Periodontal diseases: to protect or not to protect is the question?

Jeffrey L. Ebersole, David Cappelli and Stanley C. Holt

Center for Oral Health Research, College of Dentistry, Lexington, Kentucky; The University of Texas Health Science Center at San Antonio, San Antonio, Texas; and The Forsyth Institute, Boston, Massachusetts; USA

Ebersole JL, Cappelli D, Holt SC. Periodontal diseases: to protect or not to protect is the question? Acta Odontol Scand 2001;59:161–166. Oslo. ISSN 0001-6357.

For decades, investigations have identified local and systemic humoral immune responses to microorganisms comprising the supra- and subgingival biofilms in the oral cavity. Inflammation and tissue destruction in the periodontium are accompanied by alterations in the quantity, quality, and specificity of antibody. The conundrum in this scenario is the existence of a substantial plasma cell infiltrate at sites of periodontal lesions and a seemingly robust antibody response in the oral cavity and the serum, apparently coincident with progressing disease. Consequently, much effort has been expended to elucidate the critical characteristics of protective humoral responses and to develop strategies for enhancing these unique features. We and others have conducted studies attempting to distinguish disease susceptibility associated with: i) variations in response levels—significantly increased to some species with disease, minimal response to others; ii) functional comparisons of antibody—subclass differences, genetic regulation, and maturation of responses; iii) microbial and antigenic specificity of the antibody—focus on specific pathogens and identification of selected antigens as targets for immunoprotection; and, iv) kinetics of responses during disease and therapeutic interventions—linking immune changes with infection and as a measure of treatment success. This report summarizes varied research designs and results, to provide a profile of antibody in health, gingivitis, and periodontitis. These profiles may be used to provide a framework focusing on the humoral response to commensal microorganisms and likely pathogens, as they emerge in the biofilm—etiologic for or in response to disease processes. Models for antibody as a diagnostic adjunct and for predicting protective antibody responses are suggested. These concepts are likely relevant for considering vaccine approaches to periodontitis. □ *Antibody; antigens; biofilm; infection; inflammation; periodontitis*

Jeffrey L. Ebersole, Center for Oral Health Research, College of Dentistry, D544, Chandler Medical Center, University of Kentucky, Lexington, KY 40536-0297, USA.

During the past 2 decades studies of periodontal disease have been oriented towards an infectious process with specific pathogens. This concept resulted in investigations to develop immunological strategies for interfering with tissue destruction in the periodontium. Since most proposed periodontopathogens appear to be extracellular pathogens that, in some cases, produce toxins, the humoral immune response has been emphasized in these studies. The recognition of both local and systemic humoral immune responses in this disease also prompted studies to elucidate mechanisms for protection or destruction of host tissues. Investigations of both humans and animal models have also shown a sequence of cellular infiltrates into the inflamed tissues, which appears to reflect the critical nature of polymorphonuclear leukocytes (PMNs) in providing primary protection, and T lymphocytes as the local immunoregulators in maintaining a healthy periodontium. These data have also provided comparisons of: i) the characteristics of local inflammatory responses at the cellular and mediator level; ii) the characteristics of the local humoral response; and iii) the characteristics of the systemic inflammatory/immune response, which have been primarily associated with different forms of periodontal disease. It is now clear that specific pathogens or consortia of microorganisms initiate an immunoinflammatory process, which is the major contributor to tissue destruction in periodontitis (1). These concepts have been formulated into a consideration of 'high-risk' patients for periodontitis within the population (2). One concept underlying this characterization has focused on variations in host response capacity.

Variations in response levels

Bacteria contain an array of antigens with various chemical and antigenic compositions. The antibody response elicited by any specific infection will be a composite of antibodies to the various epitopes. All subclasses of IgG have been identified as present in gingival crevicular fluid (GCF) (3). The antibody variances in IgG subclass responses are presumably indicative of the dominant antigens associated with the bacteria and should have an effect on the mechanisms by which the host interacts with these pathogens (4). For example, the relative predominance of IgG2, a subclass that lacks strong complement fixation and opsonic properties, and the low avidity of patient anti-*Porphyromonas gingivalis* antibodies suggested that the humoral response to infection may be ineffective in clearing this microorganism (5).

