Published in final edited form as:

J Periodontol. 2009 July ; 80(7): 1154–1165. doi:10.1902/jop.2009.080199.

Periodontitis in Pregnancy: Clinical and Serum Antibody Observations From a Baboon Model of Ligature-Induced Disease

D. Cappelli^{*}, M.J. Steffen[†], S.C. Holt[‡], and J.L. Ebersole[†]

^{*}Dental School, The University of Texas Health Science Center at San Antonio, San Antonio, TX

[†]Center for Oral Health Research, College of Dentistry, University of Kentucky, Lexington, KY

[‡]The Forsyth Institute, Boston, MA

Abstract

Background—Chronic oral infections that elicit host responses leading to periodontal disease are linked with various sequelae of systemic diseases. This report provides seminal information on the clinical and adaptive immunologic characteristics of a baboon model of ligature-induced periodontitis during pregnancy.

Methods—Female *Papio anubis* were evaluated for periodontal health at baseline. Ligatures were tied around selected teeth to initiate oral inflammation and periodontitis. Then the animals were bred. At midpregnancy (~90 days), a clinical evaluation was performed, and additional ligatures were tied on teeth in the contralateral quadrants to maintain progressing periodontitis throughout pregnancy. A final clinical evaluation was done for all experimental teeth after delivery, and ligatures were removed. Serum was collected at all sampling intervals for the determination of antibody levels to a group of 20 oral bacteria. Unligated animals served as controls.

Results—At baseline, 16% of animals exhibited minimal plaque and gingival inflammation without periodontal disease. The remaining baboons demonstrated varying levels of inflammation/bleeding, and ~20% of the population had periodontal pocketing (>3 mm). Ligated animals expressed increased levels of inflammation and increased probing depths and clinical attachment loss (AL) and could be stratified into multiple subsets postligation based upon changes in clinical parameters at midpregnancy and at delivery. Baboons were categorized into disease susceptibility groups (periodontal disease susceptibility 1 through 4) that described the extent/severity of induced disease during pregnancy. Control animals showed minimal periodontal changes during gestation. Significant differences in serum antibody to multiple oral bacteria were found in animals presenting with periodontitis at baseline and during the 6 months of ligature-induced disease. A significant correlation to antibody to *P. gingivalis*, which was sustained throughout ligation and pregnancy, was observed with disease presentation.

Conclusions—The clinical presentation at baseline, reflecting the natural history of oral disease in these animals, suggests individual variation that is reflected in the characteristics of the adaptive

Correspondence: Dr. Jeffrey L. Ebersole, Center for Oral Health Research, HSRB422 Bosomworth Building, College of Dentistry, University of Kentucky, Lexington, KY 40536. Fax: 859/257-6566; jleber2@uky.edu..

The authors report no conflicts of interest related to this study.

immune responses to oral bacteria. The variability in the response to ligation with resulting periodontal disease provides a model to document prospectively the relationship between oral and systemic health outcomes.

Keywords

Antibody; bacteria; periodontitis; pregnancy; primate studies; serum

Periodontitis is a polymicrobial disease, in which a complex microbial ecology that matures in biofilms in the subgingival sulcus triggers a chronic immunoinflammatory lesion that destroys soft and hard tissues of the periodontium.^{1,2} The extent and severity of tissue destruction are affected by the magnitude and characteristics of the host response and may be modulated by environmental, systemic, or genetic factors.³⁻⁵ The importance of periodontal disease as a model of host–bacterial interactions and inflammatory disease lies in our ability to isolate and characterize bacterial and host factors from the oral cavity in a non-invasive manner and correlate these changes with pathologic changes occurring in host tissues. The immune responses to oral bacteria are complicated by the fact that these commensal opportunistic pathogens provoke a local⁶ and systemic immune response;^{7,8} levels of antibody to suspected periodontopathogens are significantly elevated in periodontitis patients,^{7,9} and decreases are seen with the successful clinical management of the disease.^{8,10,11}

Evidence from recent studies supported the likelihood that chronic oral infections can trigger systemic changes that contribute to various general health consequences,¹²⁻¹⁶ including adverse pregnancy outcomes.¹⁷⁻²⁶ The association between periodontal inflammation and pregnancy is well documented and occurs in ~30% to 100% of pregnant women, related to plaque bacteria and hormonal alterations.²⁷⁻²⁹ Recent studies^{18,19,21,24,30-32} also support an association between periodontal infections and gestational age and neonatal birth weight, particularly in pregnant women who exhibit severe or generalized periodontal disease.

The primate model has provided a bridge for understanding the interaction of the subgingival microbiota with the inflammatory/immune response to selected members of this microbiota to protect against disease progression or to exacerbate the inflammatory process, leading to the progression of disease.³³⁻⁴⁰ We and others have shown that characteristics of the inflammatory response and systemic humoral immune responses that accompany ligature-induced periodontitis in various non-human primate species^{35-39,41-43} parallel those observed in human periodontitis.^{6,8,44,45} The baboon has been identified as a useful non-human primate model for periodontal disease investigations.⁴⁶⁻⁴⁹

This report hypothesizes that ligation of teeth of pregnant baboons will induce varying levels of destruction of the periodontium that is reflected in the characteristics of host adaptive immune responses to oral microorganisms associated with progressing periodontitis. It is part of an experimental design to evaluate the potential contribution of periodontal disease to systemic changes that may predispose to preterm labor and birth. These initial studies will enable a subsequent evaluation of the relationship of oral infection and systemic host responses to birth outcomes in this non-human primate model of an oral–systemic disease linkage and provides an opportunity to examine potential causal relationships among chronic

periodontal infections, inflammation, and the subsequent risk for preterm birth/low birth weight pregnancy outcomes.

MATERIALS AND METHODS

Animal Husbandry and Experimental Design

The 237 female *Papio anubis* in this project (n = 156 experimental; n = 81 control) were part of a larger colony of baboons housed at the Southwest National Primate Research Center at the Southwest Foundation for Biomedical Research (SFBR), San Antonio, Texas, from 2003 to 2007. The animals were housed in corrals with ~200 females and offspring in each corral. Females (8 to 13 years of age) were selected based upon a history of successful pregnancies and were culled from the larger population in groups of 25 to 30 animals. The working groups were randomly assigned to experimental (i.e., ligated) or control cohorts for the study. Baboons were sedated using a regimen of ketamine, xylazine, and valium that was administered based upon the weight of the animal and the required length of sedation. Blood was drawn by femoral venipuncture for serum, clinical periodontal status was measured, and subgingival plaque samples were collected at baseline prior to any intervention.⁵⁰ Ligatures were tied, using 3-0 silk sutures, around the second premolar and the first and second molar teeth in the maxillary and mandibular quadrants on the right side of the animal.^{41,51} Then each group of 25 to 30 females was bred to a male. Pregnancy was determined via observation of cessation of menses and confirmed by obstetric ultrasound. At approximately midpregnancy (MP; 90 days), samples and evaluations were obtained as described above for the baseline collection. Ligatures were tied on the maxillary and mandibular teeth in the contralateral quadrants of the experimental animals to maintain a consistent periodontitis challenge to the animals. Generally, from 24 hours to ~2 weeks postdelivery, the final samples (delivery [D]) were collected and clinical evaluations were performed on sedated animals, all ligatures were removed, and the mother and infant were returned to the general population. Control animals were managed in an identical fashion; however, no ligatures were placed. All procedures and the experimental protocol were approved by the Animal Care and Utilization Committee (ACUC) at the SFBR.

