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Effects of a *Porphyromonas gingivalis* Infection on Inflammatory Mediator Response and Pregnancy Outcome in Hamsters

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This study examines the effects of various localized, nondissemination challenges of *Porphyromonas gingivalis* on inflammatory mediator production and pregnancy outcome in the golden hamster. Live or heat-killed (HK) organisms were inoculated into a previously implanted subcutaneous tissue chamber on the 8th day of gestation to determine the effects on fetal weight, viability, and resorption. In one group of animals, HK organisms were inoculated prior to mating to determine the effects of previous exposure on day-8 gestational challenges. Chamber contents were assayed at 1 and 5 days after challenge for prostaglandin E_2 (PGE_2) and tumor necrosis factor alpha (TNF-α). All *P. gingivalis* challenges caused a significant increase in chamber PGE_2 and TNF-α at $P < 0.01$ in the following order of potency: HK < Live < HK+Live. For example, following the HK+Live challenge, PGE_2 levels increased from 4.7 pg/ml at baseline to 362 pg/ml at day 5 and TNF-α increased from 26.4 pg/ml to 724 pg/ml at day 5. The same order of potency of the various challenges was maintained with regard to the toxic effects of *P. gingivalis* on pregnancy outcome. For the HK+Live challenge, fetal weight was decreased 24%; embryolethality increased to 26.5% and the percent fetal resorption increased to 10.6% compared with control animal levels. There was a statistically significant association between increasing levels of both PGE_2 and TNF-α and fetal growth retardation and embryolethality at $P < 0.001$. These data suggest that infections with gram-negative periodontal pathogens can elicit adverse pregnancy outcomes and that the levels of PGE_2 and TNF-α produced as a result of challenge are associated with the severity of fetal effect.

Porphyromonas gingivalis is a gram-negative, black-pigmented, nonfermentive anaerobe originally isolated from the oral cavity and described by Gibbons and MacDonald (9). *P. gingivalis* has been implicated as an oral pathogen because of its frequent association with destructive periodontal disease (5, 20). The frequency of *P. gingivalis* isolation from periodontally healthy individuals is sufficiently rare that *P. gingivalis* is not considered a member of the normal oral flora.

Previous investigations have described infection models using periodontal pathogens in rodents. Genco and colleagues (7, 8) described the virulence properties of *P. gingivalis* A7436 in the subcutaneous tissue chamber model. The chamber not only serves as a site for bacterial inoculation but also provides a serum-filled reservoir for repeated sampling and analyses of inflammatory mediators. In this investigation, we have utilized a hamster timed-pregnancy model to simultaneously examine the effects on both pregnancy outcome and mediator production in response to a *P. gingivalis* infection.

Genitourinary tract infections, which are capable of eliciting low birth weight and spontaneous abortions in humans, rarely involve direct bacterial invasion of the fetal-placental unit (10). Recent reviews on the pathogenic mechanisms by which genitourinary infections induce low birth weight have suggested that the effects of infection on the fetus are not mediated directly by the bacteria per se but rather are mediated by the maternal cytokines. These cytokines and lipid mediators are produced either at the infectious site or at the placenta in response to the infection. Several mediators produced during gram-negative infection have been proposed to elicit a deleterious effect on the developing fetus, including tumor necrosis factor alpha (TNF-α; cachectin) and prostaglandin E_2 (PGE_2). In various pregnant animals, exogenous treatment with these molecules will induce rupture of membranes, cervical dilation, uterine contraction, and abortion (2, 6). However, both TNF-α and PGE_2 increase in the amniotic fluid during normal pregnancy and are believed to mediate routine parturition. It has been suggested that during infection, abnormally high levels of these mediators can lead to premature termination of pregnancy with concomitant low birth weight. Supporting this concept, Heyborne (11) has recently shown a relationship between intra-amniotic levels of TNF-α and intrauterine growth retardation in humans.

In this investigation we have examined the effects of *P. gingivalis* infection in a subcutaneous chamber on TNF-α and PGE_2 production in the hamster. Using pregnant hamsters, we have demonstrated that a *P. gingivalis* infection within the chamber is capable of producing fetal growth retardation and can be embryolethal. Pretreatment with heat-killed (HK) organisms prior to mating enhances mediator production and embryotoxicity of *P. gingivalis* infection during pregnancy. There is good agreement between mediator concentrations within the chamber and abnormal pregnancy outcomes (APOs).

