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Aging Effects on Humoral Immune Responses in Chronic Periodontitis

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Abstract

Periodontal disease is a dominant global bacterial infection that increases with aging.

Aim—This report focuses on host adaptive immune responses in periodontitis. While experimental models and humans diagnosed with periodontitis demonstrate an antigenic specificity for particular oral bacteria, we have a limited understanding of how aging affects: (i) the adaptive immune responses to these bacteria that chronically colonize the oral cavity for decades prior to disease expression, and (ii) how the magnitude and specificity of the response interfaces with pathogens that emerge within the bacterial ecology during exacerbations of disease.

Materials and Methods—Serum antibody levels to a group of pathogenic and commensal oral bacteria was measured in a population of individuals from 21–74 years of age, stratified based on clinical status of the periodontium, smoking, and sex.

Results—Clinical parameters were not significantly different within health, gingivitis, or periodontitis groups related to age. Antibody to oral pathogens and commensals were similar in different age groups in each of the clinical categories, with no age correlation noted in the periodontitis patients.

Conclusions—The adaptive immune responses to oral bacteria that chronically colonize the oral cavity appear generally unaffected by age, but clearly are linked to the extent of disease.

Keywords

aging; periodontitis; antibody; oral bacteria

INTRODUCTION

A wealth of literature has been produced over the last 4 decades documenting immune responses, immune response genes, and genetic polymorphisms in susceptibility, shifts in

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host-microbial equilibrium, and protective versus destructive outcomes of host responses to the complex microbial ecology of the oral cavity. Since the two most prevalent oral diseases of mankind are bacterial infections, *i.e.* dental caries and periodontal disease, a better understanding of the role of the immune system in clinical disease activity, disease progression, stabilization and remission is warranted. Investigations of adaptive immunity have included both T cell-based cellular immune responses and the B cell-based humoral immune responses (Saraiva et al., 2014, Hwang et al., 2014, Ebersole et al., 2013, Di Benedetto et al., 2013, Garlet et al., 2010, Garlet, 2010). Evidence has shown activation of T and B cells in experimental models and in humans diagnosed with periodontitis, and demonstrate an antigenic specificity for particular bacteria suggested to hallmark the periodontopathic microbial biofilms, *e.g.* *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* (Ebersole et al., 2013, Holt and Ebersole, 2005, Rams et al., 2006, Wang et al., 2005, Pussinen et al., 2005). These immune responses have been shown to develop both systemically and within the local oral environment, *e.g.* gingival crevicular fluid, saliva (Ebersole, 2003, Salminen et al., 2014). Epidemiological studies and clinical observations have reported that the prevalence and severity of periodontitis increases with age (Eke et al., 2015, Baelum and Lopez, 2013, Eke et al., 2012). However, we have a rather limited understanding of how aging affects: (i) the adaptive immune system responses to the bacteria that chronically colonize the oral cavity prior to disease, and (ii) how the magnitude and specificity of the response interfaces with pathogens that emerge within the bacterial ecology during exacerbations of disease.

Age-related decreases in the innate and adaptive immune systems are defined as immunosenescence (O'Connor et al., 2014). Increases in the incidence of inflammation, autoimmunity, cancer and susceptibility to infections coincide with this decline in host immunity and the efficacy of vaccinations in aging individuals (Mabbott et al., 2015, Taverna et al., 2014). Thus, a large body of work suggests that immune capabilities are dysregulated in aging, including T cells, PMNs, and B cells (Sato et al., 2015, Muller and Pawelec, 2015, Castelo-Branco and Soveral, 2014, Shaw et al., 2013, Fulop et al., 2014). The adaptive immune system with its broad range of antigen receptors creating specificity for these immune responses requires highly regulated cellular interactions among cells carrying specific antigenic receptors, cellular activation, and molecular effector functions. Thus, aging appears to functionally impair dendritic cell uptake of antigens, phagocytosis of apoptotic cells, and migration and priming of both CD4⁺ and CD8⁺ T cells (Gupta, 2014). To avoid adverse responses such as autoimmunity and immunopathology check points are built into the system for controlling both the responses and the production of effector molecules and appear to be compromised with aging, resulting in inadequate immune control and unwanted responses. Deterioration of the immune system (*ie.* immunosenescence) in humans has generally been documented at the population level, although a clear increase in variation of response profiles is noted with aging. As such, reports use group averages of immune phenotypes, while this variation in immune system capacity and functions needs to be more clearly delineated at the individual level to discover critical regulators of altered immune states that reflect biologic and chronologic age (Shen-Orr and Furman, 2013).