Whereas these bacteria can exist in the subgingival ecology of the human, the host recognition of these periodontal bacteria, which leads to systemic antibody,

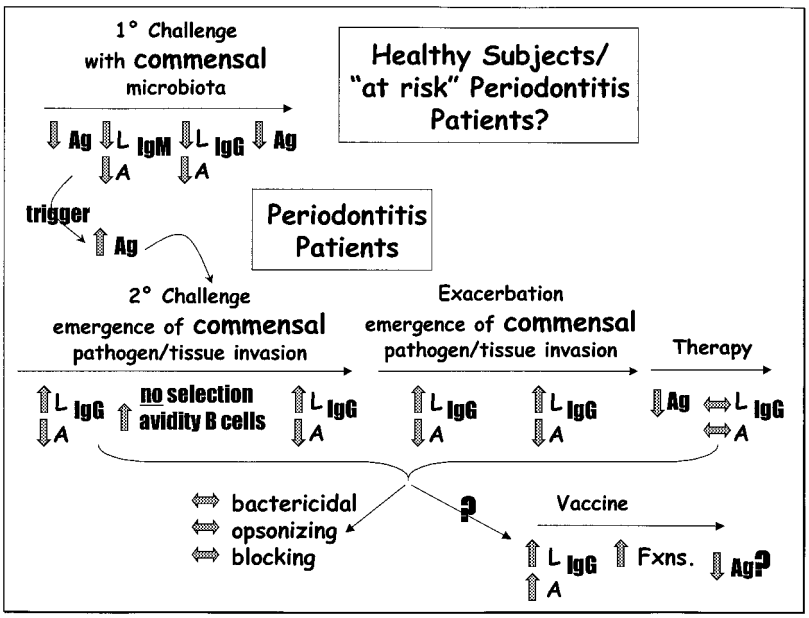

Fig. 1. Schematic representation of host-pathogen interactions associated with the opportunistic commensal challenge in periodontal disease. The schema provides thoughts on changes in antibody level, avidity and antimicrobial functions related to primary and secondary challenge, exacerbation, and the effects of standard therapy. Potential impacts of a successful vaccination strategy are also included. Ag denotes antigen (that is, microorganism), L denotes level of antibody, A denotes avidity of antibody, IgM/IgG are antibody isotypes, Fxns denotes the group of antibacterial functions of the antibody.

may vary, and the findings of differences in isotype and subclass responses suggest that either the variation is in response to different types of dominant antigens stimulating the host, or the variation is due to differences in ecological niche and/or tissue interactions of the different bacteria with the host. It is also feasible that certain subjects have specific subclass responses, which are reflected in these studies and which are instrumental in rendering them susceptible to the disease. More detailed studies of these variations may enable a clearer perspective of antigens of interest for interference with the virulence properties of the bacteria and a clarification of the host-parasite interactions that contribute to disease.

Functional comparisons of antibody

Generally, two concepts are evaluated related to antibody function: i) the ability of the antibody to interact with the pathogen or its components, and ii) the ability of the antibody to express anti-bacterial effector mechanisms including: toxin neutralization, opsonization, complement fixation, and blocking activities. Antibody avidity has been correlated to acute stages of systemic bacterial infections, diagnosing disease, and differentiating disease active states with viral and protozoan infections, and to determination of the onset and duration of infections (6). These findings may have relevance in investigations of the phasic destructive episodes of chronic periodontitis, with the possibility that acute exacerbation and quiescent periods could be differentiated. Numerous studies support the contention that in periodontal diseases gingival plasma cells provide for a specific immune response to certain microorganisms in the oral microbiota (7). Therefore, questions have arisen as to why there exists this coincidence of chronic infection with accompanying periods of exacerbated disease and an active, often substantial, local and systemic antibody response, and, furthermore, why certain individuals appear to be susceptible to certain pathogens.

Recent reports have suggested the need to investigate the functional capabilities of these antibodies (8). Studies of antibody avidity in *Actinobacillus actinomycetemcomitans*-infected subjects suggest that the antibody avidity may be a very dynamic process with fluctuations more closely integrated with alterations in the host–parasite interactions in the periodontium (9). Studies examining antibody avidity to *P. gingivalis* in periodontal diseases suggested that relative avidity may be related to the extent of disease in these patients. For example, it has been suggested that many patients with generalized early-onset periodontitis (GEOP) do not produce protective levels of biologically functional antibody as a result of natural infection but that treatment may induce the production of such antibodies (10).