MATERIALS AND METHODS

In the first series of experiments, nonpregnant female hamsters were used to study the effects of *P. gingivalis* challenge on PGE_2 and TNF-α response. After placing subcutaneous chambers, four groups of six animals received one of the following treatments: (i) control (culture broth only), (ii) HK *P. gingivalis* (HK), (iii) live *P. gingivalis* (Live), or (iv) HK *P. gingivalis* initially, followed by live *P. gingivalis* 21 days later (HK-Live).

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Chamber fluids were collected at baseline and 1 and 5 days postchallenge for the analysis of PGE_2 and TNF-α.

In the second series of experiments, these same four treatments were performed on timed-pregnancy animals. The effects of *P. gingivalis* challenges on mediator levels in pregnant hamsters were determined and compared with those of nonpregnant animals. In addition, the effects of bacterial challenge on three pregnancy outcomes, fetal weight, embryolethality, and percent resorptions, were assessed.

Bacteria. *P. gingivalis* A7436 was used in these studies. Strain A7436 was initially isolated from a refractory periodontitis patient and characterized by V. R. Dowell, Anaerobic Microbiology Laboratory, Centers for Disease Control, Atlanta, Ga. (7, 8). *P. gingivalis* was grown on anaerobic blood agar plates in a Coy anaerobic chamber. Following incubation at 37°C for 1 to 2 days, the bacterial cells were inoculated into Wilkins-Chalgren anaerobic broth and grown for approximately 18 h until the culture reached an A_{660} of 0.16, as read on the LKB spectrophotometer. This absorbance corresponded to approximately 10^8 CFU/ml. All data reported were generated from animals challenged with *P. gingivalis* A7436 at a dosage of 10^7 CFU.

The hamster pregnancy model has recently been described in detail (3). Briefly, hamsters were mated and the pregnancy was terminated at day 15 to allow inspection of the uterus, fetuses, and resorption sites. *P. gingivalis* (live or HK) was presented as a challenge on day 8. Other animals received culture broth (no-bacterium control) on day 8, and one group received a HK challenge 21 days prior to mating. Golden Syrian hamsters (*Mesocricetus auratus*) were maintained in the UNC Dental Research Center in an American Association for Accreditation of Laboratory Animal Care-approved facility. Housing consisted of plastic, solid-bottom cages with wood shavings for bedding. The male hamsters for breeding were housed individually, and the nonpregnant females were three to a cage. Pelleted Rodent Laboratory Chow 5001 (Purina) and water were provided ad libitum.

Hamsters are polyestrous and can be bred year-round with artificial lighting conditions, with a 14:10 (light:dark) lighting cycle providing optimal breeding. The animals in these studies were maintained on this lighting cycle, and body weight was utilized to determine sexually mature females. The sexually mature females usually have a 4-day estrous cycle. Day 1 represents ovulation, and on day 2, which is postovulatory, a characteristic vaginal discharge was used to stage the cycle. On the evening of the third day following the discharge, the female was introduced into the male's cage. The pair was left undisturbed overnight, and the female was moved to a separate cage the next morning. The day following evening breeding was considered day 1 of the gestation period, which is typically 15 to 18 days. The females were sacrificed on the afternoon of day 15 of pregnancy. Euthanasia was performed by CO_2 asphyxiation. The uterus was dissected, and fetal weight, viability, and resorptions were determined. The sites of implantation and resorption were counted. The viability of each fetus was assessed visibly. Each fetus was removed from the chorioamniotic sac and weighed to the nearest microgram. The mean fetal weight was determined for each pregnant dam, pooling six dams to compute a group mean standard error. Embryolethality was expressed as a percentage of total implantation sites per litter which had neither a resorption site nor a nonvital fetus, again pooling dams for each treatment group.

