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Gender differences in systemic inflammation and atheroma formation following *Porphyromonas gingivalis* infection in heterozygous apolipoprotein E-deficient mice

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Background and Objective: Men are at higher risk for periodontal and cardiovascular diseases compared with women, although they have lower serum levels of risk markers, including lipids and acute phase proteins. In this study, we investigated whether infection with a major periodontal pathogen, *Porphyromonas gingivalis*, affected the inflammatory and atherosclerotic response of male and female mice differently.

Material and Methods: Forty-eight heterozygous apolipoprotein E-deficient mice (24 males and 24 females), maintained on normal diet, were infected twice by intrasubcutaneous chamber injections of *P. gingivalis* or vehicle at weeks 11 and 14 of age. Serum samples were collected before the first infection and bi-weekly thereafter, to quantify levels of high-density lipoprotein (HDL) cholesterol and the murine acute phase protein, serum amyloid A (SAA). Mice were killed at week 17 to evaluate aortic atheroma lesion score.

Results: Males had significantly higher baseline HDL cholesterol levels ($p < 0.01$, factorial ANOVA). Following *P. gingivalis* infection, HDL cholesterol levels decreased over time in infected males only [$p < 0.05$, generalized estimating equation (GEE)], whereas SAA levels increased and remained elevated over time in both male and female infected mice ($p < 0.01$, GEE). Lesion scores were significantly higher in infected mice (3-fold, $p < 0.01$, factorial ANOVA), and lesion scores of all mice were positively correlated with SAA levels at the time of killing (Spearman correlation coefficient = 0.40, $p < 0.01$).

Conclusion: In these young mice, *P. gingivalis* infection induced sex-specific changes in serum lipids but no gender differences in acute phase proteins and atheroma lesion score.

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Two different meta-analyses of existing literature have concluded that periodontal diseases are associated with cardiovascular diseases (1,2). What still remains uncertain is whether this association is due to a common underlying predisposition or whether periodontal diseases independently contribute to cardiovascular diseases. Some of the recognized common risk factors for both conditions include older age, male gender, cigarette smoking, diabetes and low socio-economic status. The majority of epidemiological studies either stratify, match or adjust for these risk factors, including gender. Among the studies that look at the effect of gender, most confirm that there is a higher risk for periodontal disease and cardiovascular disease in men (3–8). Few studies have explored the biological basis for this differential risk by gender (4,5,8), which still remains unexplained.

Mildly elevated serum levels of acute phase proteins and altered serum lipid profiles are associated with several morbid conditions, including obesity, the metabolic syndrome, hypertension, type 2 diabetes and cardiovascular diseases (9). While the source of this low-grade chronic inflammatory response in these conditions is unknown, chronic exposure to infectious agents might represent a contributing factor. Periodontal disease is a relatively common infectious and inflammatory condition of the adult population, caused by the overgrowth of microorganisms invading the periodontal space between the gums and the roots of the teeth and by the destructive inflammatory response of the gingival tissue. Remarkably, this localized oral response has a measurable systemic impact. Indeed, elevated serum levels of C-reactive protein (CRP) and other acute phase markers are reported in periodontitis patients (3,10–15), as well as elevated serum levels of total cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides, accompanied by decreased serum levels of high-density lipoprotein (HDL) cholesterol (3,5,7,11,16). These observed systemic changes might explain part of the increased risk for atherogenesis and cardiovascular diseases in patients with periodontal diseases. However, in the general population, serum CRP levels are higher in women than in men (17,18) and the prevalence of individuals with elevated levels of CRP and total cholesterol is higher in women than in men (19), suggesting that women should be at higher risk for cardiovascular diseases than men. This inconsistency is the premise for our investigation.

One primary etiological agent of periodontal disease is *Porphyromonas gingivalis*, which has also been shown to initiate and/or exacerbate the atherogenic process in animals (20–22). Since there are no previous data available in an animal model on the sex-specific association between infection and atherosclerosis, we investigated whether *P. gingivalis* infection would affect male and female mice differently in terms of their inflammatory, metabolic and atherosclerotic response. We used a model of localized distant infection by means of a subcutaneous chamber, which was previously shown to mimic the sequence of localized inflammatory events observed in a diseased human periodontal pocket (23–25). This infection was superimposed on an attenuated genetic background of susceptibility to atherogenesis, namely mice heterozygous for the apolipoprotein gene ($ApoE^{+/-}$), maintained on a normal diet (26). Infection with *P. gingivalis* represented the major risk factor for the development of early atherosclerotic lesions in these mice.