Clinical Measurements

A periodontal examination, measuring supragingival plaque (plaque index [PI]),⁵² probing depth (PD), recession, and bleeding on probing (BOP), was performed on each study tooth at four sites per tooth (disto-buccal, buccal, mesio-buccal, and lingual). The methodology for the determination of recession and PD measurements, calculated attachment level value (cemento-enamel junction to the base of the pocket), and BOP was similar to that described in the literature.⁵² Missing teeth or teeth that could not be scored were noted. A single examiner collected all clinical measurements.

The composite index of periodontal disease (CIPD) was developed to provide a single index value that would incorporate measures of disease extent and severity (unpublished data). For the CIPD, we weighted the variables such that the measure of destructive disease (clinical attachment loss [AL]) and the extent of destruction (percentage of sites with clinical AL >2 mm) provided a weighted increase to the CIPD. The final formula for CIPD was: CIPD =

0.5 (mean BOP) + 0.1 (% sites with BOP >0) + (mean clinical AL) + 2 (% sites with clinical AL >2 mm).

The CIPD results demonstrated substantial heterogeneity in the clinical presentation of the baboons, not dissimilar from that reported in human populations. The CIPD was capable of stratifying the population into five groups based upon the interaction of inflammation and destructive disease. A CIPD <20 is reflective of a reasonably healthy periodontium for this age group of non-human primates. A CIPD of >20 to 50 describes the animals with gingivitis and represents a measure primarily related to the extent and severity of bleeding. A CIPD >50 is a measure of periodontitis, with >50 to 75 categorized as mild periodontitis, >75 to 100 categorized as moderate periodontitis, and >100 categorized as generalized periodontitis with extensive bleeding and loss of attachment contributing to an increased extent and severity of disease.

Serum Antibody Levels

Blood (~10 ml) was obtained for serum and stored at -70°C after separation into 0.5- to 0.75-ml aliquots. Immunoglobulin G (IgG) antibody levels to 20 bacteria were determined by quantitative enzyme-linked immunosorbent assay using formalin-killed whole bacterial antigens.⁵³⁻⁵⁵ The microorganisms included *Actinomyces naeslundii* (An) (American Type Culture Collection [ATCC]49340, *Aggregatibacter actinomycetemcomitans* (Aa; previously *Actinobacillus actinomycetemcomitans*) JP2, *Campylobacter rectus* (Cr) ATCC33238, *Capnocytophaga gingivalis* (Cg) ATCC33624, *Capnocytophaga ochracea* (Co) ATCC33596, *Capnocytophaga sputigena* (Cs) ATCC33624, *Eikenella corrodens* (Ec) ATCC23834, *Eubacterium nodatum* (En) ATCC33099, *Fusobacterium nucleatum* (Fn) ATCC49256, *Parvimonas micra* (Pm; previously *Peptostreptococcus micros* or *Micromonas micros*) ATCC33270, *Porphyromonas gingivalis* (Pg) FDC381, *Prevotella intermedia* (Pi) ATCC25611, *Prevotella loescheii* (Pl) ATCC15930, *Prevotella nigrescens* (Pn) ATCC33563, *Streptococcus gordonii* (Sg) ATCC49818, *Streptococcus mutans* (Sm) ATCC25175, *Streptococcus sanguis* (Ss) ATCC10556, *Treponema denticola* (Td) ATCC35405, *Tannerella forsythia* (Tf; previously *T. forsythensis*) ATCC49307, and *Veillonella parvula* (Vp) ATCC10790. A purified macaque IgG[§] was included on each plate to generate a gravimetric standard to enable comparison of antibody levels across the various bacteria. Biotinylated goat antisera to macaque IgG^{||} and streptavidin-alkaline phosphatase[¶] were used to determine the antibody concentration in the baboon sera. A preliminary set of studies showed that by using this development system, a similar dilution of baboon serum to cynomolgus monkey sera could be used to quantify the antibody, with absorbance values falling in the linear range of the standard curve. General estimates from these initial studies indicated ~75% to 80% reactivity with the baboon IgG. Because all baboon antibody levels were determined in an identical system and using the same standard, this differential did not significantly impact the comparisons across the animals.

[§]Antibodies, Davis, CA.

^{||}Antibodies

[¶]Sigma, St. Louis, MO.

Statistical Analyses

For the clinical parameters, an analysis of variance (ANOVA) was performed with the post hoc Holm-Sidak method for multiple comparisons for normally distributed data. Data that were not normally distributed were evaluated using a Kruskal-Wallis one-way ANOVA by ranks with a post hoc Dunn method for multiple comparisons.

Because of the extensive variability in antibody levels across the population, the antibody data were transformed using a $\log_2$ transformation. Antibody data were standardized using the antibody baseline mean and SD. As an example, the standardized baseline *Pg* antibody (z_{B_PG}) was used to analyze the baseline *P. gingivalis* antibody (B_PG). The standardization formula for baseline *Pg* antibody is: $z_{B_PG} = \log_2(B_PG) - \text{mean}(\log_2[B_PG]) / \text{SD}(\log_2[B_PG])$.

To analyze *Pg* antibody at the various times points of the study (i.e., MP and D), the following standardization formula was used: $z_{MP_PG} = \log_2(MP_PG) - \text{mean}(\log_2[B_PG]) / \text{SD}(\log_2[B_PG])$.

Using this z-statistic, a negative value denotes that the antibody level in the individuals in a particular subset at a time point was low with regard to the overall population level at baseline. Conversely, a positive z-statistic demonstrates that the group antibody level or change at a particular time point was increased with respect to the overall population at baseline. An ANOVA was used to determine antibody differences among the baseline disease categories with a post hoc Holm-Sidak assessment for individual group differences. The Spearman rank correlation was used to determine the relationship among the clinical measures, CIPD, and antibody levels and changes during the protocol.