This study examined specific antibody responses in serum of a population of patients defined as periodontally healthy, or expressing gingivitis or periodontitis at various levels. Including subjects from 21–74 with health and disease, we hypothesized that these systemic responses to chronic oral colonization/infection across the lifespan would be impacted by the aging process, independent of disease.

MATERIAL and METHODS

Patients and clinical measures

The demographics of the 447 patient population is depicted in Table 1. Clinical measures included sites bleeding on probing (BOP), mean probing pocket depth (PPD), frequency of sites with PPD ≥4mm and ≥5mm, mean clinical attachment loss (CAL), and frequency of sites with CAL ≥2mm (Preshaw et al., 2008, Novak et al., 2008a). Healthy subjects were defined by <10% of sites with BOP ≥1, <5% sites with PPD ≥4mm, and no sites with PPD ≥5mm. Gingivitis was defined by >10% sites with BOP ≥1, <10% sites with PPD ≥4mm, and no sites with PPD ≥5mm. Periodontitis was defined by >10% BOP ≥1, ≥10% of sites with PPD ≥4 mm, and ≥3% sites with CAL ≥2 mm. Periodontitis was categorized into mild (mean PPD <3 mm), moderate (mean PPD 3–4 mm), and severe (mean PPD >4 mm).

Samples

Specimens collected from the patients included subgingival plaque samples derived from a periodontally diseased site and a healthy site (Novak et al., 2008b, Kirakodu et al., 2008) in each of 132 subjects. These were collected, DNA prepared, and qPCR conducted for *Porphyromonas. gingivalis*, *Treponema denticola*, and *Tannerella forsythia*, as we have described previously and total bacteria in the samples were determined using a universal prokaryotic primer set (Kirakodu et al., 2008).

Venipuncture blood was collected (10 ml) and serum prepared, aliquoted, and stored frozen at –80C.

Bacterial antigens and serum antibody measures

Whole formalin-fixed bacteria were used as antigens to detect serum IgG antibody levels (Ebersole et al., 1987). The antigens included, *P. gingivalis* (ATCC33277), *Aggregatibacter actinomycetemcomitans* (JP2), *T. denticola* (ATCC35405), *T. forsythia* (ATCC49307), *Actinomyces naeslundii* (ATCC39340), *Capnocytophaga ochracea* (ATCC33596), *Prevotella loescheii* (ATCC15930), *Streptococcus sanguinis* (ATCC10556), and *Veillonella parvula* (ATCC10790) cultured as we have described previously (Hayman et al., 2011). The quantification of the antibody was based upon parallel standard IgG curves incorporated into each plate of the experimental samples (Ebersole et al., 2009).

Statistical analysis

Analyses of any differences among inflammatory mediators and IgG antibody levels, was conducted via a Kruskal-Wallis ANOVA with *post hoc* testing of paired groups using a Dunn's method (SigmaStat, Systat Software, Inc., Richmond, CA). Evaluation of the significance of correlation data was performed using the Spearman Correlation test. Results

with an alpha of <0.05 (after being adjusted for the multiple comparisons) were accepted as statistically significant.

RESULTS

While existing epidemiological data demonstrates clear increases in the prevalence of periodontitis with aging (Eke et al., 2016a, Eke et al., 2015, Eke et al., 2012), generally this is reflected by increases in the proportion of moderate periodontitis, with only slight changes in mild and severe periodontitis over the 30–80 year age range. However, we wanted to more clearly define the characteristics of clinical periodontal parameters in our cohort of healthy, gingivitis, and periodontitis over the 5 decade age range. Figure 1A–D provides a summary of these findings and demonstrate no significant difference in % BOP, mean probing pocket depth, or frequency of pockets with >4 mm or >5 mm pockets related to age stratification of the patients in each clinical category. As would be expected, the periodontitis group exhibited higher levels of these parameters versus the healthy and gingivitis subjects.