Material and methods

Animal procedures

The University of North Carolina Institutional Animal Care and Use Committee approved all animal protocols. Colonies of homozygous $ApoE^{-/-}$ (back-crossed for at least 10 generations in the C57BL/6J background) and wild-type C57BL/6J mice were maintained at the School of Dentistry animal facility using breeder pairs originally obtained from the Jackson Laboratory (Bar Harbor, ME, USA). Experimental heterozygous $ApoE^{+/-}$ mice were obtained from $ApoE^{-/-}$ males paired with wild-type females. We used heterozygous $ApoE^{+/-}$ mice fed a normal diet because preliminary studies conducted in our laboratory had shown that the atherogenic effect of *P. gingivalis* infection was overwhelmed in homozygous $ApoE^{-/-}$ mice or in heterozygous $ApoE^{+/-}$ mice fed a high-fat diet (data not shown). Our choice of heterozygous $ApoE^{+/-}$ mice is supported by the findings of Li *et al.* (20), who described a significant atherogenic effect of *P. gingivalis* infection in heterozygous $ApoE^{+/-}$ mice regardless of the diet they were fed. Mice were enrolled in the experiment as they were weaned from six separate breeder pairs of our in-house breeding colony, and subjected to the experimental procedure on a weekly basis, in three consecutive groups of 23, eight and six mice each. Mice were randomly assigned to control and infected groups and kept mixed in cages with up to four mice per cage. All mice were fed regular mouse chow.

Figure 1 represents the experimental protocol. At 4 weeks of age, $ApoE^{+/-}$ mice were weaned and randomly assigned to different experimental groups: 12 males and 12 females to the *P. gingivalis*-infected group (although 1 mouse died in each sex group, resulting in 11 males and 11 females), and 12 males and 12 females to the control group. Chambers measuring 1 cm in length and 0.5 cm in diameter were constructed from a cylindrical coil spring made of surgical wire. Two chambers were surgically implanted subcutaneously, one on each side, in the dorsal lumbar region of each mouse at 6 weeks of age (anesthesia = intra peritoneal injection of ketamine/xy lazine mixture 124/8.8 mg/kg body weight; preoperative analgesic = subcutaneous infiltration of 0.25% bupivicaine; post-operative analgesic = topical applications of 2.5% of iodine/2.5% prilocaine cream as needed for up to 48 h). Late exponential phase [optical density at 660 nm (OD_{660}) = 0.8–1.2] cultures of *P. gingivalis* strain A7436 grown in Wilkins–Chalgren anaerobe broth (WC broth) were prepared fresh at the desired concentration [OD_{660} = 1.0 corresponding to

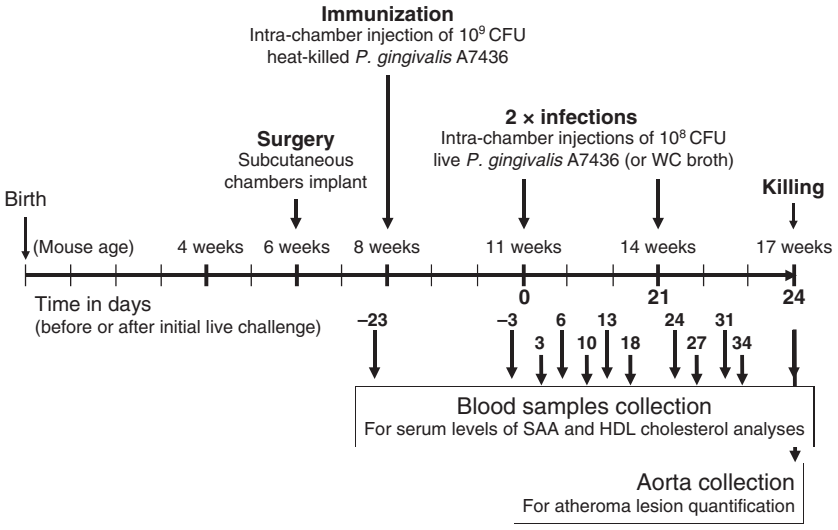

Fig. 1. Experimental time line. The abbreviation CFU indicates colony-forming units.