STC. We employed the subcutaneous tissue chamber (STC) model as described by Genco et al. (7). This model provides facile chamber access for mediator analyses. At the levels of *P. gingivalis* used in this study, chamber inoculation does not affect maternal weight or cause visible signs of systemic infection or sepsis. The model consists of a cylindrical coil spring (approximately 5 by 10 mm) prepared from 0.5-mm-diameter surgical stainless steel wire. Coils are implanted in the dorsolumbar region of each female hamster and allowed to heal for 10 days before the chamber is inoculated. Anesthesia for this procedure consisted of sodium pentobarbital (60 to 90 mg/kg of body weight). After 10 days, chambers were inoculated with 0.1 ml of live *P. gingivalis*, HK *P. gingivalis*, or Wilkins-Chalgren anaerobic broth media as a control. *P. gingivalis* was heat killed at 95°C for 10 min (7). A total of 48 hamsters were challenged: four groups of 6 nonpregnant hamsters and four groups of 6 timed-pregnancy (day 8) animals. One group from the nonpregnant and pregnant subsets each received a pretreatment with HK organisms 21 days prior to the primary challenge. Thus, there were four treatment groups for both pregnant and nonpregnant animals consisting of (i) control media, (ii) HK *P. gingivalis*, (iii) live *P. gingivalis*, and (iv) HK-Live.

Measurement of mediator response. The chamber contents were sampled at baseline (prior to inoculation) and on day 1 and day 5 postinjection of the challenge. Chamber fluid was aseptically removed from each chamber with a 25-gauge hypodermic needle. A total of 100 μl of chamber fluid aspirate was diluted 1:10 with radioimmunoassay buffer and stored at −70°C for inflammatory mediator analysis. The lipid metabolite PGE_2 was quantitated by radioimmunoassay procedures described previously by Offenbacher (17). The chamber contents were analyzed for the release of cytokine TNF-α by a commercial immunoabsorbent assay system (Endogen Inc., Boston, Mass.). Schlenker and colleagues have demonstrated cross-reactivity with this murine kit and hamster TNF antigens as well as Western blot (immunoblot) reactivity with the murine anti-TNF antibody (18a, 19). The manufacturer's instructions were followed to assay duplicate 50-μl aliquots of diluted chamber aspirate in 96-well plates. The plates were read at a wavelength of 490 nm on a Molecular Devices kinetic microplate reader. The amount of each mediator in the chamber was determined by using duplicate samples, interpolating from standard curves which were generated by plotting log concentration versus cumulative normal proportion bound. Mediator levels are expressed as mean concentrations ± standard errors by pooling six animals per group at each time point.

Analyses of data. Time-dependent changes in inflammatory mediator levels and group differences were tested for statistical significance with repeated-measure analysis of variance. When group- or time-dependent differences were present, pairwise comparisons were made by using nonpaired or the paired *t* test. An alpha level of less than 0.05 was considered statistically significant. Simple regression correlations were performed by using nontransformed data to examine for an association between mediator level and APO testing for slope $\neq 0$ at $P < 0.05$.

RESULTS

In the first series of experiments using nonpregnant animals, the temporal changes in PGE_2 and TNF-α within the chamber fluid were determined in response to the various *P. gingivalis* challenges. The PGE_2 levels in the chamber fluid challenged with *P. gingivalis* or media control are shown in Fig. 1. The PGE_2 concentrations were determined at baseline and at 1 and 5 days after intrachamber challenge with the various *P. gingivalis* preparations. Control animals, which received media only, produced PGE_2 levels comparable to those of the baseline on

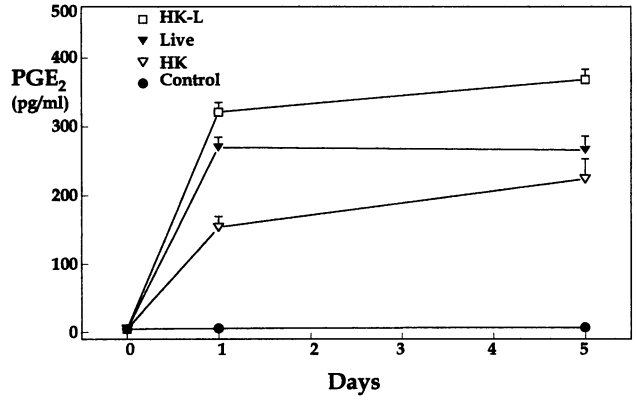

FIG. 1. Chamber fluid PGE_2 response in nonpregnant hamsters. PGE_2 concentration is shown at the baseline and at 1 and 5 days postintrachamber challenge with media (Control) and the various *P. gingivalis* preparations. The mean and standard error for each group (n = 6) are shown.

