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Periodontitis in Pregnant Baboons: Systemic Inflammation and Adaptive Immune Responses and Pregnancy Outcomes in a Baboon Model

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Abstract

Chronic periodontal infections have been suggested to contribute to the risk of adverse pregnancy outcomes. This study describes the relationship of patterns of systemic inflammatory mediators and IgG antibody to 20 oral bacteria in pregnant female baboons (*Papio anubis*) coupled with clinical features of ligature-induced periodontitis, as risk indicators for adverse pregnancy outcomes. Animals showing a preterm delivery and/or low birth weight newborns, as well as those pregnancies resulting in spontaneous abortion, stillbirth, or fetal demise were tabulated as adverse pregnancy outcomes. A significantly greater frequency of the periodontitis group neonates had a low birth weight (18.1%; $p=0.008$) and decreased gestational age (9.8%). Spontaneous abortion/stillbirth/fetal demise were increased in the periodontitis (8.7%) versus the control group (3.8%) ($p=0.054$). The baseline oral clinical presentation of the experimental animals did not relate to the adverse pregnancy outcomes. Animals with the greatest extent/severity of periodontitis progression during the initial ½ of gestation (ie. to mid-pregnancy) had the greatest risk for adverse pregnancy outcomes. Baseline biological parameters indicating historical responses of the animals to periodontal challenge demonstrated individual variation in selected mediators, some of which became more differential during ligature-induced periodontitis. The relationship of clinical parameters to systemic inflammatory responses was consistent with a temporal contribution to adverse pregnancy outcomes in a subset of the animals. These results support a link between periodontitis and adverse pregnancy outcomes in the baboons and provide a prospective experimental model for delineating the biologic parameters that contribute to a causal relationship between chronic oral infections and birth events.

Keywords

baboons; pregnancy; periodontitis; inflammation

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INTRODUCTION

Preterm birth (PTB; <37 weeks gestation) and low birth weight (LBW; <2,500 g) deliveries continue to increase in the U.S. resulting in substantial economic and societal costs (1, 2). However, a substantial proportion of the general overall increase in incidence of PTB and LBW, including severe PTB (<32 weeks) and very low birth weight (<1,500 g), cannot be explained by classical risk factors for these negative birth outcomes (3–7). Thus, a broader view of the potential interrelationships leading to adverse pregnancy outcomes, including biologic markers or processes could provide some predictive value allowing earlier intervention to reduce this burden in the population.

Infections are an important risk factor for premature rupture of membranes (PROM) resulting in PTB often accompanied by a low birth weight (4, 8, 9) triggers a local production of various inflammatory mediators and matrix metalloproteinases (MMP). This results in amnionitis that negatively impacts placental function and potentially contributing to fetal infections (2, 4, 10, 11). This finding is consistent with observations demonstrating that prostaglandins and MMPs are necessary components of the normal birth process (11, 12). Thus, any challenge to the mother that would dysregulate the sequence of production of these mediators of gestation might be expected to alter the pregnancy. However, <50% of PTB births have been identified related specifically to an infectious challenge (4, 13, 14).

Periodontitis is a chronic oral infection with a polymicrobial biofilm generating an immunoinflammatory lesion that results in soft tissue alterations, both epithelium and connective tissue, and stimulates a net resorption of alveolar bone (15–18). As the localized lesions progress, the chronic infection leads to a translocation of bacteria into the systemic circulation (19). These bacteria can “lodge” in distant tissues/organs resulting in diminished functions either via a direct effect of bacterial factors, or more often related to the elicitation of a tissue destructive inflammatory response (20–24). Additionally, the distribution of these oral bacteria into the systemic circulation can generate a systemic inflammatory response, as well as an adaptive immune response ((25). Thus, the host reaction to these oral commensal opportunistic pathogens encompasses both a localized and systemic response (26, 27). Systemic inflammatory responses have been documented in periodontitis patients and appear to be related to the severity/extent of disease, with a parallel decrease following periodontal therapy (20, 28, 29).

Periodontal disease in the nonhuman primate provides a bridge to understanding human disease, since this animal model reflects the commensal subgingival microbiota, characteristic local and systemic responses, and histological and clinical presentation of disease processes identified in humans (30–34). Similarly, the baboon model has been used in obstetrical research (35–38), which makes it an ideal model to study the link between periodontitis and negative birthing outcomes. This study hypothesized the female baboons during their pregnancy would exhibit increased levels of various inflammatory mediators in serum resulting from ligature-induced periodontitis. The targeted mediators would be those that could contribute to adverse pregnancy outcomes and might be predictive of the biologic risk and causal link of periodontal disease with these events.