10^9 colony-forming units/mL] on the challenge days. Mice were 'primed' at 8 weeks of age by a single intra-chamber injection of 10^9 colony-forming units heat-killed (10 min at 100°C, challenged group) *P. gingivalis* or WC broth (control group). During the 21 days following priming, mice develop a circulating antibody response to *P. gingivalis*, which is not part of their natural flora, similar to that observed in humans (27). These primed mice are more representative of the general human condition, since the human oral cavity is colonized early in life with *P. gingivalis* (28) and humans of all ages present with measurable levels of serum antibodies specific to *P. gingivalis* (29). Mice were then infected twice, at weeks 11 (right chamber) and 14 (left chamber), by intra-chamber injection of 10^8 colony-forming units live *P. gingivalis* (infected group) or WC broth (control group). Primed mice tolerate this live infection well, with the establishment of a localized and contained intra-chamber infection representative of a typical pathogen load observed in a diseased human periodontal pocket. Mice were monitored daily for signs of fever, cachexia and chamber swelling, and every 3 weeks for weight gain or loss. Serum samples were collected from tail veins prior to immunization (day −23), prior to initial live infection (day −3) and bi-weekly thereafter (days 3, 6, 10, 13, 18, 24, 27, 31 and 34) until killing at week 17 (day 41; Fig. 1). Mice were killed by intracardiac puncture for blood collection after being overdosed with a ketamine–xylazine mixture (180/12 mg/kg). The chest was opened to perfuse the heart and vascular tree through a left ventricular cannula as previously described (30). The heart and aorta up to the renal bifurcation were dissected and processed for *en face* analysis. Mice were monitored daily for signs of fever, cachexia and chamber swelling, and every three weeks for weight gain or loss.

Serum HDL cholesterol and serum amyloid A (SAA) levels

Serum HDL cholesterol levels (in mg/dL) were determined by colorimetric assay (Sigma-Aldrich Co., St Louis, MO, USA). A baseline mean (average of values measured at days 3 and 6), post-first live *P. gingivalis* infection mean (average of values measured at days 3, 6, 10, 13 and 18) and post-second live *P. gingivalis* infection mean (average of values measured at days 24, 27, 31, 34 and 41) were calculated for each mouse.

Serum amyloid A is the major murine acute phase protein (31). Serum SAA levels were measured by ELISA (BioSource™, Invitrogen Corp., Carlsbad, CA, USA). When measured values were below the lower detection limit (< 0.059 μg/mL), the value was imputed to 0.029 μg/mL (half lower detection limit). When measured values were above the higher detection limit (> 1.9 μg/mL), samples were reanalyzed at a higher dilution. When no sample was left for re-analysis, the value was imputed at the upper detection limit multiplied by the highest dilution tested. The percentage of imputed values was less than 20% at all time points except at day 31 (27%). This imputation scheme was conservative, since it underestimated the out-of-range high SAA values.

En face morphometric analysis of aortic atheroma lesion

The extent of aortic atheroma lesion was evaluated by *en face* morphometric quantification as previously described by Palinski and colleagues (32). Aortas were opened longitudinally, pinned on dark-colored wax and stained with Sudan IV (specific for lipids). Total aortic surface area and atherosclerotic lesion area (Sudan IV stained) were measured on captured images using ImageJ Software (Rasband WS, ImageJ, US National Institutes of Health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/, 1997–2005). Atheroma lesion scores were calculated as percentages of aortic surface area covered by atheroma lesions.

Statistical analyses

The values of serum SAA levels which were highly skewed were normalized with the decimal logarithm (log SAA). Mean values of mouse weight, serum HDL cholesterol levels and log-transformed serum SAA levels were calculated for each experimental group and each time point. The baseline mean values were compared separately for weight, HDL cholesterol and log SAA using factorial ANOVA with sex (male or female), infection [*P. gingivalis* or WC broth (control)], and an interaction term, sex-by-infection, in each model (STATA 9.0 software from

StataCorp. LP, College Station, TX, USA). Changes over time for each variable were assessed using Generalized Estimating Equation (GEE) models with two between-subject factors (sex and infection) and one within-subject factor (time; three levels for weight and HDL cholesterol, and 11 for log SAA). In initial models, the three-way interaction between time, sex and infection, as well as all possible pairwise interactions, were included. The three-way interaction and the sex-by-infection interaction were not statistically significant and were not included in the final models. Factorial ANOVA was used to assess whether the average atheroma lesion score was affected by sex and infection, and whether the difference between control and *P. gingivalis*-infected mice was the same for males and females. Finally, the bivariate associations between log SAA, HDL cholesterol and atheroma lesion score at time of death were evaluated by Spearman rank correlation. Level of significance for all analyses was set at 0.05.