RESULTS

Natural Variation in the Periodontium in Female Baboons

Initially, we evaluated the natural variation in the clinical presentation of the female baboons. Assessing the various clinical parameters in 237 female baboons (156 experimental and 81 control), we observed a broad variation in plaque, inflammation, and pocketing, which reinforced our previous observations in rhesus⁵⁶ and cynomolgus^{41,57} monkeys. Figure 1A provides a depiction of the cumulative baseline distribution of BOP, plaque, PD, and clinical AL for all animals presented as mouth means for the 12 study teeth (three teeth/quadrant). There was a broad heterogeneity in the general population of baboons that mimics a similar observation that is routinely seen in human populations and was independent of age in this group of animals. Additionally, individual females had previously experienced between one and five successful births. No relationship was identified between the number of previous pregnancies and the periodontal clinical parameters at baseline. Figure 1B presents distributions of the baseline clinical measurements across the population. The frequency distribution of animals is shown relative to mean clinical scores of BOP and clinical AL and the percentage of sites demonstrating BOP >0 or clinical AL >2 mm. It is clear that the majority of animals at baseline presented with minimal BOP and clinical AL. However, a small, but distinct, population of animals presented with increased baseline BOP and clinical AL.

Using the clinical parameters of BOP and AL, we stratified the animals into subsets consistent with periodontal “health” (bleeding <30% of sites and no AL >2 mm), “gingivitis” (bleeding >30% of sites and no AL >2 mm), and “periodontitis” (bleeding >30% of sites and at least one pocket with AL >2 mm). Based upon these criteria, this baseline stratification defined ~16% of the baboons as healthy, 64% as having gingivitis, and 20% with periodontitis. The results presented in Figure 2 show the mean clinical parameters in the stratified groups at baseline and demonstrate significant differences between the three groups for PI, BOP, PD, and AL. These differences are commensurate with the existence of three distinct populations at baseline: a population of periodontally healthy animals with low PI and BOP and minimal PD and AL; a population of animals with gingivitis with greater PI and BOP than the healthy group; and a group with naturally occurring periodontitis that had significantly greater BOP, PD, and clinical AL than the other two groups.

Clinical Response to Ligation in the Periodontium in Female Baboons

Figure 3 demonstrates that significant increases in BOP and clinical AL were observed within these three groups. Increases in the mean levels and frequency of affected sites were observed in the experimental animals following 3 months (MP) and 6 months of ligation (D). These changes were noted in the animals independent of the clinical presentation at baseline, although the healthy group generally showed less clinical change and greater variability. In contrast, the control baboons exhibited fewer changes in the clinical measurements over time, although a subset of the animals seemed to exhibit increased bleeding by MP, which reflected an oral response to the hormonal changes associated with pregnancy. These differences between the groups would have been predicted based upon our previous findings in other non-human primate models,^{41,57} with ligation eliciting significant changes in periodontal disease measures. However, there is considerable individual variation in disease expression and heterogeneity in the experimental population that may be overlooked if the data are evaluated only at the group level.

Based upon the individual changes in clinical parameters from baseline, through MP after ligation of two quadrants, and further changes by delivery after ligation of all four quadrants, we were able to divide the experimental group of animals into four distinct susceptibility groups (Fig. 4A). Periodontal disease susceptibility (PDS)-1 group (n = 49) demonstrated a low level of change through MP, not substantially different from control animals, with a significant increase in periodontal measurements by delivery. PDS-2 baboons (n = 42) showed minimal disease progression through MP; however, the ligation of additional quadrants (measured at D) demonstrated substantial clinical progression reflected by bleeding and tissue destruction. A third group of baboons (PDS-3; n = 39) showed significantly increased clinical changes through MP, maintaining that level of disease expression through D. Finally, the fourth subset of animals (PDS-4; n = 26) showed significantly increased clinical measurements through MP, with the continuing progression of disease severity through D. However, we were unable to differentiate the nuances of disease severity because of the limited range of periodontal clinical measurements, e.g., PI, BOP, PD, and AL. This limitation impaired our ability to effectively describe the heterogeneity of clinical presentation in the population, as well as to efficiently document

changes in conditions in the periodontal environment that occur during ligature-induced disease. To be better able to describe this phenomenon, we developed a CIPD that incorporates the severity and extent of inflammation and tissue destruction into a single clinical index (unpublished data). Figure 4B shows the results of PDS stratification as related to the CIPD measure of periodontitis. The CIPD provides an enhanced periodontal disease profile of these groups and uses a systematic approach to identify and choose females who responded quite differently to ligature placement in the level of periodontal changes, as well as the kinetics of these changes during ligation and pregnancy. These findings may be able to differentiate those baboons that are at increased risk for systemic complications.

Serum Antibody Levels in Pregnant Baboons

Previous investigations^{7,8,45,54} identified elevated serum antibody to periodontal pathogens in the human population, with some relationship to the severity/extent of disease, category of disease, and treatment outcomes. The results presented in Figure 5 demonstrate the presence of serum IgG antibody in the experimental and control female baboons to various oral microorganisms commonly associated with periodontal disease in humans. Although antibody to a wide range of oral bacteria was detected, generally, antibody to the early colonizers in control animals was higher or similar to levels in the periodontitis animals. Responses to *P. nigrescens* and *C. gingivalis* were significantly elevated in the animals with periodontitis (CIPD >50) at baseline (Fig. 5A). Comparison of the antibody levels to oral bacteria, considered to be late colonizers in periodontopathic biofilms, in the various clinical groupings of the baboons at baseline revealed an increased level of the total antibody (i.e., Σ) in the subset of animals with periodontal disease (Fig. 5B). This sum was affected by significantly increased serum antibody to *P. gingivalis*, *P. intermedia*, *T. forsythia*, and *E. nodatum* in the periodontitis group (CIPD >50).

Based upon the extent of variation in antibody levels across the population of animals and individual variation in antibody levels during the ligation phase of the study, we calculated an antibody z-score to enhance the ability to evaluate antibody differences and changes among the individual animals over time. This transformation was dependent upon population antibody levels at baseline (Table 1) and explained differences in the antibody within the subsets stratified by clinical presentation at baseline. The results demonstrated significant differences in antibody to select bacteria, particularly those associated with diseased sites in humans, e.g., late colonizers. Moreover, the positive z-statistics, especially for the late colonizer group, demonstrate a unique pattern of antibody responses in the groups with CIPD >50 compared to the other animals.

Table 2 describes the magnitude of antibody change in the animals during ligature-induced periodontitis and pregnancy. These depict the changes in antibody through MP (i.e., two quadrants ligated), comparing the groups of animals based on baseline clinical characteristics. Changes in antibody were noted across the baseline clinical subsets. No significant changes in serum antibody to any of the bacteria were observed in the control animals. Significant differences were noted between the subsets with regard to the antibody responses to multiple bacteria representing bridge organisms and late colonizers. The pattern

of antibody changes clearly demarcates the animals entering the study with some periodontal disease (CIPD >50) from the other animals. Although the results in Figure 5 indicate that the periodontitis subset of animals generally exhibited higher antibody to many of the oral bacteria at baseline, by MP, their change in the antibody responses to the oral bacteria were generally less than in the other subsets of animals (i.e., negative z-score).