The maturation of the host response via antibody avidity measures could potentially determine active or recent infection versus quiescent or convalescent stages in

the disease process. The existing paradigm describes increased antibody levels and a maturation of the immune response towards antibody populations with increased avidity in both acute infections and active immunization. This combination of increased levels and somatic mutations leading to increased avidity synergize to eliminate the antigen. In contrast, there is substantial evidence that sera from most, if not all, healthy individuals contains antibody to oral microorganisms, including putative periodontopathogens. This presumably occurs via specific responses to low levels of these bacteria within the commensal microbiota (11), albeit this could reflect a cross-reactive response to other common antigens (12). Predictably, the antigen (for example, microorganism) remains low in healthy subjects, resulting in low levels and avidity of IgM and IgG, since no selective pressure exists to expand B cells for increasing levels or avidity. The initial challenge, in patients ('at risk') who will develop periodontitis, would be similar to that in healthy subjects, with a low level and avidity of antibody to the pathogens existing in the commensal microbiota. After some biological 'triggering' mechanism, specific microorganisms emerge in the subgingival plaque (13) or potentially invade the host tissues (14, 15), at diseased sites of periodontitis patients (Fig. 1). This process leads to increased antigen availability that stimulates increased levels of IgG antibody but does not appear to select for high avidity antibody, yielding rather consistent observations of increased levels but rather meager avidity. This process also appears to occur associated with subsequent exacerbations of disease, maintaining or increasing levels of antibody, without a concomitant maturation of the response. Finally, therapeutic intervention would be predicted to decrease antigen; however, with existing high levels of IgG antibody, some selection for antibody maturation (for example, higher avidity) may or may not occur. Therefore, at the individual patient level both antibody levels and avidity may contribute to the variation in host resistance to infection and disease associated with primary periodontopathogens. Lastly, the schematic summarizes information on the functional capacity of the antibody elicited during disease. In this respect, there is little information contributing to our understanding of factors controlling individual variation in the ability of antibody to affect antibacterial host resistance, resulting in control but not elimination of the pathogens. Accordingly, the concept of a vaccination strategy to increase levels, avidity, and function of the antibody, with an outcome of pathogen (Ag) elimination, has been put forth. Clarification of target pathogens, antigens, and specific populations still presents a challenge with regard to benefit/risk considerations in this approach.

Evidence describing the anti-bacterial functional capabilities of human antibodies suggest: i) inhibition of *A. actinomycetemcomitans* leukotoxin; ii) opsonization of *A. actinomycetemcomitans* in vitro; iii) anti-lipopolysaccharide (LPS) specificity; iv) in vitro bactericidal activity of serum antibodies in combination with complement appears to be limited against *P. gingivalis*; and v) antibodies in sera from periodontitis patients are not particularly effective in opsonizing *P. gingivalis*. Consequently, individual patient variation in these functions and the multiple strategies used by the pathogens to avert the host response decreases our understanding of pressures to select for these functions.

The above studies have generally emphasized the ability of antibody to augment the immune repertoire of the host for controlling these pathogens and their virulence components. However, data are available showing that they still have the capacity to bind to, invade, alter cellular structure, and stimulate resident host cells, including epithelial cells and gingival fibroblasts. Moreover, *P. gingivalis* appears to be well equipped to disarm the host by blocking microbicidal activities and neutralizing humoral elements of host defense, by proteolytic degradation of immunoglobulin and complement components. Many of the divergent symptoms and the tissue destruction associated with bacterial infections, including periodontitis, thus appear to be a consequence of the microorganisms stimulating host inflammatory mediators and cytokines (1).

Clearly, there is a multitude of potential mechanisms that may be used by *A. actinomycetemcomitans* and *P. gingivalis* to colonize the host, obtain nutritional materials for replication, and evade host responses. We have seemingly only scratched the surface in clarifying the functional specificity of serum antibody in preventing the deleterious aspects of these bacterial virulence factors.

Microbial and antigenic specificity of the antibody

Response to A. actinomycetemcomitans

Studies of the humoral immune response to gram-negative microorganisms in periodontitis have tended to target microorganisms such as *P. gingivalis* and *A. actinomycetemcomitans* because of their marked association with periodontal disease. Thus, numerous investigations of many ethnic and geographically disparate groups tend to confirm that *A. actinomycetemcomitans* is frequently associated with, and probably an etiologic agent in, the vast majority of localized early-onset periodontitis (LEOP) patients and a substantial proportion of other EOP patients (8, 16). Therefore, antibody response characteristics suggest that *A. actinomycetemcomitans* should not be perceived to be limited as a pathogen to only young individuals but may have the capacity to initiate disease in an at-risk adult group (17).