MATERIALS & METHODS

Animal Husbandry, Experimental Design, and Clinical Measures

An experimental cohort of 397 *Papio anubis* were enrolled in this study for a prospective assessment of the effect of periodontitis on pregnancy outcomes. Inclusion in the study was dependent upon the following criteria: (i) had a minimum of twenty teeth; (ii) were in good general health based upon an examination by the veterinarian; (iii) ranged in age from 6–13

years; and, (iv) had produced previous offspring. Potential mothers were excluded if they demonstrated systemic illness that required veterinary treatment during the course of the project that would adversely impact the pregnancy outcome (*ie.* infection) and/or administration of antibiotic and/or anti-inflammatory therapy, which could confound the onset and severity of periodontitis. Loss of body weight $\geq 15\%$ also excluded the baboon from further participation in this project. Nulliparous dams (*eg.* previous births increase likelihood of successful breeding for this study), dams of extreme ages, either younger or older, and those dams having less than 20 teeth were excluded. No animals were pregnant at baseline sampling, prior to tooth ligation and mating. One hundred and thirty-eight control animals and 181 experimental animals completed the study with birth outcomes yielding a study cohort of 319 who completed the clinical and birth aspects of the study. Of the 78 that did not complete the study, 21 in the ligature group and 19 of the controls never got pregnant during their experimental interval. An additional 7 were exited during the study due to injuries occurring with the group housing and treatment that required surgical intervention and extended use of antibiotics. Finally, the last group of 31 control animals had to be excluded from the study due to a confounding infection in this cohort (##). The protocol and all procedures for the study were approved by the Animal Care and Use Committee at the Texas Biomedical Research Institute.

Experimental and control animals were sampled at baseline for periodontal clinical measures and serum was obtained. Teeth in the right maxillary and mandibular quadrants (1 & 4) were then ligated (39) in the experimental animals only. Immediately after samples were collected from the cohort and ligatures tied on the experimental animals, the male was introduced into the harem. A second sampling took place at mid-gestation (~3 months) and ligatures were tied on the contralateral maxillary and mandibular quadrants (2 & 3). The third sample was obtained from 2–10 days after delivery and the ligatures were removed. A complete periodontal evaluation was performed at each of the 3 sampling intervals for supragingival plaque, pocket depth, recession, and bleeding upon probing (39) at four sites on each tooth: distobuccal, buccal, mesiobuccal, and lingual in each quadrant. Attachment level values were calculated from the pocket depth and recession measures (39). Missing teeth or teeth that could not be scored were noted. A gingival bleeding score, following determination of the pocket depth measure, was obtained. Ligatures were tied on the first and second molar and second premolar teeth (teeth 5, 6, and 7) using 3-0 silk sutures. To promote inflammation, the animals were placed on a soft chow diet, consisting of commercial chow biscuits soaked in warm water for 10 minutes and drained (30). Control animals were managed in the same fashion during the pregnancy and sampled identically; however, the control animals were fed a regular commercial diet and were not ligated.

The Composite Index of Periodontal Disease (CIPD) was developed to provide a single index value that would incorporate measures of both periodontal disease extent and severity. The calculated index included weighted measures of gingival bleeding and attachment loss (40) as described in the following algorithm. With these variables as a basis, we determined that using an equal weighting for the 4 variables did not provide a sufficiently robust discrimination of the level of destructive disease, nor separation of animals with varying combinations of inflammation (BOP) and tissue destruction (CAL). Thus, for the final CIPD we weighted the variables such that the measure of destructive disease (CAL) and the extent of destruction (% of sites with CAL >2 mm) were increased in contribution to the CIPD. The final formula for the CIPD was:

$$\text{CIPD} = 0.5(\text{mean BOP}) + 0.1(\% \text{BOP} > 0) + (\text{mean CAL}) + 2(\% \text{CAL} > 2)$$

A CIPD of <20 is consistent with relative gingival health in nonhuman primates, $20\text{--}50$ represents gingivitis, $50\text{--}75$ mild periodontitis, $75\text{--}100$ moderate periodontitis, and >100 severe periodontitis.

Serum Inflammatory Mediators and Antibody

Blood (approximately 10 ml) was obtained by femoral venipuncture into red top vacutainer tubes. The blood was allowed to clot for 1 h, centrifuged for 15 min. at 3000 × g and the serum removed and the serum prepared and stored at −70°C after separation into 0.5–0.75 ml aliquots.