The results in Table 3 provide a similar assessment of antibody changes through delivery, after ligation of teeth in all four quadrants. Again, significant differences in antibody levels were noted among the baseline CIPD groups, particularly with respect to antibody levels to the early colonizers and bridge organisms. As at MP, the profile of antibody changes was different for the subset presenting with periodontitis at baseline (CIPD >50) compared to the other animals. These data also generally reflected a diminished change in antibody levels in the baseline periodontitis subset through delivery compared to the changes in antibody in the remaining population of animals.

The data in Table 4 describe the correlation of antibody levels at baseline or changes in antibody levels from baseline to MP or from baseline to D in the experimental animals. Significant relationships were noted during the ligature-induced disease process. In particular, antibody to *P. gingivalis* was significantly correlated with the level of disease throughout the study. Additionally, other black-pigmented bacteria (e.g., *P. intermedia*, *P. loescheii*, and *P. nigrescens*) demonstrated similar relationships with the disease characteristics of the animals.

DISCUSSION

Periodontitis is a polymicrobial disease triggered by commensal opportunistic pathogens.^{2,5,62} This disease leads to the destruction of connective tissue and bone as a consequence of an oral infection eliciting chronic inflammation and the resulting collateral damage to the host tissues attempting to eliminate the noxious challenge in the subgingival milieu.² The extent and severity of disease are related to the quality and quantity of the host response, which seems to be modified by environmental, systemic, and genetic factors.^{4,5,62}

During the last two decades, retrospective epidemiological studies and prospective longitudinal, as well as interventional, studies supported that chronic oral infections of periodontal disease, with the accompanying chronic inflammation, are associated with various systemic diseases and their sequelae.^{15,16,63-65} Studies^{13,17-21,32} continue to support that the oral cavity functions as a nidus for a microbial infection that can contribute to a variety of general health sequelae. Additional data indicate that chronic periodontal disease can trigger/increase the acute-phase response^{33,66-68} and a systemic antibody response in periodontitis patients, revealing the specific induction of antibody to the oral infection with these microorganisms.^{8,69,70} We evaluated the use of the non-human primate to provide a model of the polymicrobial infection and host response portfolio that occur in human disease. This article provides seminal information about the periodontal conditions of breeding-age female baboons, changes in the clinical parameters of disease, and an assessment of adaptive immune responses related to these clinical parameters initiated by

ligation of the teeth during pregnancy, to enable the examination of oral disease and links to systemic disease sequelae.

The non-human primate, which manifests periodontal disease that is clinically, microbiologically, and immunologically similar to humans, can provide an extremely useful model to examine mechanistic studies of periodontitis.^{34,39} This use of the model is related to the ability to control the initiation and extent/severity of disease. It can be used to develop prospective studies with the potential to elucidate mechanistic pathways and provide causal linkages of this chronic local disease to general health effects. We initiated the use of a baboon model to provide prospective data to examine the linkage of oral disease and adverse pregnancy outcomes to help overcome some limitations of these studies in human populations^{18,30} and described the clinical characteristics of the cohort in this article.

This study documented variations in the natural occurrence of gingival inflammation and periodontal disease expression in breeding-age female baboons, similar to that noted in humans.⁷¹⁻⁷³ A subset of the cohort had minimal inflammation and no destructive disease, consistent with health, even in the absence of routine oral hygiene. The largest subset of the baboons demonstrated gingival inflammation, with bleeding and minimal periodontal destruction, consistent with a diagnosis of gingivitis. Finally, ~20% of the female baboons presented with extensive gingival inflammation and measurable pocketing and AL, indicative of periodontitis, although within this cohort the extent of disease could be considered generally mild. As expected, ligation of teeth elicited significant changes in plaque, bleeding, PD, and AL in the treated baboons. An interesting outcome of these changes was that the severity of clinical changes at MP and D within the experimental group seemed to be generally unrelated to the initial clinical presentation of the individual baboons. This observation was surprising. Extrapolating from human studies,^{74,75} the literature suggests that subsets of patients who develop more severe disease are often less responsive to standard therapy. We would have predicted some differences in disease progression related to inherent risk (i.e., clinical presentation at baseline).

The changes in inflammation and destruction reflected by the clinical measures enabled us to discriminate three groups: baboons who seemed rather resistant to clinical inflammation and destructive disease; animals who clearly responded to the ligation with progressing periodontitis (however, their response system seemed to adequately control the challenge and limit the amount of destruction); and baboons that could be considered a hyperresponse phenotype, in whom more extensive disease continued throughout the pregnancy at all ligated teeth. We used these measures to stratify the baboons into four PDS groups, representing variations in the risk for local disease to contribute to systemic sequelae.

Changes in disease in response to the ligation exhibited significant individual variation and were reflected by a substantial individual variation in host responses. The use of a z-score transformation⁷⁶ of the antibody levels enabled a profiling of antibody changes among the animals. The findings demonstrated that animals entering the study with periodontitis exhibited elevated antibody to six of 20 bacterial species, as well as to the sum of antibody levels to the entire battery. Periodontitis in the baboons elicited by ligature placement was accompanied by changes in the subgingival microbial ecology (unpublished data), with

bacterial species similar to those in human disease.^{58,77} Extensive studies^{8,45,53,54,56,57} in humans and non-human primates demonstrated a relationship between the level of serum antibody to many of these oral species and the extent, severity, and progression of periodontitis. The results of this study demonstrated significant systemic antibody responses to a battery of oral bacteria in female baboons that were related to the oral clinical presentation of the animals at entry into the study. The relationship of the profile of antibodies in the primates was similar to that observed in human subjects with varying levels of oral health or disease. In particular, elevations in serum antibody to *P. gingivalis* and a select group of other bacteria in the animals with periodontitis paralleled levels identified in chronic periodontitis.^{44,78} The changes in the responses of the baboons were noted through MP (two quadrants ligated) and D (four quadrants ligated). The results demonstrated clear differences in the pattern of serum antibody responses between animals stratified according to their baseline clinical presentation. In particular, the subset of animals with periodontitis at baseline was quite distinctive from the other animals, with antibody patterns unique to this group at MP and D. These patterns were most striking when comparing antibody levels to the group of oral bacteria historically associated with periodontitis in humans, often described as late colonizers contributing to the pathogenicity of the biofilms.⁵⁸ Additionally, although antibody levels to a number of these species increased significantly in the ligated animals, our findings suggested that those animals entering the study with disease demonstrated the fewest change in response to ligation. Our data found a subset of the animals with naturally occurring periodontitis that seemed to respond less well to the ligation process. This finding may be indicative of animals that are inherently more susceptible to dysregulation of inflammatory responses with an inferior ability to control the ramifications of the infection and chronic inflammation.

