossmann, P., Bartova, J., Sokol, D., Funda, D. P., Borovska, D., Rehakova, Z., Sinkora, J., Hofman, J., Drastich, P. & Kokesova, A. (2004) Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. *Immunology Letters* **15**, 97–108.

Van Tilburg, M. L., Kozarov, E. V., Progulske-Fox, A. & Brady, L. J. (2001) The effect of monoclonal antibody and route of immunization on the humoral immune response against *Porphyromonas gingivalis*. *Oral Microbiology and Immunology* **16**, 153–162.

Vasel, D., Sims, T., Bainbridge, B., Houston, L., Darveau, R. & Page, R. C. (1996) Shared antigens of *Porphyromonas gingivalis* and *Bacteroides forsythus*. *Oral Microbiology and Immunology* **11**, 226–235.

Verma, J. N., Rao, M., Amselem, S., Krzych, U., Alving, C. R., Green, S. J. & Wassef, N. M. (1992) Adjuvant effects of liposomes containing lipid A: enhancement of liposomal antigen presentation and recruitment of macrophages. *Infection and Immunity* **60**, 2438–2444.

Weibel, R. E., Carlson, A. J., Villarejos, V. M., Buynak, E. B., Mclean, A. A. & Hilleman, M. R. (1980) Clinical and laboratory studies of combined live measles, mumps and rubella vaccines using the RA 27/3 rubella virus. *Proceedings of the Society for Experimental Biology and Medicine* **165**, 323–326.

Yamazaki, K., Ohsawa, Y., Itoh, H., Ueki, K., Tabeta, K., Oda, T., Nakajima, T., Yoshie, H., Saito, S., Oguma, F., Kodama, M., Aizawa, Y. & Seymour, G. J. (2004a) T-cell clonality to *Porphyromonas gingivalis* and human heat shock protein 60s in patients with atherosclerosis and periodontitis. *Oral Microbiology and Immunology* **19**, 160–167.

Yamazaki, K., Ueki-Maruayama, K., Honda, T., Nakajima, T. & Seymour, G. J. (2004b) Effect of periodontal treatment on the serum antibody level to heat chock proteins. *Clinical Experimental Immunology* **135**, 478–482.

Young, S.-H., Ye, J., Frazer, D. G., Shi, X. & Castranova, V. (2001) Molecular mechanism of tumor necrosis factor-α production in 1 → 3-β-glucan (zymosan)-activated macrophages. *Journal of Biological Chemistry* **276**, 20781–20787.

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