A panel of acute phase reactants, including C-reactive protein (CRP), bactericidal permeability inducing factor (BPI), and lipopolysaccharide binding protein (LBP) were quantified using an ELISA developed in our laboratory (*ie.* CRP) or obtained commercially (BPI, LBP; Hycult Biotechnology, Cell Sciences, Canton, MA). Various serum cytokines/chemokines, including interleukin (IL)-1 β , IL-6, IL-8, tumor necrosis factor (TNF) α , MIP-1 α (CCL3; Macrophage Inflammatory Protein-1 α), RANTES (CCL5; Regulated upon Activation, Normal T-cell Expressed, and Secreted), IL-12p40, and MCP-1 (CCL2; Macrophage Chemotactic Protein-1) were measured using a multiplex beadlyte assay on a Luminex IS-100 (Millipore, Billerica, MA). Prostaglandin E₂ (PGE₂) levels were assessed using a commercial ELISA kit (Assay Design, Ann Arbor, MI).

IgG antibody levels to twenty bacteria were determined by quantitative ELISA using formalin-killed whole bacterial antigens as we have described previously (41): *Actinomyces naeslundii* ATCC49340, *Actinobacillus actinomycetemcomitans* JP2, *Campylobacter rectus* ATCC33238, *Capnocytophaga gingivalis* ATCC33624, *Capnocytophaga ochracea* ATCC33596, *Capnocytophaga sputigena* ATCC33624, *Eikenella corrodens* ATCC23834, *Eubacterium nodatum* ATCC33099, *Fusobacterium nucleatum* ATCC49256, *Parvimonas micra* ATCC33270, *Porphyromonas gingivalis* FDC381, *Prevotella intermedia* ATCC25611, *Prevotella loeschii* ATCC15930, *Prevotella nigrescens* ATCC33563, *Streptococcus gordonii* ATCC49818, *Streptococcus mutans* ATCC25175, *Streptococcus sanguis* ATCC10556, *Treponema denticola* ATCC35405, *Tannerella forsythia* ATCC49307, and *Veillonella parvula* ATCC10790.

Statistical Analyses

A Student's t-test was used to evaluate differences in the gestational age and birthweights between the experimental and control groups, and between the subgroups with adverse pregnancy and normal birth outcomes. An ANOVA was used to evaluate differences in GA or BW related to baseline, mid-pregnancy, and delivery disease categories, with a *post hoc* Holm-Sidak (SigmaStat 3.5, Systat Software, San Jose, CA) assessment for individual group differences. A Fisher's exact test or Chi-square analysis was used to evaluate differences in the frequency of shortened gestation and low birthweights between the experimental and control groups. Relative risk and confidence intervals were determined using MedCalc Software (Mariakerke, Belgium).

Due to the extensive variability in mediator levels across the population, the host response data were all transformed using a log transformation to create a normal distribution. A Student t-test was used to compare the group means throughout the pregnancy for each analyte. Spearman's correlation on ranks was used to determine relationships between the various host response variables, as well as to the periodontal presentation of the animals.

RESULTS

Effect of Periodontitis on Pregnancy Outcomes

Table 1 presents the average gestational age (GA) and birth weight (BW) for each of the groups and demonstrates that there were no significant differences in the mean GA and BW between the experimental and control groups. Nevertheless, we observed inherent clinical

variation in the periodontal health of the animals as they entered the study and based upon this variability, Table 1 also presents the GA and BW for subsets of animals that were stratified based upon baseline clinical presentation into a healthy (CIPD <20), gingivitis (CIPD 20–<50) and periodontitis (CIPD >50). These oral clinical health/disease differences at baseline were not predictive of the pregnancy outcomes.

Fig. 1A describes the distribution of preterm birth (PTB) and low birth weight (LBW) deliveries in the experimental group when compared to the control animals in the study. The first strategy determined the frequency of gestational age and birth weight that fell below one standard deviation of the mean of the control population. Since this assessment would categorize approximately 5% of the control animals into a low gestational age or birth weight, the finding that 18.1% of the experimental animals exhibited a LBW ($p<0.008$) and 9.8% showed a preterm delivery indicated an effect of oral disease on the pregnancy outcomes. The second strategy paralleled that routinely used for humans in which a birth weight of <2,500 g and gestational age of <37 weeks, representing approximately 80% (birth weight) and 92% (gestational age) of normal, are considered abnormal. The results in the baboon model (Fig. 1B) demonstrated that 4.0% and 3.2% of the control animals exhibited a PTB or LBW delivery, respectively. This observation was in contrast to experimental group where 5.0% had a PTB delivery and 9.3% had an LBW delivery ($p<0.027$). These assessments included a description of the live births in these groups of animals. However, a portion of the animals demonstrated birth outcomes defined by spontaneous abortion/stillbirth/fetal demise, which could be considered an extreme endpoint for an adverse pregnancy outcome affected by periodontitis. Fig. 1C provides the summary of the incidence of these pregnancy outcomes in the experimental and control animals. The results demonstrated that 8.7% of the experimental group and 3.9% of the control animals had pregnancy outcomes of spontaneous abortion/stillbirth/fetal demise, which approached the $p<0.05$ level for statistical significance. The extent and severity of periodontal disease was associated with lower infant birth weights (RR=3.24, 95% CI=1.12–9.41, $p=0.07$); however, periodontal disease was not associated with preterm birth outcomes (RR=1.37, 95% CI=0.47–4.00, $p=0.56$). While periodontitis was not significantly associated with stillbirth or fetal demise only (RR=2.44, 95% confidence interval, 0.92–6.50, $p=0.07$), the risk of having a negative birthing outcome (composite of all negative pregnancy experiences) was positively related to periodontal disease (RR=2.82, 95% CI=1.45–5.47, $p=0.002$). We can conclude that ligature-induced periodontitis during pregnancy increased the likelihood of a poor birth outcome by over 2.8 fold in this model.