Of the 20 microorganisms examined, only the serum antibody level to *P. gingivalis* was significantly correlated with CIPD, indicating greater antibody levels with increasing periodontal disease. The relationship of baseline CIPD with antibody to *P. gingivalis* was also noted at MP and D. We also found significant correlations of CIPD with antibody changes to a number of bacteria normally associated with periodontitis. We observed a negative correlation, suggesting that a more severe disease presentation at baseline was related to less change in antibody during the subsequent 6 months of periodontitis. This finding also supports that those animals with naturally occurring periodontitis may be less able to mount an effective adaptive immune response, increasing the likelihood of more severe progressing periodontitis. Additional analyses will enable us to relate the changes in inflammatory and adaptive responses in this periodontal disease model as they relate to variations in clinical disease parameters during ligation.

CONCLUSIONS

This study provides an initial evaluation of the clinical and systemic immune response characteristics of female baboons to ligature-induced periodontitis during pregnancy. The results provide a number of observations that will enable us to examine, in greater detail, the relationship among acquired immunity to oral bacteria, local disease parameters, and the systemic sequelae of adverse pregnancy outcomes. We propose that our model will elucidate

individual differences and allow a better definition of biologic risk factors for oral-systemic disease linkages.

Acknowledgments

This work was supported by United States Public Health Service (USPHS) grant DE013598 from the National Institute for Dental and Craniofacial Research, Bethesda, Maryland. The authors thank Drs. Lianrui Chu, Scott Eddy, and Robert Ayala, Department of Community Dentistry, University of Texas Health Science Center at San Antonio, and Lakshmya Kesavalu and Malini Bharadwaj, University of Kentucky, for technical support in developing and managing these data. We acknowledge the crucial contribution of Drs. Kathleen Brasky and Karen Rice and the scientific and technical staff at the Southwest Foundation for Biomedical Research and the contribution from USPHS grant 13986 in support of the Southwest National Primate Research Center at the foundation. We also thank Dr. M.J. Novak, Center for Oral Health Research, University of Kentucky, for the critical review of the manuscript.

REFERENCES

1. Salvi GE, Lang NP. Host response modulation in the management of periodontal diseases. *J Clin Periodontol.* 2005; 32(Suppl. 6):108–129. [PubMed: 16128833]
2. Tatakis DN, Kumar PS. Etiology and pathogenesis of periodontal diseases. *Dent Clin North Am.* 2005; 49:491–516. [PubMed: 15978238]
3. Kornman KS, di Giovine FS. Genetic variations in cytokine expression: A risk factor for severity of adult periodontitis. *Ann Periodontol.* 1998; 3:327–338. [PubMed: 9722717]
4. Skaleric U, Kovac-Kavcic M. Some risk factors for the progression of periodontal disease. *J Int Acad Periodontol.* 2000; 2:19–23. [PubMed: 12666982]
5. Preshaw PM, Seymour RA, Heasman PA. Current concepts in periodontal pathogenesis. *Dent Update.* 2004; 31:570–572. 574–578. [PubMed: 15656071]
6. Ebersole JL. Humoral immune responses in gingival crevice fluid: Local and systemic implications. *Periodontol 2000.* 2003; 31:135–166. [PubMed: 12657000]
7. Ebersole JL, Taubman MA. The protective nature of host responses in periodontal diseases. *Periodontol 2000.* 1994; 5:112–141. [PubMed: 9673165]
8. Ebersole JL, Cappelli D, Holt SC. Periodontal diseases: To protect or not to protect is the question? *Acta Odontol Scand.* 2001; 59:161–166. [PubMed: 11501885]
9. Ebersole, JL. Immune responses in periodontal diseases. In: Wilson, TG., Jr.; Kornman, KS., editors. *Fundamentals of Periodontics.* Quintessence Publishing; Chicago: 2003. p. 111–143.
10. Rams TE, Listgarten MA, Slots J. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* subgingival presence, species-specific serum immunoglobulin G antibody levels, and periodontitis disease recurrence. *J Periodontol Res.* 2006; 41:228–234. [PubMed: 16677293]
11. Pocolos DK, Lerche-Sehm J, Abron A, Fine JB, Papapanou PN. Infection patterns in chronic and aggressive periodontitis. *J Clin Periodontol.* 2005; 32:1055–1061. [PubMed: 16174268]
12. Novak KF, Taylor GW, Dawson DR, Ferguson JE 2nd, Novak MJ. Periodontitis and gestational diabetes mellitus: Exploring the link in NHANES III. *J Public Health Dent.* 2006; 66:163–168. [PubMed: 16913241]
13. Mealey BL, Oates TW. Diabetes mellitus and periodontal diseases. *J Periodontol.* 2006; 77:1289–1303. [PubMed: 16881798]
14. Spahr A, Klein E, Khuseynova N, et al. Periodontal infections and coronary heart disease: Role of periodontal bacteria and importance of total pathogen burden in the Coronary Event and Periodontal Disease (CORODONT) study. *Arch Intern Med.* 2006; 166:554–559. [PubMed: 16534043]
15. Mattila KJ, Pussinen PJ, Paju S. Dental infections and cardiovascular diseases: A review. *J Periodontol.* 2005; 76:2085–2088. [PubMed: 16277580]
16. Beck JD, Offenbacher S. Systemic effects of periodontitis: Epidemiology of periodontal disease and cardiovascular disease. *J Periodontol.* 2005; 76:2089–2100. [PubMed: 16277581]