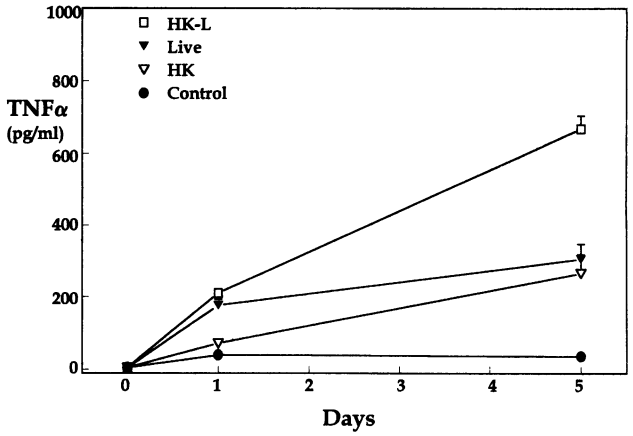

FIG. 2. Chamber fluid TNF-α response in nonpregnant hamsters. TNF-α concentration is shown at the baseline and at 1 and 5 days postintrachamber challenge with media (Control) and the various *P. gingivalis* preparations. The mean and standard error for each group (n = 6) are shown.

both day 1 and day 5. At baseline, the PGE_2 chamber level was 4.7 ± 0.3 pg/ml, which slightly increased to 6.8 ± 1.3 pg/ml by day 5 after media challenge, but this increase was not statistically elevated over baseline levels. All *P. gingivalis* preparations induced a significant rise in PGE_2 compared with the media control, but there were quantitative differences in the response to the various preparations. The PGE_2 levels in the HK group increased significantly to 154.6 ± 15.8 pg/ml at day 1 and 224.8 ± 29.2 at day 5. Animals receiving live *P. gingivalis* had a significantly greater PGE_2 response at day 1 compared with the HK group (P = 0.0004), but there was no difference by day 5. Animals which received HK followed by live *P. gingivalis* challenge showed the largest increase in PGE_2 levels on day 1 and 5 postchallenge compared with all groups ($P < 0.04$). The PGE_2 levels of all three test groups did not change significantly from day 1 to day 5, appearing to reach an early plateau. However, all of the *P. gingivalis* challenges elicited a dramatic PGE_2 response with the following ranked potency: HK < Live < HK-Live.

Figure 2 shows the chamber fluid TNF-α response to *P. gingivalis* challenge. Control chambers showed low levels of TNF-α at day 0 (26.4 ± 2.1 pg/ml) which did not increase over baseline levels through day 5. There was a small but statistically significant increase in the response to HK by day 1 compared with the control (71.1 ± 9.0 versus 39.2 ± 7.2 pg/ml; P = 0.02). By day 5 there was a significant rise in TNF-α following the HK challenge relative to control levels. The same rank potency for TNF-α secretion was observed for these three groups, with HK < Live < HK-Live. In contrast to the PGE_2 responses, which reached an apparent plateau by day 1, both the HK and HK-Live challenges resulted in significant increases in day 5 relative to day 1 at $P < 0.005$. By day 5, the HK-Live TNF-α production is significantly increased to a level of 668.2 ± 35.3 pg/ml. Pretreatment with HK resulted in a greater than twofold increase in TNF-α levels within the chamber relative to those of nonexposed animals. Thus, previous exposure with HK resulted in a greater early PGE_2 rise and a later TNF-α increase. The maximal TNF-α response to *P. gingivalis* challenge appears to be delayed compared with those for PGE_2 in all groups, as can be seen by comparing Fig. 1 and 2. Note that by 1 day postchallenge, PGE_2 levels are from 68 to 87% of the maximum values determined on day 5, while day 1 TNF-α levels are from 26 to 31% of day 5 values, suggesting that the TNF-α levels in the chamber fluid continue to rise while PGE_2 levels reach an earlier maximum concentration.

The second series of experiments tested the effects of *P. gingivalis* challenge on pregnancy outcome and determined whether pregnancy altered the inflammatory mediator response within the chamber compared with that of the nonpregnant hamster. Table 1 shows the TNF-α and PGE_2 levels at day 5 in pregnant and nonpregnant hamsters following the various challenges. As can be seen, there is remarkable similarity in PGE_2 and TNF-α levels within the chambers comparing pregnant with nonpregnant animals. Furthermore, the day 1 mediator responses were not altered by pregnancy (data not shown). The magnitude and direction of both mediator responses are maintained during pregnancy, indicating that pregnancy does not significantly alter the inflammatory response induced by *P. gingivalis* in this model. These data also demonstrate the consistency and reproducibility of the mediator response within the chambers following these different *P. gingivalis* challenges.