Clinical Periodontal Disease Heterogeneity of the Pregnant Baboons Related to Birth Outcomes

Fig. 2 graphically represents an assessment of the periodontal clinical presentation (CIPD) for the experimental group of animals at baseline (B), mid-pregnancy (MP) and delivery (D) stratified to compare normal births to adverse pregnancy outcomes. The results show no differences in periodontal disease at baseline or at the time of delivery. However, the APO subset of animals showed higher levels of periodontal disease extent/severity at the mid-pregnancy time point compared to the normal birth animals.

Examination of the characteristics of the individual animal's responses to ligature placement leading to the induction of inflammation and tissue destruction, demonstrated clinical heterogeneity within the population. Because of this observation, we stratified the animals into 4 periodontal disease susceptibility (PDS) groups based upon changes in clinical presentation from baseline through mid-pregnancy and delivery. At baseline the composite scores (CIPD) of the entire cohort of animals (prior to ligation) was skewed towards a healthy/gingivitis score, since the majority of animals at this age do not demonstrate broad

and substantial bleeding and pocketing. Thus, we determined the level of change in CIPD at mid-pregnancy that would be required from baseline to reach statistical significance. The animals were then stratified into those with elevations in CIPD that were either similar too or significantly greater than baseline. This stratification separated PDS-1/PDS-3 (similar to baseline) from PDS2/PDS-4 (greater than baseline) subgroups. We then determined the animals with significant changes in CIPD at delivery compared to mid-pregnancy disease levels. PDS-1 and PDS-3 showed no difference from MP to D. A similar analysis was conducted to separate PDS-2 from PDS-4, thus these groupings were made empirically based on the data distribution. The characteristics of the groups are summarized in Table 2. Thus, this stratification provides an overview for the way that each animal responded to the challenge of ligature-induced periodontitis. The results in Table 2 suggest that animals in PDS-3 and PDS-4 showed a trend toward a lower gestational age and birth weight than animals in the other 2 groups. Since these 2 categories demonstrated a greater clinical change from baseline to mid-pregnancy, this outcome is consistent with the previous results suggesting the oral clinical changes early in pregnancy may have the greatest impact on birth outcomes. Additionally, the results in Table 3 present an analysis of the PDS groups as they relate to the distribution of adverse pregnancy outcomes in the experimental animals. The results support that the animals in PDS-3 and PDS-4, demonstrating the greatest clinical change during the baseline to mid-pregnancy time points, displayed a significantly increased risk ($p=0.043$) for the range of adverse pregnancy outcomes observed in the study.

Serum Inflammatory Mediators Levels and Pregnancy Outcomes

Fig. 3 shows the levels of these mediators in the experimental population at baseline, and after ligation of teeth in 2 quadrants (MP) or 4 quadrants (D) stratified based upon birth outcomes. The results in Fig. 3A shows that only PGE₂ was elevated and LBP levels in serum were decreased at baseline in animals with subsequent APO. By mid-pregnancy (Fig. 3B) levels of PGE₂ and IL-6 were elevated in the APO animals. Finally, Fig. 3C demonstrates that at the time of delivery the pro-inflammatory mediators, IL-6 and IL-8 remained elevated in the females with APO birth outcomes. Additionally, at the time of delivery, elevated levels of BPI were also observed in the APO females.

Serum Antibody Levels and Pregnancy Outcomes

Table 4 summarizes the serum antibody responses to the 20 oral bacteria in the ligated (periodontitis) animals at baseline, mid-pregnancy and delivery stratified into normal and adverse birth outcomes. The results demonstrate elevations in antibody levels to *F. nucleatum* in the animals with APO birth outcomes at each of the sampling points through gestation. Similarly, antibody to *P. gingivalis* was significantly increased at baseline in the APO animals. Interestingly, the animals with normal births demonstrated increased systemic antibody responses to *P. gingivalis* at both mid-pregnancy and at delivery. A similar elevation in antibody to *T. forsythia* was also observed in normal birth animals at delivery. We also calculated a summation of antibody responses to an important consortium of species that has been implicated consistently in adult periodontitis in humans (42) and included *P. gingivalis*/*T. denticola*/*T. forsythia*. The results showed higher levels of antibody to this consortium at baseline in the APO animals, with little change in response to the ligature-induced disease. In contrast, antibody to this bacterial consortium increased by nearly 50% in the normal birth animals from baseline through delivery during the periodontal challenge to the animals.