Response to P. gingivalis

The host responses to *P. gingivalis* provide compelling evidence for the etiological contribution of this microorganism to periodontitis. Whereas there is some overlap among the various clinical categories with regard to the

contribution, exclusivity generally appears within individual patients; although the basis for this exclusivity is unknown. Finally, recent studies have supported the capacity of *P. gingivalis* to express variations in virulence between subjects in a population and across patient populations, potentially contributing to its distribution and pathogenicity (18).

Choice of antigen in immunological studies of periodontitis

The extensive literature on antibody studies of medically significant pathogens emphasizes the importance of knowledge on responses to individual antigens presented by the bacteria. This information can provide details on specific bacterial virulence determinants, natural protective responses, and strategies for vaccine development. In this regard, some studies have investigated the systemic humoral response to particular antigens of putative oral pathogens rather than to the whole bacterial cells. Recent genotypic studies have indicated that *A. actinomycetemcomitans* isolates with an altered promoter and enhanced leukotoxin production are most associated with disease sites in selected populations (19). It is not clear whether the antibody titer against the leukotoxin is more discriminatory than the antibody titer to the whole bacteria, and the biologic relevance of the leukotoxin as a virulence factor has not been clearly shown in vivo. Therefore, the ability to evaluate risk of disease with antibody to the leukotoxin as a biomarker still remains under active investigation. The response to LPS is a significant marker of *A. actinomycetemcomitans* infection and existing periodontitis. However, the relationship of this response to amelioration of the infection and disease process also remains to be determined. Irrespective of the patient population investigated or the antigen preparation examined, there appear distinct similarities in the characteristics of the human serum antibody to protein antigens of *A. actinomycetemcomitans*. Thus, in *A. actinomycetemcomitans* infections in which this microorganism continually colonizes the subgingival ecology in the presence of an active host immune response with accompanying active or remitted disease, the interplay of the host responses that regulate this pathogen remains to be delineated.

As in most bacterial infections, various investigations have identified increased systemic antibody responses to *P. gingivalis* in periodontal disease. Recently, examination of *P. gingivalis* has shown that the microorganism contains various potential virulence factors and structures that may be crucial to its pathogenicity (15). Various studies have also identified human antibody responses in periodontitis to some of these factors and a large number of additional surface antigens of *P. gingivalis* (20). As an example, Laine et al. (21) have identified serotypic capsular antigens among *P. gingivalis* and suggested a linkage of the capsule to virulence expression by this pathogen and the potential for anti-capsular antibody to interfere with its virulence.

Phenotypic changes in bacteria grown in vitro are paralleled by the dramatic ability of certain pathogens to alter the surface characteristics that they present to their environment(s). Variation in antigenic composition of pathogens generally comes in two forms, which can be defined as antigenic 'variation' (shift) and 'diversity' (drift). With regard to periodontopathogens, reports have been published on the concept of antigenic heterogeneity of various oral microorganisms. However, the available literature is primarily limited to genetic polymorphisms in sites and patients, with negligible information on antigenic diversity and its potential role in virulence, although recent evidence supports antigenic diversity among *A. actinomycetemcomitans* antigens (22). Genco et al. (23) have also reported on the antigenic heterogeneity of *P. gingivalis*. However, the in vivo significance of antigenic diversity in these pathogens remains to be determined.

The host response in differentiation of disease states

It is now accepted that periodontitis is a biological process with characteristics of an infectious disease and resultant host inflammatory and immunologic responses; studies have been instituted to examine the use of clinical and laboratory variables for prediction or risk assessment of periodontal disease. Evidence describing a microbial progression in the subgingival microbial ecology associated with periodontitis has suggested the potential for microbiological discrimination of periodontal risk (24). An outcome of these investigations was also the identification of significant increases in serum antibody in periodontitis patients.

The process of utilizing host immune responses (that is, antibody) to detect diseases caused by specific infectious agents is widespread in medically important infections. Diagnoses based on host antibody responses have been used to delineate the course of an infection by i) differentiating between specific agents that induce similar symptoms; ii) assessment of the onset of an infection; iii) determination of remission from an infection; and iv) defining the success of directed treatment regimens. Periodontitis is clearly a multifactorial disease that appears to result from a combination of factors, including genetic susceptibility, challenge with a specific pathogen, and characteristics of the host inflammatory/immune response. Current thoughts on host-parasite interactions in periodontal disease(s) emphasize a specific bacterial etiology rather than a nonspecific incitement directly related to plaque mass, although the concepts of 'microbial consortia' and 'biofilms' as more representative of the pathogenic plaque environment have generated substantial discussion of the etiology of these diseases. Variations in onset, severity, and clinical characteristics support the