TABLE 1. STC PGE_2 and TNF-α levels in nonpregnant and pregnant hamsters at day 5 following challenge with media or *P. gingivalis* preparations[a]

Hamsters	STC levels (pg/ml)							
	PGE_2				TNF-α			
	Control	HK	Live	HK-Live	Control	HK	Live	HK-Live
Nonpregnant	6.8 (1.3)	224.8 (29.2)	266.8 (20.4)	368.0 (15.2)	35.6 (4.5)	266.0 (52.0)	305.2 (41.8)	668.2 (35.3)
Pregnant	10.3 (1.4)	242.9 (17.6)	320.8 (11.0)	362.2 (6.9)	42.0 (2.6)	358.1 (109.7)	365.2 (103.9)	724.8 (27.6)

[a] The mean and standard error for each group (n = 6) are shown.

TABLE 2. Pregnancy outcome data for the control group and the *P. gingivalis* challenge groups[a]

Group[b]	Fetal wt (g)	Embryolethality (%)	Resorption/litter (%)
Control	1.91 ± 0.020	0	0
HK	1.69* ± 0.019	0.83* ± 0.12	0.16 ± 0.15
Live	1.58* ± 0.024	2.0* ± 0.36	0.50 ± 0.22
HK-Live	1.45* ± 0.027	3.5* ± 0.22	1.50* ± 0.22

[a] All data are represented as means plus or minus the standard error. *, $P < 0.05$.
[b] $n = 6$ for all groups.

The effects of *P. gingivalis* challenge on pregnancy outcome in the hamster are shown in Table 2. The pregnancy outcome variables of fetal weight, embryolethality, and fetal resorptions are reported plus or minus the standard error. The mean fetal weight in control animals was 1.91 g, with no embryolethal events. Thus, as the challenge to the pregnant dam increases in severity from HK < Live < HK-Live, there is a parallel increase in fetal growth retardation, embryolethality, and fetal resorptions. Fetal weight decreased by 11.4, 17.2, and 24.0% in the animals challenged with HK, Live, and HK-Live, respectively. These dramatic decreases in fetal weight represent significant deviations from the control animals at $P = 2.0 \times 10^{-5}$, 4.2×10^{-7}, and 1.8×10^{-7}, respectively. The effects of the various challenges on fetal weight mimic the rank order of mediator responses, in that HK < Live < HK-Live. Embryolethality is defined as the number of nonvital fetuses and resorption sites per total implantation sites. The percent embryolethality increased from 5.9% with the HK challenge to 14.3% with the Live challenge to 26.5% with the group receiving the HK-Live challenge. These increases are all significantly elevated over control levels at $P < 0.05$ and from each other at $P < 0.03$. The percentage of fetal resorptions increased from 1.2% with the HK challenge to 3.7% with the Live bacteria and 10.6% with the HK-Live challenge. The HK-Live challenge has a statistically significant effect on the prevalence of resorptions at $P = 0.0009$. These data demonstrate that by increasing the embryotoxic traits of the challenge from HK to Live to HK-Live followed by Live, the result on pregnancy outcome is fewer and smaller surviving fetuses.

The relationship between chamber fluid PGE_2 levels and pregnancy outcome is shown in Fig. 3. The maternal chamber fluid PGE_2 levels at day 5 in all animals including controls are plotted against fetal weight (Fig. 3A) and embryolethality (Fig. 3B). There is a statistically significant association between increasing PGE_2 levels and decreasing fetal weight. This is shown as a regression slope significantly less than zero at $P = 6.3 \times 10^{-12}$. The correlation between elevated PGE_2 levels at day 5 and decreased hamster fetal weight is excellent, as shown by the regression coefficient $r = 0.939$ and an r^2 value of 0.881. Figure 3A demonstrates that the pregnant animals with the highest PGE_2 chamber fluid concentration produced fetuses with the lowest mean weight. There is a similar relationship between chamber PGE_2 levels and fetal death. The regression of embryolethality on PGE_2 concentration (Fig. 3B) demonstrates a statistically significant association (slope $\neq 0$ at $P = 3.2 \times 10^{-6}$, $r^2 = 0.629$). Higher chamber PGE_2 levels were associated with the dams having a higher percentage of nonvital fetuses. The agreement with linearity is fair to good. Thus, there is a significant relationship between increasing maternal PGE_2 and fetal growth retardation and lethality.