Finally, in Table 5, we provide a summary of the clinical and host response interactions that were related to the adverse pregnancy outcomes in the model of oral disease and related these differences to gestational time points. The results suggest that even in the absence of discernible clinical differences, biological parameters provide some distinguishing

characteristics, which include both inflammatory and adaptive immune responses. Importantly, primary clinical differences were observed by mid-gestation.

DISCUSSION

Expanding the scientific knowledge base to describe the broad impact of chronic diseases in the human population has stimulated studies to elucidate the biologic processes that result in alterations in tissues and organs contributing to the etiology of these diseases (43, 44). Periodontal infections trigger chronic inflammation resulting in damage to soft and hard tissues of the periodontium (45, 46). Due to the inability in human studies to provide information suggesting a “cause-and-effect” relationship regarding periodontitis and systemic diseases, we used a nonhuman primate ligature-induced periodontal disease to provide a model of this polymicrobial disease effects on pregnancy outcomes. We have reported previously that the female baboons demonstrate an heterogeneity in periodontal clinical presentation as a natural variation in the population, which reflect similar findings in human populations (47, 48). We have also demonstrated that experimental baboons demonstrated significant increases in inflammation and destruction of the periodontium following placement of ligatures around the teeth during pregnancy (40). Periodontitis in the baboons elicited by ligature placement is accompanied by changes in the subgingival microbial ecology with bacterial species similar to those in human disease, coupled with changes in systemic inflammatory mediators (49) and increases in antibody specific for various oral bacteria (40), as has been observed in humans.

Examination of the distribution of gestational age and birth weights between the control animals and the group with ligature-induced periodontitis demonstrated no significant differences in mean GA or BW for the populations. This finding is not inconsistent with a number of reports from human studies that find no differences in the incidence of PTB or LBW in expectant women with periodontitis (50). However, we noted both an heterogeneity in oral clinical presentation of the baboons, and a variation in both GA and BW in the experimental group. Thus, we were able to identify a subset of animals with periodontitis and having a higher frequency of shortened gestation and/or lower birth weights. The results demonstrated that over 18% of the experimental animals presented a birth weight at delivery that was outside a standard deviation of the control population. This contrasted with <10% of the animals exhibiting a GA shorter than the controls. A similar comparison focused on the estimation of the incidence of PTB and LBW in the experimental and control groups based upon a similar threshold used in human populations, a cutoff for PTB of 166 days (92% of normal gestation) and for LBW of 711 grams (20% below normal) for baboon births. These results demonstrated a significantly greater frequency of LBW deliveries in the experimental (periodontitis) animals (9.4%) compared to the control group (2.9%), although no differences were noted in the frequency of PTB. Consequently, using either approach to analyze the distribution of GA and BW in this model, we identified an effect of periodontitis on the incidence of LBW deliveries.

Various epidemiologic studies of periodontitis and pregnancy in humans indicated that women with periodontitis had a higher frequency of stillbirth/abortion/fetal demise compared with periodontally healthy women (51–53). Additional data from the Obstetrics and Periodontal Therapy (OPT) clinical trials demonstrated a decrease in spontaneous abortion/stillbirth/fetal demise in the women treated for periodontal disease during the 2nd trimester, compared to women with periodontitis treated after delivery (54). Therefore, one could consider this adverse pregnancy outcome as the most severe end of a continuum of effects on parturition. In this study, we identified a significant increase in the prevalence of spontaneous abortion/stillbirth/fetal demise in the experimental group compared to the control animals that was 3 times the rate of the controls. A range of adverse pregnancy

outcomes was documented in the experimental group with ligature-induced periodontitis, supporting a direct relationship between the clinical features of the oral disease and this systemic complication. We also examined the characteristics of periodontitis in the baboons exhibiting an adverse pregnancy outcome (APO; PTB/LBW + spontaneous abortion/stillbirth/fetal demise), compared to those with normal pregnancies. The results in both the experimental and control groups suggested that the APO subset of animals showed greater periodontal disease at mid-pregnancy than the normal birth subset in each group. Moreover, there were no differences in periodontal disease measures at baseline or at delivery between these birth outcome subsets. The lack of relationship of the baseline periodontal disease classification to significantly forecast an increased likelihood of an adverse pregnancy outcomes is suggested to result from both the low number of animals in both groups having periodontitis on entry into the study (~20%), and our findings that among the cohort, there exists what appears to be a subset of animals that are truly susceptible to periodontitis altering the pregnancy outcomes. Thus, too few animals had baseline would have had existing periodontitis and sufficient risk to demonstrate a statistical relationship to APO. It appeared that periodontal infections with associated chronic inflammation occurring during the early stages of the pregnancy (*ie.* first trimester and early second trimester in humans) were most related to birth outcomes, in contrast to previous concepts suggesting that infections/inflammation later in pregnancy might be expected to provide a greater risk (55, 56).