17. Bobetsis YA, Barros SP, Offenbacher S. Exploring the relationship between periodontal disease and pregnancy complications. *J Am Dent Assoc.* 2006; 137(Suppl.):7S–13S. [PubMed: 17012730]
18. Paquette DW. Periodontal disease and the risk for adverse pregnancy outcomes. *Grand Rounds in Oral-Systemic Medicine.* 2006; 1:14–26.
19. Vettore MV, Leal M, Leao AT, da Silva AM, Lamarca GA, Sheiham A. The relationship between periodontitis and preterm low birthweight. *J Dent Res.* 2008; 87:73–78. [PubMed: 18096898]
20. Pitiphat W, Joshipura KJ, Gillman MW, Williams PL, Douglass CW, Rich-Edwards JW. Maternal periodontitis and adverse pregnancy outcomes. *Community Dent Oral Epidemiol.* 2008; 36:3–11. [PubMed: 18205634]
21. Zadeh-Modarres S, Amooian B, Bayat-Movahed S, Mohamadi M. Periodontal health in mothers of preterm and term infants. *Taiwan J Obstet Gynecol.* 2007; 46:157–161. [PubMed: 17638624]
22. Xiong X, Buekens P, Vastardis S, Yu SM. Periodontal disease and pregnancy outcomes: State-of-the-science. *Obstet Gynecol Surv.* 2007; 62:605–615. [PubMed: 17705886]
23. Vergnes JN, Sixou M. Preterm low birth weight and maternal periodontal status: A meta-analysis. *Am J Obstet Gynecol.* 2007; 196:135.e1–7. [PubMed: 17306654]
24. Toygar HU, Seydaoglu G, Kurklu S, Guzeldemir E, Arpak N. Periodontal health and adverse pregnancy outcome in 3,576 Turkish women. *J Periodontol.* 2007; 78:2081–2094. [PubMed: 17970674]
25. Sanchez AR, Bagniewski S, Weaver AL, Vallejos N. Correlations between maternal periodontal conditions and preterm low birth weight infants. *J Int Acad Periodontol.* 2007; 9:34–41. [PubMed: 17506382]
26. Lopez R. Periodontal treatment in pregnant women improves periodontal disease but does not alter rates of preterm birth. *Evid Based Dent.* 2007; 8:38. [PubMed: 17589481]
27. Barak S, Oettinger-Barak O, Oettinger M, Machtei EE, Peled M, Ohel G. Common oral manifestations during pregnancy: A review. *Obstet Gynecol Surv.* 2003; 58:624–628. [PubMed: 12972838]
28. Davenport J. Your patient is pregnant ... now what? *Hawaii Dent J.* 2004; 35:8–9. [PubMed: 15032050]
29. Laine MA. Effect of pregnancy on periodontal and dental health. *Acta Odontol Scand.* 2002; 60:257–264. [PubMed: 12418714]
30. Yeo BK, Lim LP, Paquette DW, Williams RC. Periodontal disease - The emergence of a risk for systemic conditions: Pre-term low birth weight. *Ann Acad Med Singapore.* 2005; 34:111–116. [PubMed: 15726229]
31. Michalowicz BS, DiAngelis AJ, Novak MJ, et al. Examining the safety of dental treatment in pregnant women. *J Am Dent Assoc.* 2008; 139:685–695. [PubMed: 18519992]
32. Sharma R, Maimanuku LR, Morse Z, Pack AR. Preterm low birth weights associated with periodontal disease in the Fiji Islands. *Int Dent J.* 2007; 57:257–260. [PubMed: 17849684]
33. Ebersole JL, Cappelli D. Acute-phase reactants in infections and inflammatory diseases. *Periodontol 2000.* 2000; 23:19–49. [PubMed: 11276764]
34. Madden TE, Caton JG. Animal models for periodontal disease. *Methods Enzymol.* 1994; 235:106–119. [PubMed: 8057890]
35. Moncla BJ, Braham PH, Persson GR, Page RC, Weinberg A. Direct detection of *Porphyromonas gingivalis* in *Macaca fascicularis* dental plaque samples using an oligonucleotide probe. *J Periodontol.* 1994; 65:398–403. [PubMed: 8046554]
36. Persson GR, Engel D, Whitney C, et al. Immunization against *Porphyromonas gingivalis* inhibits progression of experimental periodontitis in nonhuman primates. *Infect Immun.* 1994; 62:1026–1031. [PubMed: 8112836]
37. Persson GR, Engel LD, Moncla BJ, Page RC. *Macaca nemestrina*: A non-human primate model for studies of periodontal disease. *J Periodontal Res.* 1993; 28:294–300. [PubMed: 8393106]
38. Roberts FA, Houston LS, Lukehart SA, Mancil LA, Persson GR, Page RC. Periodontitis vaccine decreases local prostaglandin E₂ levels in a primate model. *Infect Immun.* 2004; 72:1166–1168. [PubMed: 14742568]

39. Schou S, Holmstrup P, Kornman KS. Non-human primates used in studies of periodontal disease pathogenesis: A review of the literature. *J Periodontol.* 1993; 64:497–508. [PubMed: 8336250]
40. Wirthlin MR. Natural and experimental periodontal diseases in non-human primates. *J West Soc Periodontol Periodontol Abstr.* 1991; 39:89–108. [PubMed: 1813656]
41. Holt SC, Ebersole J, Felton J, Brunsvold M, Kornman KS. Implantation of *Bacteroides gingivalis* in nonhuman primates initiates progression of periodontitis. *Science.* 1988; 239:55–57. [PubMed: 3336774]
42. Ebersole JL, Cappelli D, Mott G, Kesavalu L, Holt SC, Singer RE. Systemic manifestations of periodontitis in the non-human primate. *J Periodontal Res.* 1999; 34:358–362. [PubMed: 10685361]
43. Ebersole JL, Cappelli D, Holt SC, Singer RE, Filloon T. Gingival crevicular fluid inflammatory mediators and bacteriology of gingivitis in nonhuman primates related to susceptibility to periodontitis. *Oral Microbiol Immunol.* 2000; 15:19–26. [PubMed: 11155160]
44. Kinane DF, Podmore M, Murray MC, Hodge PJ, Ebersole J. Etiopathogenesis of periodontitis in children and adolescents. *Periodontol 2000.* 2001; 26:54–91. [PubMed: 11452906]
45. Ebersole JL, Cappelli D, Steffen MJ, Willmann DE, O'Dell DS. Host response assessment in recurring periodontitis. *J Clin Periodontol.* 1996; 23:258–262. [PubMed: 8707986]
46. Engel GA, Jones-Engel L, Schillaci MA, et al. Human exposure to herpesvirus B-seropositive macaques, Bali, Indonesia. *Emerg Infect Dis.* 2002; 8:789–795. [PubMed: 12141963]
47. Aufdemorte TB, Fox WC, Miller D, Buffum K, Holt GR, Carey KD. A non-human primate model for the study of osteoporosis and oral bone loss. *Bone.* 1993; 14:581–586. [PubMed: 8363909]
48. Avery BE, Simpson DM. The baboon as a model system for the study of periodontal disease: Clinical and light microscopic observations. *J Periodontol.* 1973; 44:675–686. [PubMed: 4127025]
49. Miller DR, Aufdemorte TB, Fox WC, Waldrop TC, Mealey BL, Brunsvold MA. Periodontitis in the baboon: A potential model for human disease. *J Periodontal Res.* 1995; 30:404–409. [PubMed: 8544104]
50. Moritz AJ, Cappelli D, Lantz MS, Holt SC, Ebersole JL. Immunization with *Porphyromonas gingivalis* cysteine protease: Effects on experimental gingivitis and ligature-induced periodontitis in *Macaca fascicularis*. *J Periodontol.* 1998; 69:686–697. [PubMed: 9660338]
51. Kornman KS, Holt SC, Robertson PB. The microbiology of ligature-induced periodontitis in the cynomolgus monkey. *J Periodontal Res.* 1981; 16:363–371. [PubMed: 6459435]
52. Cappelli D, Holt SC, Singer RE, Pickrum HM, Ebersole JL. Effects of 0.12% chlorhexidine gluconate on experimental gingivitis in non-human primates: Clinical and microbiological alterations. *Oral Dis.* 2000; 6:124–131. [PubMed: 10702791]
53. Ebersole JL, Kornman KS. Systemic antibody responses to oral microorganisms in the cynomolgus monkey: Development of methodology and longitudinal responses during ligature-induced disease. *Res Immunol.* 1991; 142:829–839. [PubMed: 1665573]
54. Ebersole JL, Cappelli D, Steffen MJ. Characteristics and utilization of antibody measurements in clinical studies of periodontal disease. *J Periodontol.* 1992; 63:1110–1116. [PubMed: 1336049]
55. Ebersole JL, Taubman MA, Smith DJ, Frey DE, Haffajee AD, Socransky SS. Human serum antibody responses to oral microorganisms. IV. Correlation with homologous infection. *Oral Microbiol Immunol.* 1987; 2:53–59. [PubMed: 10870468]
56. Ebersole JL, Steffen MJ, Reynolds MA, et al. Differential gender effects of a reduced calorie diet on systemic inflammatory and immune parameters in nonhuman primates. *J Periodontal Res.* 2008; 43:500–507. [PubMed: 18565132]
57. Ebersole JL, Brunsvold M, Steffensen B, Wood R, Holt SC. Effects of immunization with *Porphyromonas gingivalis* and *Prevotella intermedia* on progression of ligature-induced periodontitis in the nonhuman primate *Macaca fascicularis*. *Infect Immun.* 1991; 59:3351–3359. [PubMed: 1894349]
58. Socransky SS, Haffajee AD. Periodontal microbial ecology. *Periodontol 2000.* 2005; 38:135–187. [PubMed: 15853940]
59. Kolenbrander PE, Eglund PG, Diaz PI, Palmer RJ Jr. Genome-genome interactions: Bacterial communities in initial dental plaque. *Trends Microbiol.* 2005; 13:11–15. [PubMed: 15639626]