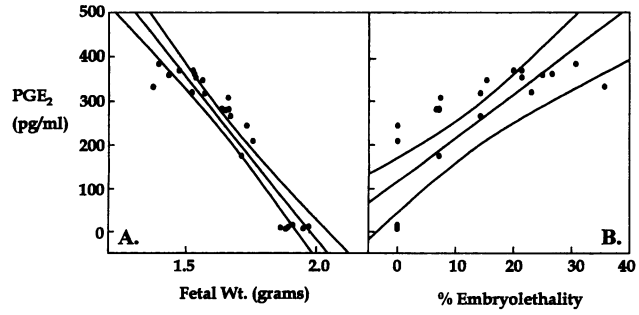

FIG. 3. Relationship between chamber fluid PGE_2 levels and pregnancy outcome. Maternal chamber fluid PGE_2 levels at day 5 in all animals are plotted against fetal weight (A) and percent embryolethality (B). Shown are linear regression lines and 95% confidence intervals.

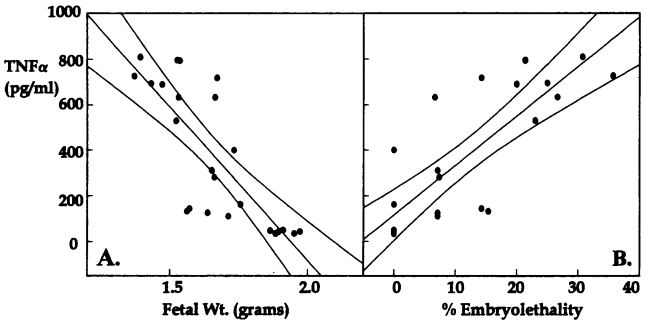

FIG. 4. Relationship between chamber fluid TNF-α levels and pregnancy outcome. Maternal chamber fluid TNF-α levels at day 5 in all animals are plotted against fetal weight (A) and percent embryolethality (B). Shown are linear regression lines and 95% confidence intervals.

The associations of chamber fluid TNF-α levels with fetal weight and embryolethality are shown in Fig. 4. Again, a significant negative association exists for TNF-α levels and fetal weight (Fig. 4A) with a P value of 2.6×10^{-6}, a regression coefficient of $r = 0.796$, and an r^2 value of 0.635. The regression of embryolethality on TNF-α levels (Fig. 4B) demonstrates a positive association at $P = 1.2 \times 10^{-6}$, $r = 0.811$, and $r^2 = 0.658$. There is also a significant relationship between TNF-α chamber levels at day 5 and fetal resorptions (data not shown). Although the percent embryolethality includes resorption sites as well as nonvital developed fetuses, the association of TNF-α levels and fetal resorption sites (slope $\neq 0$ at $P = 0.002$, $r^2 = 0.634$) indicates that TNF-α levels may also help explain variations in the number of fetal resorption sites, not just fetal death.

Independently, both PGE_2 and TNF-α chamber levels are associated with APO in this model. Intrachamber levels of PGE_2 and TNF-α are also significantly related at $P = 3.2 \times 10^{-6}$, $r = 0.793$, and $r^2 = 0.629$. The lack of statistical independence between inflammatory mediator levels and pregnancy outcomes indicates that the coordinated expression of both PGE_2 and TNF-α is associated with decreasing fetal weight and increased lethality.

DISCUSSION

This study examined the effects of a localized *P. gingivalis* infection on pregnancy outcome in the golden hamster. The ability to simultaneously monitor the host response to the localized infection in the STC model and relating this to the

pregnancy outcome is a unique application of this model. Unlike previous animal models such as the murine abscess model (MAM), the use of subcutaneous chambers provided the ability to quantitate and monitor PGE_2 and TNF-α production during the course of the *P. gingivalis* infection. In the STC model, the bacteria are exposed to antibodies, complement, and phagocytic cells (7, 8). The chamber infection at the levels of challenge tested in this report do not lead to systemic dissemination. The animals remain afebrile and do not experience weight changes or malaise. The STC allows continuous analysis of locally produced host factors in response to the *P. gingivalis* challenge. The selection of the golden hamster for our experiments was based on the regular gestation period of 15 to 18 days and the rapid differentiation of the embryo during the eighth day of gestation (6). These qualities make the hamster a standard model for teratology studies.