Serum antibody levels were evaluated related to the age of the patient. As shown in Fig. 2A–C, no differences were noted in antibody to *P. gingivalis*, *T. denticola*, or *T. forsythia* related to age categories, or antibody to a group of pathogens or commensal bacteria within the periodontitis group. As would be expected from the literature, serum antibody to individual bacteria, particularly the pathogens were significantly different in periodontitis patients compared to age-matched subsets with gingivitis or a healthy periodontium. Interestingly, the youngest group of gingivitis patients showed significantly elevated antibody to *P. gingivalis*, *T. denticola*, *A. actinomycetemcomitans*, and the sum of antibody to the pathogens compared to healthy individuals, which was not observed in the older gingivitis groups. This generally reflected the lowest level of antibody to these bacteria in healthy younger individuals, with no differences in antibody levels across age groups within the gingivitis population. Supplementary Fig. 1 presents the distribution of antibody across the age range in the periodontitis patients to three principal periodontal pathogens and shows no significant correlation of antibody with age to any of these oral pathogens associated with chronic adult periodontitis. Also noted in Fig. 2B, antibody to some of the bacteria commonly considered as nonpathogenic commensal bacteria were also different with disease or aging. Specifically, antibody to *C. ochracea* was significantly elevated in the periodontitis patients irrespective of age, while antibody to both *A. naeslundii* and *Pr. loescheii* were elevated in the younger periodontitis patients compared to other age groups.

The antibody levels were then related to clinical parameters within the patient populations. As shown in the heat map in Fig. 3, antibody to each of the pathogens demonstrated a negative correlation with the clinical parameters of disease specifically in the youngest age group of patients. In contrast, with aging and particularly in the oldest age group, the relationship reversed with elevated antibody being significantly positively correlated with the severity of periodontitis.

Fig. 4 presents the data on the level of colonization by each of the bacterial species, as well as total bacteria, in diseased and healthy sites from the patients categorized according to age.

The results demonstrated actually lower levels of total bacteria and *P. gingivalis* in diseased sites and lower *P. gingivalis* in healthy sites in the oldest age group.

The results in Table 2 indicate that while levels of probing pocket depth (PPD) and clinical attachment loss (CAL) are minimal in the healthy subjects, these are still measurable and the data indicate a positive correlation of these clinical measures with aging in periodontally healthy subjects. A similar positive correlation with PPD and CAL with aging was seen in the gingivitis patients. Importantly to the consideration of age effects on disease, there was a loss of age correlation with all of the clinical parameters when the periodontitis patient category is sequestered from the overall population. Table 2 also depicts the relationship of the serum antibody levels to the bacteria in disease sites stratified on patient age. The results showed significant positive correlations in antibody level with *P. gingivalis* and *T. denticola* bacterial levels, but only in the youngest group. This specific antibody response and bacterial burden correlation was lost in the older patients.

Since sex and smoking have been implicated as additional factors in the development and severity of periodontitis (Nagarajan et al., 2016, Eke et al., 2016a, Reynolds, 2014, Schulze and Busse, 2016, Timmerman and van der Weijden, 2006), we evaluated the impact of smoking and sex within age categories on the antibody levels. As shown in Table 1, there were more smokers in the periodontitis group and a consistent trend for the more severe disease group to have a higher proportion of smokers and lower proportion of females. Fig. 5A summarizes sex effects on antibody responses in periodontitis patients across the age categories. Frequently females showed elevated antibody to the pathogens with significantly greater levels to *A. actinomycetemcomitans*, *P. gingivalis*, and the average antibody response to the group of pathogens, but only in the oldest cohort. The results in Fig. 5B show that antibody levels in smokers was elevated compared to nonsmokers in multiple age categories, which paralleled the increased disease severity with smoking, but no trends that reflected an interaction of age and smoking in the antibody response levels was noted.

DISCUSSION

While substantial literature is available documenting levels of serum, gingival crevicular fluid, and salivary antibody to oral bacteria as they relate to periodontitis, evidence is sparse regarding how these antibody responses are regulated with aging (Ebersole et al., 2013). However, even early studies of these responses that implied an increase in antibody levels with age, really did not effectively distinguish the effect of aging directly on the immune response system versus response profiles that reflected increased extent and severity of periodontitis, *e.g.* oral infection, with aging (Mouton et al., 1981). This study evaluated the systemic antibody responses to an array of oral bacteria including both putative pathogens and microorganisms considered part of the normal commensal ecology. Importantly, for the first time, we provided data that attempted to evaluate the impact of aging on these adaptive immune responses isolated from the natural age-associated prevalence of periodontitis. The results demonstrated that aging had a minimal effect on overall antibody levels to the range of oral bacteria that were tested, when patients were stratified based upon condition of the periodontium. While the gingival tissue exists within a mucosal environment in the oral cavity, and clearly saliva and the mucosal immune system bathes these tissues, the general