Our previous studies in various nonhuman primate models demonstrated a substantial individual variation in the kinetics and severity of progressing periodontitis (39, 57), which was also observed in the pregnant baboons. Consequently, we hypothesized that those animals with adverse pregnancy outcomes, *ie.* PTB, LBW, spontaneous abortion/stillbirth/fetal demise, would demonstrate a more severe periodontitis that is indicative of animals more susceptible to dysregulation of chronic inflammation and resulting destructive disease. Thus, we identified that within this population of female baboons there was a variation in the clinical presentation of the periodontium at entry into the study (baseline). At the individual animal level there existed significant variability in the magnitude of the response to ligature placement and measures of progressing periodontitis. We noted that the baseline clinical presentation was not a predictor of subsequent adverse pregnancy outcomes. This finding is not particularly surprising, since the majority of the animals exhibited a relatively healthy periodontium with localized gingivitis. Only about 20% of the animals demonstrated periodontitis with <2% exhibiting a more severe level of disease. However, following ligation, all animals demonstrated some presentation of increased inflammation and tissue destruction. Thus, we stratified the experimental cohort into 4 somewhat distinct clinical groups reflecting their response to challenge, *ie.* ligation, with approximately 25% of the animals showing a moderate increase in disease at the MP and D time points compared to baseline; defined as Periodontal Disease Susceptibility (PDS-1) group. The PDS-2 group animals (~40% of the cohort) demonstrated a small change in disease by MP with a substantial increase in disease between MP and D. The PDS-3 group (~26%) exhibited substantial clinical changes within the first three months (*ie.* MP), with less change from MP to D. Finally, ~27% of the females showed significant increases in periodontal disease from baseline to mid-pregnancy that continued to increase significantly through delivery. Based upon this stratification, we were able to examine both the distribution of adverse pregnancy outcomes related to the kinetics and magnitude of oral clinical disease expression. The results indicated that the subsets of animals with the greatest response to the ligature challenge between baseline and mid-pregnancy (*ie.* PDS-3, PDS-4) appeared at greatest risk for APO, while substantial clinical changes later in pregnancy did not appear to significantly impact the birth outcomes. These findings are consistent with those of Jeffcoat and colleagues (58, 59) who demonstrated a relationship between periodontitis severity and likelihood of PTB/LBW birth outcomes.

Many chronic diseases have been identified to have components of chronic inflammation, both locally and systemically, that contribute to inducing collateral damage of the host resulting in the clinical symptoms (60–63). The systemic acute phase response with increases in various inflammatory mediators is an important component of innate immunity and is fundamentally designed to protect the non-immune host from noxious challenge and to reestablish homeostasis (64). However, it is now recognized that the chronic stimulation of the systemic inflammatory response provides markers for risk of disease, as well as the probability that the biomolecules of this response actually can contribute to the disease processes. Current theory holds that gingival tissue destruction around affected teeth in periodontitis results from the local release of inflammatory mediators secondary to colonization by complex biofilms, generally containing characteristic pathogenic opportunistic microorganisms (15, 16, 65). The responses to oral bacteria are complicated, as these commensal opportunistic pathogens provoke both a localized and systemic response during the disease (66–68). Systemic inflammatory responses are generally low in individuals with a healthy periodontium or in subjects with reversible gingival inflammation (*ie.* gingivitis). The levels of these mediators are generally associated with the severity/extent of periodontal disease (57, 66, 69, 70), frequently decrease significantly with periodontal therapy (28, 71–73), and are decreased in patients that become edentulous (74–76).