60. Rickard AH, Gilbert P, High NJ, Kolenbrander PE, Handley PS. Bacterial coaggregation: An integral process in the development of multi-species biofilms. *Trends Microbiol.* 2003; 11:94–100. [PubMed: 12598132]
61. Palmer RJ Jr, Wu R, Gordon S, et al. Retrieval of biofilms from the oral cavity. *Methods Enzymol.* 2001; 337:393–403. [PubMed: 11398445]
62. Ritchie CS, Kinane DF. Nutrition, inflammation, and periodontal disease. *Nutrition.* 2003; 19:475–476. [PubMed: 12714106]
63. Li X, Kolltveit KM, Tronstad L, Olsen I. Systemic diseases caused by oral infection. *Clin Microbiol Rev.* 2000; 13:547–558. [PubMed: 11023956]
64. Page RC. The microbiological case for adjunctive therapy for periodontitis. *J Int Acad Periodontol.* 2004; 6:143–149. [PubMed: 15536782]
65. Mombelli A. Periodontitis as an infectious disease: Specific features and their implications. *Oral Dis.* 2003; 9(Suppl. 1):6–10. [PubMed: 12974524]
66. Adonogianaki E, Mooney J, Kinane DF. The ability of gingival crevicular fluid acute phase proteins to distinguish healthy, gingivitis and periodontitis sites. *J Clin Periodontol.* 1992; 19:98–102. [PubMed: 1376333]
67. Ebersole JL, Cappelli D, Mathys EC, et al. Periodontitis in humans and non-human primates: Oralsystemic linkage inducing acute phase proteins. *Ann Periodontol.* 2002; 7:102–111. [PubMed: 16013223]
68. Ebersole JL, Machen RL, Steffen MJ, Willmann DE. Systemic acute-phase reactants, C-reactive protein and haptoglobin, in adult periodontitis. *Clin Exp Immunol.* 1997; 107:347–352. [PubMed: 9030874]
69. Kinane DF, Mooney J, Ebersole JL. Humoral immune response to *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in periodontal disease. *Periodontol* 2000. 1999; 20:289–340. [PubMed: 10522229]
70. Schenkein HA, Gunsolley JC, Best AM, et al. Antiphosphorylcholine antibody levels are elevated in humans with periodontal diseases. *Infect Immun.* 1999; 67:4814–4818. [PubMed: 10456935]
71. Tanner AC, Kent R Jr, Van Dyke T, Sonis ST, Murray LA. Clinical and other risk indicators for early periodontitis in adults. *J Periodontol.* 2005; 76:573–581. [PubMed: 15857098]
72. Heitz-Mayfield LJ. Disease progression: Identification of high-risk groups and individuals for periodontitis. *J Clin Periodontol.* 2005; 32(Suppl. 6):196–209. [PubMed: 16128838]
73. Borrell LN, Papapanou PN. Analytical epidemiology of periodontitis. *J Clin Periodontol.* 2005; 32(Suppl. 6):132–158. [PubMed: 16128835]
74. Haffajee AD, Uzel NG, Arguello EI, Torresyap G, Guerrero DM, Socransky SS. Clinical and microbiological changes associated with the use of combined antimicrobial therapies to treat “refractory” periodontitis. *J Clin Periodontol.* 2004; 31:869–877. [PubMed: 15367191]
75. Handal T, Olsen I, Walker CB, Caugant DA. Detection and characterization of beta-lactamase genes in subgingival bacteria from patients with refractory periodontitis. *FEMS Microbiol Lett.* 2005; 242:319–324. [PubMed: 15621454]
76. Janda, K. [Accessed December 2007] Statistical inference: Normal distribution. Available at: <http://jandaorg/c10/Lectures/topic05/L15-Normal.htm>
77. Teles RP, Haffajee AD, Socransky SS. Microbiological goals of periodontal therapy. *Periodontol* 2000. 2006; 42:180–218. [PubMed: 16930311]
78. Papapanou PN, Neiderud AM, Disick E, Lalla E, Miller GC, Dahlen G. Longitudinal stability of serum immunoglobulin G responses to periodontal bacteria. *J Clin Periodontol.* 2004; 31:985–990. [PubMed: 15491314]

Figure 1.

A) Depiction of the variation in plaque, bleeding, PD, and AL across a population of 237 female baboons at baseline, which was prior to pregnancy and ligature-induced periodontitis. PD and AL are presented as mouth means. B) Histograms demonstrating mean BOP and clinical AL and frequency of sites for BOP >0 and clinical AL >2 mm. The bars denote the percentage of animals at each value interval.

Figure 2.

Characteristics (mean $\pm$ SD) of PI, BOP, PD, and AL in baboons stratified at baseline into generally periodontally healthy (H), gingivitis (G) and periodontitis (P) subsets. **A)** mouth means; **B)** the percentage of affected sites. *H versus other groups ($P < 0.01$); †P versus other groups ($P < 0.01$).

Figure 3.

Changes in BOP and clinical AL level (mean $\pm$ SD) from baseline (B) through MP (two quadrants ligated) and D (four quadrants ligated) in animals stratified based on baseline clinical measurements. Control (Cont) similar values from non-ligated animals. H = healthy; G = gingivitis; P = periodontitis. ‡P < 0.05 (B/MP/D within a category); §P < 0.05 (baseline within controls); ||P < 0.05 (MP versus G and P); ¶P < 0.05 (D across categories).