paradigm in the literature is that the immune responses that impact the gingiva and subgingival sulcus are derived from the systemic immune system (Ebersole et al., 1993, Lamster and Novak, 1992, Reinhardt et al., 1989). Thus, our focus was on examination of this arm of the immune response as related to aging and periodontitis (Liljestrand et al., 2014). Nevertheless, there is some literature suggesting variations in aging effects on mucosal immune responses primarily related to the gastrointestinal and respiratory tracks and generally focusing on the impact of aging on vaccinations and viral infections (Sato et al., 2015, Fujihashi and Kiyono, 2009, McDonald et al., 2017, Fulton and Cuff, 2004). Thus, there may exist a role for this immune system in aging and periodontitis, albeit, negligible data is available documenting alterations in mucosal immune responses to oral bacteria involved in the periodontopathic microbiome as affected by aging processes.

However, some isolated observations from the data provided interesting insights into the dynamics of the host-bacterial interactions in disease. Within the periodontitis group, we observed no correlation in antibody levels to *P. gingivalis*, *T. denticola*, or *T. forsythia* with aging. Evaluation of these antibody levels within the age categories and stratifying the patients based on periodontitis severity demonstrated positive correlations of antibody to the oral pathogens in the younger group of individuals who exhibited more severe disease. Existing data support that in patients with more severe disease, generally the level of putative periodontopathogens in diseased sites are increased. As this elevated level of the bacteria also presents as an elevation in immune stimulation to the host, concomitantly increased antibody might be an expected outcome as was noted in younger individuals. However, this relationship reversed to a negative correlation in severe disease in older individuals, in which more clinical disease associated with lower antibody to the oral pathogens. One interpretation of this finding would be that while overall antibody levels were not affected by aging, the quality of the response at the individual level was not adequate to manage the chronic infection enabling more frequent/extended episodes of active disease.

Also noted, antibody to some typical commensal bacteria varied across the cohort with antibody to *C. ochracea* significantly elevated in the periodontitis patients irrespective of age, and antibody to both *A. naeslundii* and *Pr. loescheii* elevated in the younger periodontitis patients compared to other age groups. An interpretation of these findings could consider the current paradigm in periodontitis, in which, rather than limiting the focus of the microbial etiology of the disease on an “individual pathogen”, the trigger for disease initiation/progression is an evolving dysbiosis of the commensal microbial ecology (Kilian et al., 2016, Nibali et al., 2014, Hajishengallis and Lamont, 2012). Linked to this concept is that certain of the normal commensal bacteria are co-opted in this new environment to increase in numbers and express a portfolio of genes that would enrich for a more pathogenic biofilm leading to chronic inflammation and disease (Yost et al., 2015). These three typically commensal bacteria could reflect this dysbiotic increase, resulting in a greater antigen burden and accompanying antibody responses. The lower level of antibody in the older patients could reflect different microbial components of the dysbiosis, or may reflect features of these bacteria to which the aging host responds poorly, thus limiting the protective nature of the humoral response in managing the dysbiotic ecology.

Furthermore, we did observe that the total bacterial and pathogen burden in diseased sites were lower in the older population, which was unexpected based upon the epidemiologic data of more disease with aging. These findings are consistent with the reported “inflammaging” with aging, whereby a lower challenge to the system results in a chronically elevated and dysregulated inflammatory response (Franceschi and Campisi, 2014). Moreover, this reported loss of control of inflammation in aging that would contribute to the extent and severity of periodontitis was accompanied by a loss in the relationship between the levels of the microorganisms and the antibody levels in the older group. The combination of these alterations in the regulation of the host response to the altered oral microbiome in disease sites would be predicted to contribute to the underlying biological basis for the epidemiologic findings of increased disease in aging.