existence of different forms of periodontitis, which may have different microbial characteristics. Exacerbation and remissions in periodontitis denote a dynamic process and have required a consideration that the host responses may vary during these intervals. These concepts also have an impact on the significance of host immune responses for diagnosis of different forms of periodontitis. The succession of changes in the host–parasite relationship from periodontal health to disease has led to the emergence of a specific 'putative periodontopathogenic microbiota', which is also accompanied by an active specific host response. These host responses presumably arise by these species or their products entering the periodontal tissues and eliciting an enhanced host inflammatory/immune response (1). Data derived from periodontitis patients have provided support for a relationship between the distribution of selected members of the periodontopathic microbiota (that is, *A. actinomycetemcomitans*, *P. gingivalis*) and antibody levels to the intact bacteria in both serum and GCF, as well as a positive relationship between increased systemic antibody levels in humans and oral colonization with the homologous microorganism at active disease sites. Beck (25) has reviewed the development of strategies to develop clinical and laboratory tools as risk indicators or factors to enable modeling of risk assessment in periodontitis. At present, we lack sufficiently robust data sets to clearly define the clinical usefulness of humoral immunologic diagnostics as an adjunct in treatment decisions.

Conclusion

As in other bacterially mediated infectious diseases, infections in periodontitis would be expected to elicit a specific host antibody response (26). However, whereas most medically important bacterial diseases are acute infections (27), the relationship between the pathogens and host in periodontitis appears to be a chronic interaction with unknown factors 'triggering' a progressing disease process related to a member(s) of the indigenous ecology. Suggestions have therefore been put forth to use antibody testing to predict or provide a risk evaluation for disease episodes or recurring periodontitis. As this area of research continues to develop, studies will be implemented which will elucidate the characteristics of host responses, which fulfill the criteria of risk factors for identification of causality of periodontitis as described by Lilienfeld (28). Thus, the definition of these host responses as risk factors in periodontitis will require data that delineate i) the strength of association of the response with disease; ii) the specificity of association of the response with periodontitis; iii) a positive relationship of degree of exposure with risk of developing disease; iv) the consistency of association between the host response and disease in numerous studies; v) the temporal consistency of the host response risk factor in preceding the disease; and vi) the biological plausibility indicating that the association of the host response makes sense relative to the current knowledge of the disease process. As such, these data are consistent with systemic antibody as a reflection of the host response to an infectious process associated with an episode of disease activity. The attributes of the relationship that exists between *A. actinomycetemcomitans* or *P. gingivalis* and infected hosts, which results in a stable commensal relationship or can develop into a pathologic host–parasite interaction, remain to be defined. In summary, the current status of knowledge delineating antibody characteristics in periodontitis and changes associated with disease progression and treatment appears to be dependent on the patient selection, the antibody specificity examined, and the design of the study. Importantly, the use of immunologic diagnostic/prognostic tools in periodontal disease still remains primarily reserved to research laboratories. It is incumbent upon the scientific community to evaluate the practicality of such tools and how the findings may be utilized to enable the practitioner to more effectively manage an individual patient's disease.

Acknowledgements—We would like to specifically thank Michelle Steffen, Noel Sandoval, Sarah O'Dell, and Caleb Herrera for their technical support in generating the data summarized from our own research. In addition, Martin Taubman, Daniel Smith, Sigmund Socransky, Dennis Kinane, M. John Novak, and Robert Singer contributed immeasurably to developing some of the thoughts presented in this review.

References

1. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. Periodontol 2000 1997;14:216–48.
2. Page RC. Milestones in periodontal research and the remaining critical issues. J Periodontal Res 1999;34:331–9.
3. Ebersole JL, Cappelli D. Gingival crevicular fluid antibody to *A. actinomycetemcomitans* in periodontal disease. Oral Microbiol Immunol 1994;9:335–44.
4. Choi JI, Ha MH, Kim JH, Kim SJ. Immunoglobulin allotypes and immunoglobulin G subclass responses to *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in early-onset periodontitis. Infect Immun 1996;64:4226–30.
5. Califano JV, Gunsolley JC, Schenkein HA, Tew JG. A comparison of IgG antibody reactive with *Bacteroides forsythus* and *Porphyromonas gingivalis* in adult and early-onset periodontitis. J Periodontol 1997;68:734–8.
6. Hedman K, Lappalainen M, Seppala I, Makela O. Recent primary toxoplasma infection indicated by a low avidity of specific IgG. J Infect Dis 1989;4:736–40.
7. Kinane DF, Mooney J, MacFarlane TW, McDonald M. Local and systemic antibody response to putative periodontopathogens in patients with chronic periodontitis: correlation with clinical indices. Oral Microbiol Immunol 1993;8:65–8.
8. Kinane DF, Mooney, J, Ebersole JL. Humoral immune response to *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in periodontal disease. Periodontol 2000 1999;20:289–340.
9. Holbrook WP, Mooney J, Sigurdsson T, Kitsiou N, Kinane DF. Putative periodontal pathogens, antibody titres and avidities to them in a longitudinal study of patients with resistant periodontitis. Oral Dis 1996;2:217–23.
10. Chen HA, Johnson BD, Sims TJ, Darveau RP, Moncla BJ, Whitney CW, et al. Humoral immune responses to *Porphyromonas*