The work of Genco et al. (7, 8) provided not only an extensive description of the STC model in mice but also provided insight on virulence properties of *P. gingivalis* A7436. They found that an inoculum of 5×10^8 CFU caused severe cachexia with secondary ulcerated lesions and death on day 3. The hamsters in our experiments also experienced severe cachexia and secondary ulcerated lesions with this dosage, and obviously this was not conducive for the pregnancy model we established. We determined that 10^7 CFU resulted in a STC primary lesion with no evidence of cachexia. This STC primary lesion more closely reflects the periodontal infection process, that is, localized destruction without apparent systemic symptoms. Immunization with *P. gingivalis* A7436 followed 21 days later by live A7436 resulted in protection against secondary ulcerations and death; however, the colonization with live A7436 challenge was not inhibited within the chambers (8). These data provided the basis for our HK *P. gingivalis* A7436 followed 21 days later with live *P. gingivalis* A7436. Our goal was to determine whether previous exposure provided protection, by using conditions of immunization which may mimic those seen in chronic periodontal disease. The results suggest that the previous exposure to HK *P. gingivalis* A7436 actually potentiated the pregnancy outcomes of low birth weight, embryolethality, and fetal resorption rather than providing protection. This was accompanied by an enhanced PGE_2 and TNF-α response, suggesting that cell-mediated immunity was involved and that any antibody that may have been induced by the first challenge was insufficient to afford protection.

In addition to testing whole, live *P. gingivalis* A7436, which formed an active infection, we also tested HK bacteria to determine whether the lipopolysaccharide (LPS) content of the bacterial challenges was sufficient to elicit APOs in the absence of the infective process. Our data suggest that although HK bacteria can have deleterious effects, live bacteria were significantly more potent in eliciting an increase in abnormal pregnancy outcome. Thus, the live bacteria were more virulent in this model than the HK organisms with regard to the effects on the fetus. These findings, made by using HK challenge, are similar to those of our previous studies testing the effects of *P. gingivalis* A7436 LPS on fetal weight and malformations in the hamster (14). In toto, these data suggest that the LPS moiety of *P. gingivalis* provides the dominant virulence component in this model.

With these experiments, we sought not only to examine the effects of various *P. gingivalis* preparations on pregnancy outcome but also to correlate the magnitude of inflammatory mediator response to the severity of the APO. The selection of the host-derived mediators PGE_2 and TNF-α was based on several lines of evidence, suggesting that both periodontal disease and APO may be mediated by these molecules (10, 13, 17). Our data showed an increased production of PGE_2 and TNF-α which was directly associated with the increase in challenge virulence and severity of the APO. In addition, TNF-α levels in chamber fluid continued to rise in comparison to PGE_2 levels which reached an earlier maximum concentration following the various bacterial preparations. This may be explained by the fact that lipid metabolites such as PGE_2 are released relatively quickly while cytokines such as TNF-α require protein synthesis and are released in a slower and more prolonged manner. The chamber fluid levels of both mediators were similar in the nonpregnant and pregnant animals, suggesting that the magnitude and direction of PGE_2 and TNF-α responses are maintained during pregnancy in the STC model. This observation is in contrast to other reports which have suggested that pregnancy confers an immunotolerant state which decreases the inflammatory response to infectious challenge (21).

These experiments have demonstrated that a nondisseminating, localized subcutaneous *P. gingivalis* infection can lead to APOs in the hamster. Specific mediator responses to the various *P. gingivalis* preparations which correlate with APOs have been identified. Our data suggest that these APOs are associated with increases in host-derived PGE_2 and TNF-α produced following bacterial challenge. The embryotoxic effects of PGE_2 in the absence of infection were demonstrated by Liebgott and Wiley (12). By using the hamster model with a similar design, it was shown that PGE_2 delivered in a single intraperitoneal bolus on day 8 of gestation produced teratogenic effects comparable with the results of this study. A dose-response effect on fetal weight and fetal mortality was shown with various concentrations of PGE_2 (0.2, 0.5, 0.75, and 1 mg/kg). Fetal weight reduction produced by the maximum PGE_2 dose (1 mg/kg) was 25%, comparable to a 24% reduction in fetal weight in response to HK-Live *P. gingivalis* challenge. Further evidence of a PGE_2-mediated effect on adverse pregnancy outcome is derived from studies of cyclooxygenase inhibitors. Cort and Kindahl (4) studied the effects of endotoxin on early pregnancy in gilts and demonstrated that flunixin meglumine (a potent cyclooxygenase inhibitor) suppressed LPS-induced prostaglandin synthesis and prevented luteolysis and abortion in early pregnancy. In those studies, *Salmonella typhimurium* endotoxin served as the pregnancy challenge at concentrations which produced endotoxemia, monophasic fever, and abortion within 34 h. Flunixin meglumine (4 mg/kg) produced no antipyretic effect in the treated animals yet significantly suppressed a rapid rise in plasma $PGF_{2\alpha}$ (an analog of PGE_2) levels immediately after LPS injection and maintained gestation in five of six gilts. These data support our suggestion of a rapid rise in prostaglandin synthesis in response to bacterial challenge, which is associated with fetal toxicity and a decrease in fetal weight.