Overwhelming evidence is available identifying smoking as a crucial risk factor for periodontitis (Nagarajan et al., 2016, Eke et al., 2016b, Reynolds, 2014, Genco and Borgnakke, 2013, Hujoel, 2003), as is found in most chronic diseases (Johannsen et al., 2014). As aging would be expected to reflect an increased magnitude of smoking, often expressed as “pack years”, we previously reported that cotinine levels and pack years of smoking were specifically related to systemic inflammatory mediator and antibody levels in periodontitis (Ebersole et al., 2014, Hayman et al., 2011). However, as in most studies, we did not sequester the response profiles of smokers related to aging, independent of the increased prevalence of periodontitis. Smoking clearly impacts the prevalence and severity of periodontitis (Tomar and Asma, 2000, Grossi et al., 1997, Hujoel, 2003), as well as antibody responses (Nagarajan et al., 2017, Nagarajan et al., 2016, Hayman et al., 2011, Al-Ghamdi and Anil, 2007, Apatzidou et al., 2005, Gunsolley et al., 1997, Quinn et al., 1996), and this study did demonstrate that smokers in multiple age categories exhibited significantly elevated antibody to oral pathogens, compared to nonsmokers that paralleled the increased disease severity with smoking. However, we saw no effect of smoking on the levels of antibody responses to periodontopathogens being affected by aging. This relationship may reflect the long-term nature of the oral colonization/infection in these patients and, thus, a limited impact of the aging process on this measure of adaptive immunity.

The history of periodontitis risk and expression related to sex has been limited in gaining a clear understanding of its effects on this disease. Many studies emphasized balancing groups based upon sex, with some assumptions on sex differences in periodontitis prevalence and severity. A recent review by Grover et al. (Grover et al., 2016) emphasized disparities in existing data, albeit, males appeared more at risk than females although the underlying biologic and behavioral parameters that control this risk were unidentified. This tenet of male risk has been supported via multiple large epidemiologic studies (Eke et al., 2016b, Kongstad et al., 2017, Leite et al., 2017), which were similar in documenting an increased prevalence and severity of disease in males. We also found a higher frequency of males in the more severe disease groups, coupled with decreased antibody to periodontal pathogens in males. More specifically, females showed elevated antibody to the pathogens with significantly greater levels to *A. actinomycetemcomitans*, *P. gingivalis*, and the average antibody response to the group of pathogens, albeit this was most prominent in the oldest cohort. There is negligible data available regarding antibody responses to oral bacteria

affected by aging and sex. However, these findings were consistent with our previous report in aging nonhuman primates that demonstrated elevated systemic inflammatory mediators in aging males and elevated specific antibody in aging females who showed less periodontitis compared to the males (Ebersole et al., 2008). Furthermore, although not directly linked to aging, we observed that elevated antibody to various oral bacteria in pregnant women with periodontitis was reflective of a lower risk of adverse pregnancy outcomes (Ebersole et al., 2009). Thus, factors in males that may poorly regulate the level and characteristics of the adaptive immune response to this type of chronic infection need to be delineated.

A review of aging and periodontal tissues emphasized the clear observation of loss of teeth in older adults due to periodontitis and emphasized that this reflected a biologic process of aging changes related to altered osteoblasts and osteoclasts, dysregulated responses of periodontal tissue cells to the oral microbiota and resulting inflammatory changes, and more general biologic changes in aging that can alter bone and tissue homeostasis (Huttner et al., 2009). More recently, Lamster et al. (Lamster et al., 2016) described the importance of differentiating normal aging from disease with respect to the periodontium. Epidemiologic studies have also been conducted examining specific antibody responses to oral bacteria in periodontitis. These studies generally used large datasets derived from NHANES (Dye et al., 2005, Dye et al., 2009) 349 or the Atherosclerosis Risk in Communities (ARIC) Study (Beck et al., 2005) to examine antibody to various oral bacteria. Generally antibody to certain of the bacteria were elevated in older subjects, males, smokers, minorities, and type 2 diabetes and related to periodontitis clinical measures (Vlachojannis et al., 2010). However, while the patients are generally stratified based on age, these reports do not address the independent facet of aging on the antibody responses, and the modeling did not discriminate age from an increased prevalence/severity of periodontitis. It is clear that molecular inflammation changes with aging leading to the concept of “inflammaging”, with substantial recent emphasis on aging and innate immunity in the oral cavity (Ebersole et al., 2016a). These reports described targeted mechanisms of innate immunity that are altered with aging (Hajishengallis, 2010, Hajishengallis, 2014), including various critical transcription factors and the resulting genes regulated by these factors (Chung et al., 2011). Additionally, limited studies in animal models provide evidence for increased bone loss (Liang et al., 2010), altered dendritic cell functions in response to bacterial infections (Wu et al., 2016), and substantial effects on the biology of B cell functions in the oral cavity (Ebersole et al., 2016b) that would be considered to contribute to adaptive immune effects in the aging oral cavity. These data were accompanied by identification of an altered microbial challenge that likely also contributes to an adverse response profile (Feres et al., 2016, Wu et al., 2016). Only one recent report described antibody responses to oral bacteria in geriatric patients, albeit, all had periodontitis of various levels of severity (Shet et al., 2015). The results showed no differences in antibody responses to the “red complex” bacteria (Haffajee and Socransky, 2006) across periodontitis categories, while antibody to *F. nucleatum* increased with disease severity and levels to *A. actinomycetemcomitans* were most elevated in mild disease. This is becoming a more critical issue in the context of the expansion of the aging population, the accurate estimate of the prevalence of the disease across the lifespan extending to risk of specific systemic diseases, and finally clarifying the multidimensional nature of the etiology, disease expression, and treatment decisions that will impact the