In this report, periodontitis in the nonhuman primates elicited by ligature placement was accompanied by changes in the subgingival microbial ecology with bacterial species similar to those in human disease (unpublished data). While this chronic oral infection elicits elevated levels of local inflammatory, innate, and acquired immune mediators (15, 26), this report focused on the capacity of the oral infection and disease to trigger changes in the systemic host response apparatus, manifest by changes in various acute phase reactants, and inflammatory mediators and cytokines/chemokines. Our findings support that ligature-induced periodontitis in baboons elicits changes in systemic inflammatory and adaptive immune mediators. These data support a temporal relationship between the development, severity, and extent of periodontitis during pregnancy with adverse pregnancy outcomes. It is important to recognize that the disease of periodontitis is not the link with APO, but that periodontitis represents a chronic oral infection that can lead to systemic translocation of bacteria (19, 45) and accompanying changes in systemic host inflammatory responses (77). An existing report related a clinical observation of a patient with a placental/fetal infection with an *F. nucleatum* strain that was genetically identical to one identified in the oral cavity of the mother (22). This observation prompted studies by Han and colleagues that have demonstrated that a systemic infection of mice with *F. nucleatum* can induce a placental infection leading to adverse pregnancy outcomes, including fetal loss (24, 78). While these studies support the capacity of *F. nucleatum* as an oral bacterium to elicit this type of maternal-fetal infection, the infection of mice by systemic injection of a bolus of the infecting agent, and an infection with a human microorganism that is not a normal member of the autochthonous microbiota of mice, is substantially different than the host-bacterial relationship in humans and nonhuman primates with a continuous challenge during gestation with opportunistic pathogens within the commensal microbial ecology of the oral cavity. While the “cause and effect” relationship between individual systemic inflammatory mediators elicited by periodontitis and altering birth outcomes is difficult to unequivocally document, the breadth of evidence indicates that chronic periodontal infections may be a contributor to the burden of risk for initiating and/or sustaining inflammatory processes that occurring during gestation and parturition (50, 58, 79, 80). While this report provides a descriptive confirmation of the relationship between periodontitis and adverse pregnancy outcomes, extension of this nonhuman primate model should enable a definition of the potential contribution of both host response and direct bacterial biologic processes that

provide the most robust prospective mechanistic links between this chronic oral infection and birth outcomes.

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Summary

A prospective experimental model in baboons suggested a causal relationship between chronic oral infections of periodontitis and systemic responses with adverse pregnancy outcomes.

Figure 1.

(A) Frequency of preterm birth (PTB) or low birth weights (LBW) in the experimental animals that were >1 SD below the mean of the control group. The level of significance reflects comparison of the observed rate in the experimental animals compared to expected rate in control group if periodontitis had no effect. (B) Frequency of PTB or LBW in the experimental and control groups based upon outcomes that were comparable to the normal GA (174 days) or normal BW (644 grams) in human measures. (C) Frequency of spontaneous abortion/stillbirth/fetal demise pregnancy outcomes in experimental and control animals. Significant differences between experimental and control values are noted on the graphs. Experimental group $N=181$ and control group $N=138$.

Figure 2.

Comparison of periodontal disease severity/extent (CIPD) at baseline (B), mid-pregnancy (MP), and delivery (D), in experimental baboons with an adverse pregnancy outcome (APO = PTB + LBW + spontaneous abortion/stillbirth/fetal demise) compared to the experimental baboons with normal deliveries. The bars denote group means and the vertical line denotes 1 SD. Experimental group N=181 and control group N=138.

A

B

C

Figure 3.

Box and whisker plots of levels of systemic inflammatory mediators in baboons at baseline (A), mid-pregnancy (B), and delivery (C) in experimental animals related to pregnancy outcomes. The boxes denote 25th–75th percentile, whiskers denote the minimum and maximum values and the horizontal line denotes the median of the group. CRP levels in mg/mL, PGE₂ and BPI levels in ng/mL, LBP levels in $\mu\text{g/mL}$, and IL-6/IL-8/MCP-1/RANTES levels in pg/mL. The asterisk (*) denotes significantly different at least at $p < 0.05$. Baseline and Mid-Pregnancy experimental group N=181 and Delivery experimental group N=177.

Mean gestational age and birth weights of deliveries from experimental and control baboons and comparison to baseline periodontal health.

Table 1

Variable	Group (N)	Mean ± SD	Median (min., max.)	Baseline Periodontal Disease Stratification		
				CIPD <20 (healthy)	CIPD 20-<50 (gingivitis)	CIPD >50 (periodontitis)
Gestational Age (days)	Experimental (N=181)	180.6 ± 9.5	181.0 (137, 197)	183.4 ± 18.5 (N=29)	181.5 ± 18.1 (N=116)	179.6 ± 34.5 (N=36)
	Control (N=138)	180.4 ± 7.1	181.3 131.5, 195)	182.7 ± 26.1 (N=25)	181.0 ± 19.1 (N=93)	181.4 ± 35.2 (N=20)
Birthweight (grams)	Experimental	883 ± 157	886 300, 1260	875 ± 125	882 ± 83	826 ± 124
	Control	895 ± 122	888 (480, 1200	893 ± 127	880 ± 80	884 ± 146

Table 2

Clinical presentation (CIPD) of the Periodontal Disease Susceptibility Groups at baseline (B), mid-pregnancy (MP) and delivery (D).