gingivalis before and following therapy in rapidly progressive periodontitis patients. J Periodontol 1991;62:781–91.
11. Cole MF, Fistzsimmons SP, Sheridan MJ, Xu Y. Humoral immunity to commensal oral bacteria: quantitation, specificity, and avidity of serum IgG and IgM antibodies reactive with *Actinobacillus actinomycetemcomitans* in children. Microbiol Immunol 1995;39:591–8.
12. Quiding-Jabrink M, Nordstrom I, Granstrom G, Kilander A, Jertborn M, Butcher EC, et al. Differential expression of tissue-specific adhesion molecules on human circulating antibody-forming cells after systemic, enteric, and nasal immunizations. A molecular basis for the compartmentalization of effector B cell responses. J Clin Invest 1997;99:1281–6.
13. Socransky SS, Haffajee AD. Evidence of bacterial etiology: a historical perspective. Periodontol 2000 1994;5:7–25.
14. Fives-Taylor, PM, Meyer DH, Mintz KP, Brissette C. Virulence factors of *Actinobacillus actinomycetemcomitans*. Periodontol 2000 1999;20:136–67.
15. Holt SC, Kesavalu, L, Walker, S, Attardo Genco, C. Virulence factors of *Porphyromonas gingivalis*. Periodontol 2000 1999;20:168–238.
16. Ebersole JL, Taubman MA. The protective nature of host responses in periodontal diseases. Periodontol 2000 1994;5:112–41.
17. Preus HR, Zambon JJ, Dunford RG, Genco RJ. The distribution and transmission of *Actinobacillus actinomycetemcomitans* in families with established adult periodontitis. J Periodontol 1994;65:2–7.
18. Genco RJ, Zambon JJ, Christersson LA. The role of specific bacteria in periodontal disease: the origin of periodontal infections. Adv Dent Res 1988;2:245–59.
19. DiRienzo JM, Slots J, Sixou M, Sol MA, Harmon R, McKay TL. Specific genetic variants of *Actinobacillus actinomycetemcomitans* correlate with disease and health in a regional population of families with localized juvenile periodontitis. Infect Immun 1994;62:3058–65.
20. Ebersole JL. Systemic humoral immune responses in periodontal disease. Crit Rev Oral Biol Med 1990;1:283–331.
21. Laine ML, Appelmelk BJ and van Winkelhoff AJ. Prevalence and distribution of six capsular serotypes of *Porphyromonas gingivalis* in periodontitis patients. J Dent Res 1997;76:1840–4.
22. Ebersole JL, Hall EE, Steffen MJ. Antigenic diversity in the periodontopathogen, *Actinobacillus actinomycetemcomitans*. Immunol Invest 1996;25:203–14.
23. Genco RJ, Lee J-Y, Sojar HT, Bedi GS, Loos BG, Dyer DW. Antigenic heterogeneity of periodontal pathogens. In: Periodontal disease: pathogens and host immune responses. Tokyo: Quintessence Publishing Co.; 1991. p. 167–83.
24. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. Periodontol 2000 1994;5:78–111.
25. Beck JD. Methods of assessing risk for periodontitis and developing multifactorial models. J Periodontol 1994;65:468–78.
26. Falkow S. Host-parasite relationships. In: Ryan KJ, editor. Medical microbiology: an introduction to infectious diseases. Norwalk: Appleton & Lange; 1994. p. 141–68.
27. Drew WL. Epidemiology and prophylaxis of infectious diseases. In: Ryan KJ, editor. Medical microbiology: an introduction to infectious diseases. Norwalk: Appleton & Lange; 1994. p. 179–88.
28. Lilienfeld AM. Foundations of epidemiology. New York: Oxford University Press; 1976. p. 255–62.