epidemiology, societal and economic impact of the disease on populations (Papapanou and Susin, 2017).

In summary, the aging process clearly contributes to the incidence and severity of periodontitis (Papapanou and Susin, 2017, Lamster et al., 2016, Lamster, 2016, Hajishengallis, 2014) and has been related to altered physiology that leads to a dysregulation of normal development, maturation, replenishment, and response patterns of the immune system to the noxious challenge of the disease-associated oral microbiome (Ebersole et al., 2016a). However, our knowledge of aging effects on adaptive immune response profiles and functional impact on oral microbes that have chronically colonized the oral cavity of individuals over many decades remains somewhat murky. This report is one of the first to attempt to isolate the aging process from the disease process to assess the characteristics of antibody responses to pathogenic and commensal oral bacteria, and identified a pattern that suggested that the magnitude of the response was not impacted by aging, nor did age correlate with antibody responses to the oral pathogens in the periodontitis patients. However, the relationship of the antibody levels to the oral pathogens with the clinical disease parameters did vary with age, suggesting altered quality of the antibody may be a primary effect of aging related to increased disease expression and severity in the aged population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Clinical Relevance

Scientific rationale for the study

The effect of aging on humoral immune responses and their role in susceptibility or resistance to periodontitis is unclear. The general paradigm is that aging disrupts normal innate and adaptive immune response processes. However, previous reports have identified elevations in antibody to periodontal pathogens in older populations, but did not discriminate the effects of aging from the effects of the expression of periodontitis.

Principal findings

The data demonstrated that aging did not appear to impact the levels of antibody to a range of oral bacteria considered to be pathogens and commensals, independent of the substantial effects of periodontitis. However, the characteristics of the antibody related to the bacterial burden and the clinical measures were significantly affected with aging and disease severity.

Practical implications

As the field attempts to better delineate the biologic underpinnings of the variation age of onset, extent and severity of disease, and rapidity and continuity of disease exacerbation, understanding the parameters of humoral immunity to the established oral microbiome should contribute in modeling precision periodontal medicine in the future.

A

B

C

D **Figure 1.**

Clinical measures of periodontal disease (**A**) bleeding on probing (BOP); (**B**) mouth mean probing pocket depth (PPD); (**C**) Percentage (%) of sites with PPD ≥ 4 mm; and (**D**) Percentage (%) of sites with PPD ≥ 5 mm according to age stratification in years. The bars denote group means and the vertical brackets enclose 1 SD.

Figure 2.

Serum IgG antibody responses to (A) periodontal pathogens (*A. actinomycetemcomitans*-Aa, *P. gingivalis*-Pg, *T. denticola*-Td, *T. forsythia*-Tf); (B) oral commensal bacteria (*A. naeslundii*-An, *C. ochracea*-Co, *P. loeschii*-Pl, *S. sanguinis*-Ssa, *V. parvula*-Vp); and (C) sum of antibody levels to the group of pathogens or group of commensal bacteria. The bars denote group means and the vertical brackets enclose 1 SD. The asterisk (*) denotes significantly different than other disease categories for the same age category at least at $p < 0.05$. The hashtag (#) denotes significantly different from levels in healthy subjects in the same age category at least at $p < 0.05$.