PDS Group	Periodontal Clinical Findings (CIPD)			Birth Outcomes	
	B (mean ± SD)	MP (mean ± SD)	D (mean ± SD)	Gestational Age (mean ± SD)	Birthweight (mean ± SD)
PDS-1	43 ± 4.8	53 ± 7.3	68 ± 7.7 *	186.6 ± 21.5	898 ± 112
PDS-2	48 ± 5.1	58 ± 6.5	115 ± 12.9 *#	185.4 ± 20.0	886 ± 1.9
PDS-3	46 ± 4.2	94 ± 10.1 *	99 ± 8.9 *	173.9 ± 26.2	851 ± 119
PDS-4	54 ± 5.5	105 ± 13.6 *	146 ± 18.7 *#	176.1 ± 27.2	832 ± 123

PDS-1 animals presented with a progression of periodontal disease that while significantly greater than baseline, reflected a generally low inflammatory and tissue destructive response. PDS-2 animals showed a rather small mean clinical change by mid-pregnancy; however, the mean clinical presentation at delivery was significantly increased. The PDS-3 group exhibited a substantial change in periodontal disease by mid-pregnancy, although ligation of additional teeth appeared to only maintain this level of disease by delivery. The PDS-4 group showed significant increases in disease by mid-pregnancy with a continued significant increase in disease through delivery.

* Significantly different from baseline at least at $p < 0.05$.

Significantly different from MP values at least at $p < 0.05$. Experimental group PDS-1 N=44, PDS-2 N=53, PDS-3 N=47, and PDS-4 N=37.

Table 3

Adverse pregnancy outcomes related to Periodontal Disease Susceptibility Groups.

PDS Group	APO	Normal	Total	% APO
PDS-1	7	37	44	15.91
PDS-2	8	45	53	15.09
PDS-3	10	37	47	21.28
PDS-4	12	25	37	32.43
TOTAL	37	144	181	20.42

See description of PDS group in legend to Table 2.

* Fisher exact test: PDS-1/PDS-2 vs. PDS-3/PDS-4 $p=0.043$, 2-tailed test.

Table 4

Serum antibody levels in female baboons with normal and adverse pregnancy outcomes. Mean $\pm$ 1 SEM presented for those antibody specificities that were significantly different at least at $p < 0.05$ (*) between the birth groups.

Microorganism	Birth Group	Baseline	Mid-Pregnancy	Delivery
<i>F. nucleatum (Fn)</i>	Normal	16.63 $\pm$ 0.83*	13.08 $\pm$ 0.66*	11.33 $\pm$ 0.50*
	APO	31.31 $\pm$ 1.87	35.27 $\pm$ 1.20	21.73 $\pm$ 1.05
<i>P. gingivalis (Pg)</i>	Normal	21.87 $\pm$ 1.29*	37.82 $\pm$ 1.99*	48.23 $\pm$ 2.89*
	APO	28.85 $\pm$ 2.52	28.96 $\pm$ 2.68	36.65 $\pm$ 6.21
<i>T. forsythia (Tf)</i>	Normal	16.96 $\pm$ 0.83	19.03 $\pm$ 0.79	22.80 $\pm$ 1.18*
	APO	18.91 $\pm$ 1.53	16.44 $\pm$ 1.11	16.52 $\pm$ 1.30
Total <i>Pg/Td/Tf</i>	Normal	64.11 $\pm$ 2.43+*	78.74 $\pm$ 3.01*	90.84 $\pm$ 3.84*
	APO	73.70 $\pm$ 4.61	66.42 $\pm$ 3.72	70.53 $\pm$ 7.59

Antibody levels were also evaluated for *A. actinomycetemcomitans*, *A. naeslundii*, *C. rectus*, *C. ohracia*, *C. sputigena*, *E. corrodens*, *E. nodatum*, *P. micra*, *P. intermedia*, *P. loeschii*, *P. nigrescens*, *T. denticola*, *S. gordonii*, *S. mutans*, *S. sanguis*, and *V. parvula*. There were not significant differences between birth groups with any of these microorganisms. Experimental group Normal birth=144, APO birth N=37.

Table 5

Summary of adverse pregnancy outcomes as related to clinical periodontal disease, serum antibody to oral bacteria, and systemic inflammatory responses. Arrows denote direction of difference for APO animals compared to normal birth animals.

	Periodontal Disease	Serum Antibody	Serum Inflammatory Mediators
Baseline	No difference	<i>Fn</i> <i>Pg</i> <i>Pg/Td/Tf</i>	LBP PGE ₂
Mid-Pregnancy		<i>Fn</i> <i>Pg</i> <i>Pg/Td/Tf</i>	IL-6 PGE ₂
Delivery	No difference	<i>Fn</i> <i>Tf</i> <i>Pg</i> <i>Pg/Td/Tf</i>	IL-6 IL-8 BPI