Figure 4.

A) Characteristics of PDS groups across the baboon population. Distribution of mean ($\pm$ SD) BOP and AL (left) and percentage of affected sites (right) at baseline (B) and during tooth ligation (MP and D). **B)** PDS grouping based on the CIPD.

Figure 5.

Serum antibody levels (mean $\pm$ SEM) to oral bacteria at baseline in baboons stratified based on baseline clinical presentation. The Σ denotes a comparison of the summation of antibody to all of the bacteria tested. The bacteria are loosely grouped into early colonizers and bridge organisms (**A**) and late colonizers (**B**) based on the concepts of Socransky et al.⁵⁸ Kolenbrander et al.,⁵⁹ Rickard et al.,⁶⁰ and Palmer et al.⁶¹ The CIPD grouping denotes the categories determined at baseline for each animal. #Groups with antibody levels significantly different at least at $P < 0.05$ using a z-score transformation of the antibody levels for statistical evaluation.

Table 1

Z-Scores for Serum IgG Antibody Patterns to Oral Bacteria in Baboons at Baseline Sampling

	CIPD Category			
Antigen	<20	20 to <50	>50	P Value
Early colonizers				
<i>Sg</i>	0.286	0.306	−0.071	<0.02
<i>Sm</i>	0.175	−0.016	0.379	<0.05
<i>Ss</i>	−0.057	0.323	0.210	
Bridge organisms				
<i>An</i>	−0.099	0.367	0.095	
<i>Cg</i>	0.185	0.308	0.103	
<i>Co</i>	−0.108	0.113	0.023	
<i>Ec</i>	−0.276	0.319	−0.023	
<i>Fn</i>	0.299	0.348	0.064	
<i>Pl</i>	0.190	0.344	0.029	
<i>Pm</i>	−0.128	0.319	0.255	<0.05
<i>Vp</i>	0.192	0.031	0.079	
Late colonizers				
<i>Aa</i>	0.078	0.292	0.258	
<i>Cr</i>	0.253	−0.011	0.174	
<i>Pg</i>	−0.228	0.058	0.566	<0.01
<i>Pi</i>	−0.098	−0.082	0.456	<0.03
<i>Td</i>	−0.071	0.117	0.065	
<i>Tf</i>	−0.360	−0.105	−0.148	
<i>En</i>	−0.567	−0.453	0.409	<0.05
<i>Cs</i>	−0.031	0.302	−0.255	<0.03
<i>Pn</i>	0.067	−0.073	0.402	

Table 2

Z-Scores for Change in Serum IgG Antibody Patterns to Oral Bacteria in Baboons From Baseline to MP

	CIPD Category			
Antigen	<20	20 to <50	>50	P Value
Early colonizers				
<i>Sg</i>	0.255	0.111	−0.464	<0.01
<i>Sm</i>	0.081	0.438	−0.669	
<i>Ss</i>	−0.467	0.232	−0.186	
Bridge organisms				
<i>An</i>	0.221	0.071	−0.550	<0.01
<i>Cg</i>	0.377	−0.212	−0.982	<0.04
<i>Co</i>	0.208	−0.002	−0.564	<0.01
<i>Ec</i>	−0.415	−0.322	0.316	
<i>Fn</i>	0.212	0.015	−0.651	
<i>Pl</i>	0.738	0.129	−0.408	<0.03
<i>Pm</i>	−0.260	0.100	−0.073	
<i>Vp</i>	0.374	−0.349	−0.128	<0.03
Late colonizers				
<i>Aa</i>	−0.157	−0.167	−0.162	
<i>Cr</i>	0.143	0.028	−0.239	
<i>Pg</i>	0.701	−0.004	−0.851	<0.001
<i>Pi</i>	0.235	0.010	−0.580	<0.001
<i>Td</i>	0.110	0.087	−0.421	
<i>Tf</i>	0.039	−0.058	−0.824	<0.05
<i>En</i>	0.393	0.079	−0.481	
<i>Cs</i>	0.451	0.184	−0.449	
<i>Pn</i>	0.564	0.134	−0.655	<0.01

Table 3

Z-Scores for Change in Serum IgG Antibody Patterns to Oral Bacteria in Baboons From Baseline to Delivery

	CIPD Category			
Antigen	<20	20 to <50	>50	P Value
Early colonizers				
<i>Sg</i>	0.057	0.186	-0.443	<0.01
<i>Sm</i>	0.271	0.141	-0.749	<0.04
<i>Ss</i>	-0.553	0.088	0.096	
Bridge organisms				
<i>An</i>	0.238	0.173	-0.436	<0.005
<i>Cg</i>	0.260	-0.049	-0.549	<0.02
<i>Co</i>	0.181	0.006	-0.699	<0.02
<i>Ec</i>	-0.291	0.030	0.070	
<i>Fn</i>	-0.335	-0.221	-0.118	
<i>Pl</i>	0.634	0.084	-0.433	<0.03
<i>Pm</i>	-0.310	0.067	0.123	
<i>Vp</i>	0.455	0.015	0.139	
Late colonizers				
<i>Aa</i>	-0.058	0.076	-0.369	
<i>Cr</i>	-0.224	0.101	0.394	
<i>Pg</i>	0.402	-0.088	-0.473	<0.01
<i>Pi</i>	0.248	0.166	-0.360	
<i>Td</i>	0.223	0.026	-0.627	
<i>Tf</i>	0.143	0.114	-0.657	<0.02
<i>En</i>	0.119	0.057	-0.358	
<i>Cs</i>	0.269	0.250	-0.287	
<i>Pn</i>	0.417	0.140	-0.495	<0.04

Table 4

Correlation Coefficients for Baseline Clinical CIPD and Serum Antibody Levels or Changes in Serum Antibody Levels to Oral Bacteria by MP and D in Experimental Animals

Time Point	Species	Spearman ρ	P Value
Baseline	<i>P. gingivalis</i>	0.280	0.001
MP	<i>P. gingivalis</i>	-0.366	0.0001
	<i>P. intermedia</i>	-0.308	0.0001
	Σ	-0.276	0.001
	<i>C. gingivalis</i>	-0.257	0.003
	<i>P. loescheii</i>	-0.253	0.003
	<i>S. gordonii</i>	-0.233	0.007
	<i>P. nigrescens</i>	-0.230	0.008
	<i>T. forsythia</i>	-0.210	0.015
	<i>P. gingivalis</i>	-0.339	0.0001
D	<i>P. loescheii</i>	-0.243	0.005
	<i>S. sanguis</i>	0.229	0.008
	<i>P. intermedia</i>	-0.221	0.011
	<i>S. gordonii</i>	-0.214	0.014
	Σ	-0.200	0.021

Σ = sum of the antibody levels.