Figure 3.

Heat map of correlation of serum antibody levels in periodontitis patients stratified by age group and categorized by severity of periodontitis. The red circles highlight areas of the heat maps depicting different patterns of antibody correlations with clinical features in the age groups and disease severity.

Figure 4.

Distribution of total bacterial and individual oral pathogens in subgingival plaque samples from diseased (A) and healthy sites (B) in periodontitis patients categorized into different age groups. The bars denote group means and the vertical brackets enclose 1 SD. The asterisk (*) denotes significantly different than other age categories at least at $p < 0.05$.

A

B

Figure 5.

(A) Serum antibody levels based on sex and stratified into age categories. (B) Serum antibody levels in smokers and nonsmokers stratified into age groups. The bars denote group means and the vertical brackets enclose 1 SD. The asterisk (*) denotes significantly different than other sex within an age category at least at $p < 0.05$.

Table 1

Demographics of the population.

Group	Age Mean (range)	Gender M:F	% Smokers	% Sites BOP Mean (range)	Mean PPD mm (range)	% Sites PPD 4 mm (range)	% Sites PPD 5 mm (range)
Health (n=61)	39.5 (21–65)	26:35	8.2	1.2 (0–2.9)	2.1 (1.7–2.7)	1.8 (0–3.2)	0
Gingivitis (n=87)	34.6 (21–61)	39:48	18.4	25.1 (8.9–71.4)	2.4 (1.6–2.5)	4.8 (1.7–8.9)	0
Periodontitis/Total	40.4 (21–74)	187:112	28.1	41.1 (5.4–100)	3.5 (2.2–5.9)	29.2 (1.1–86.8)	13.6 (0–67.3)
Mild (n=85)	39.1 (22–74)	40:45	18.8	21.1 (10.1–78.6)	2.8 (2.2–3.0)	15.6 (1.1–86.8)	3.1 (0–13.7)
Moderate (n=152)	41.0 (21–62)	94:58	27.0	43.0 (5.4–96.2)	3.5 (3.0–4.0)	29.2 (2.4–84.3)	12.0 (0–40.5)
Severe (n=62)	40.8 (23–66)	45:17	43.5	62.8 (12.9–100)	4.5 (4.0–5.9)	47.2 (3.2–81.3)	31.6 (2.3–67.3)

Table 2

Age related correlations with clinical measures of periodontal disease and correlations of antibody levels to level of bacteria in subgingival plaque from diseased sites in the periodontitis patients. Total denotes correlation with the absolute bacteria counts and % of Total denotes correlation with the level of the individual species as a proportion of the total bacteria in the sample. For comparison with the homologous system (eg. *P. gingivalis/Pg*), included are results from a heterologous relationship (eg. *P. gingivalis/Aa*). Values in **BOLD** are significant at least at $p < 0.05$.

Group	% BOP	Mean PPD	% sites PPD 4mm	% sites PPD 5mm	Mean CAL	% sites CAL >2mm
Health	-0.2254	0.3194 (0.012) *	0.0162	N/A	0.5820 (<0.0001)	0.5269 (<0.0001)
Gingivitis	-0.2978 (0.016)	0.2252	0.1263	N/A	0.3312 (0.006)	0.2449 (0.046)
Periodontitis	-0.0683	0.0423	-0.0789	-0.0100	0.0882	0.0372

Bacteria/Antibody	Age Group (years)		
	35	36-50	>50
<i>P. gingivalis/Pg</i>			
Total	0.4357	0.3084	0.0306
% of Total	0.4353	0.2101	0.1886
<i>P. gingivalis/Aa</i>	0.1530	0.1115	-0.0652
<i>T. denticola/Td</i>			
Total	0.5610	0.2106	-0.1722
% of Total	0.1342	0.2043	-0.1615
<i>T. denticola/Aa</i>	0.1913	0.1303	-0.0648
<i>T. forsythia/Tf</i>			
Total	-0.1384	-0.1110	-0.1119
% of Total	0.0295	0.1092	-0.0355
<i>T. forsythia/Aa</i>	-0.0795	-0.1292	-0.0534

* denotes p-values for significance of the correlations.