Aseptic increases in TNF-α levels have also been shown to effect fetal survival in CBA × DBA/2 mice. Chaouat et al. (2) determined percent fetal resorption produced by injecting various cytokines into abortion-prone CBA/J × DBA/2 and non-abortion-prone CBA/J × BALB/c and C3H × DBA/2 mating combinations. Human recombinant TNF-α was injected intravenously at various concentrations (1,000 to 3,000 U) and gestational periods. A 1,000-U TNF-α challenge at day 7.5 enhanced fetal resorptions from 43 to 79% in the abortion-prone matings and from 7 to 89% (CBA × BALB/c) and 5 to 47% (C3H × DBA/2) in the non-abortion-prone combinations. The TNF-α effect was found to be both gestational age and dose dependent. TNF-α (1,000 U) challenges at various days of gestation (0.5, 4.5, 7.5, 9.5, and 13.5 days) produced an increase in fetal resorptions from 31% at day 0.5 to 88% at day

9.5 in the abortion-prone matings. TNF-α challenges on day 13.5 resulted in only a 52% fetal resorption rate, suggesting a window of the TNF-α effect on fetal resorption. Fetuses were examined on day 14.5 in this study; we did not report numbers of nonviable fetuses that were not resorbed. Chaouat et al. (2) reported that in addition to TNF-α, interleukin-2 and gamma interferon also were potent inducers of increased fetal resorptions. However, the T-cell-derived cytokines of the colony-stimulating factor family (interleukin-3 and granulocyte-macrophage colony-stimulating factor) increased the chances of fetal survival when injected into the abortion-prone mice and also increased fetal and placental weight. This study showed that cytokine regulation of pregnancy outcome occurs in vivo and suggests that the T-cell lineage may be gestationally protective. Recently, this effect of T-cell regulation of fetal development has been attributed to an embryo-protective T_H^2 response and a deleterious T_H^1 response (21).

The molecular mechanisms by which PGE_2 and TNF-α induce small or dead fetuses are unknown, but certain theories have been proposed. It has been suggested that bacterial products stimulate the monocyte/macrophage cells to produce cytokines like TNF-α that initiate parturition by stimulating the intrauterine tissues to produce prostaglandins (18). It is well documented that TNF-α causes ischemic necrosis of certain solid tumors (for a review, see reference 1), and there is an obvious analogy to the feto-placental unit as a foreign tumor. Thus, some of the placental pathology associated with low birth weight may be a result of TNF-α challenge. Finally, TNF-α is also cachectic and prevents lipid uptake and utilization. It is possible that intrauterine growth retardation may represent a selective TNF-α-induced nutrient deprivation. Further studies are needed to examine the specific mechanisms associated with TNF-α and PGE_2 interactions during pregnancy, as well as with chronic infections such as periodontal disease.

Whether the presence of an oral periodontal infection can lead to sufficient systemic exposure to LPS or cytokines to affect pregnancy outcome needs to be addressed. In previous studies (16, 17), we have shown that the severity of maternal periodontal disease is associated with a risk for low birth weight in humans. In this study, the specific mediator responses which correlated with pregnancy outcome may be useful as future risk markers for human APOs. For instance, in many cases of low birth weight which occur in the absence of maternal genitourinary tract infections, the levels of intra-amniotic fluid TNF-α and PGE_2 appear abnormally elevated. This observation has prompted several investigators to suggest that many preterm deliveries which result in low birth weight occur as a result of infection of unknown origin (10). In this hamster model, previous exposure to *P. gingivalis*, a common periodontal pathogen, did not afford protection. This finding suggests that humans with preexisting periodontal infections might be at a greater risk for pregnancy complications and that the apparently innocuous sustained exposure which occurs via the local periodontal infection might be an important candidate as a stealth infection.

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