Results

Clinical assessment

No evident clinical differences were noted between males and females. All *P. gingivalis*-infected mice exhibited signs of localized infection (subcutaneous chamber swelling) within 4–7 days after each infection, which usually subsided within 13–27 days. All *P. gingivalis*-infected mice rejected their first infected chamber approximately 20 days after the infection (21.4 ± 3.5 days for males and 19.2 ± 4.7 days for females, mean ± SD, $p = 0.24$, Student's unpaired *t*-test). Healing and return to general overall health coincided with exfoliation of the infected subcutaneous chamber. Not all infected mice had time to reject their second infected chamber before the time of killing. All mice presented with normal internal organ aspect at post mortem examination.

Baseline values of weight, serum HDL cholesterol levels and log-transformed serum amyloid A levels

There were no statistically significant sex-by-infection interactions for weight, HDL cholesterol or log SAA (Table 1; $p = 0.29$, 0.44 and 0.38, respectively), indicating that the average differences between the infected and control mice were similar for males and females. Moreover, prior to the first live *P. gingivalis* infection, the control and *P. gingivalis*-infected mice did not differ in their weight, HDL cholesterol or log SAA values (Table 1; $p = 0.42$, 0.40 and 0.90, respectively). However, mean baseline values of weight were significantly higher in males than in females (Table 1; $p < 0.01$ for sex, with females 5–6 g smaller). Interestingly, mean baseline values of HDL cholesterol were significantly lower in females than in males (Table 1; $p < 0.01$). Mean baseline values of log SAA were not significantly different between males and females (Table 1; $p = 0.10$).

Variations of mouse weight over time

The pattern of change in mouse weight over time was not statistically different between males and females ($\beta_{\text{sex-by-time}} = -0.01 \pm 0.01$, $p = 0.50$), although the pattern of differences between control and *P. gingivalis*-infected males increased over time. The pattern of change in mouse weight did differ between control and *P. gingivalis*-infected mice ($\beta_{\text{infection-by-time}} = -0.05 \pm 0.01$, $p < 0.01$). *Porphyromonas gingivalis*-infected mice tended to loose or not gain as much weight over time compared with control mice (Fig. 2A). On average, the weight of males was always significantly higher than the weight of females ($\beta_{\text{sex}} = 5.10 \pm 0.46$, $p < 0.01$).

Variations of serum HDL cholesterol levels over time

The pattern of change in HDL cholesterol values over time differed significantly between males and females ($\beta_{\text{sex-by-time}} = -0.24 \pm 0.09$, $p = 0.01$) and between control and *P. gingivalis*-infected mice ($\beta_{\text{infection-by-time}} = -0.19 \pm 0.09$, $p = 0.04$). *Porphyromonas gingivalis*-infected males exhibited a constant decrease in HDL cholesterol over time, while the other experimental groups did not (Fig. 2B).

Variations of log-transformed serum amyloid A levels over time

The pattern of change in log SAA values over time did not differ between males and females ($\beta_{\text{sex-by-time}} = -0.001 \pm 0.007$, $p = 0.95$), whereas it

Table 1. Comparisons of mean ± SD values of mouse weight, serum HDL cholesterol level and log-transformed serum SAA level at baseline, and aortic atheroma lesion score at the time of death, by factorial ANOVA

	Control		*Porphyromonas gingivalis* infected		Factorial ANOVA (*p* values)		
Variables	Males	Females	Males	Females	Sex	Infection	Interaction
Baseline values							
Number of mice	12	12	12	12			
Weight	27.9 ± 1.6	21.7 ± 1.7	27.4 ± 2.1	22.2 ± 1.6	< 0.01	0.42	0.29
HDL cholesterol	51.4 ± 10.9	32.3 ± 11.5	50.0 ± 6.4	36.8 ± 19.4	< 0.01	0.40	0.44
Log SAA	0.7 ± 1.1	0.1 ± 0.5	0.3 ± 1.1	0.1 ± 0.7	0.10	0.90	0.38
Time of death values							
Number of mice	12	12	11	11			
Lesion score	0.04 ± 0.05	0.05 ± 0.04	0.16 ± 0.07	0.15 ± 0.06	0.97	< 0.01	0.85

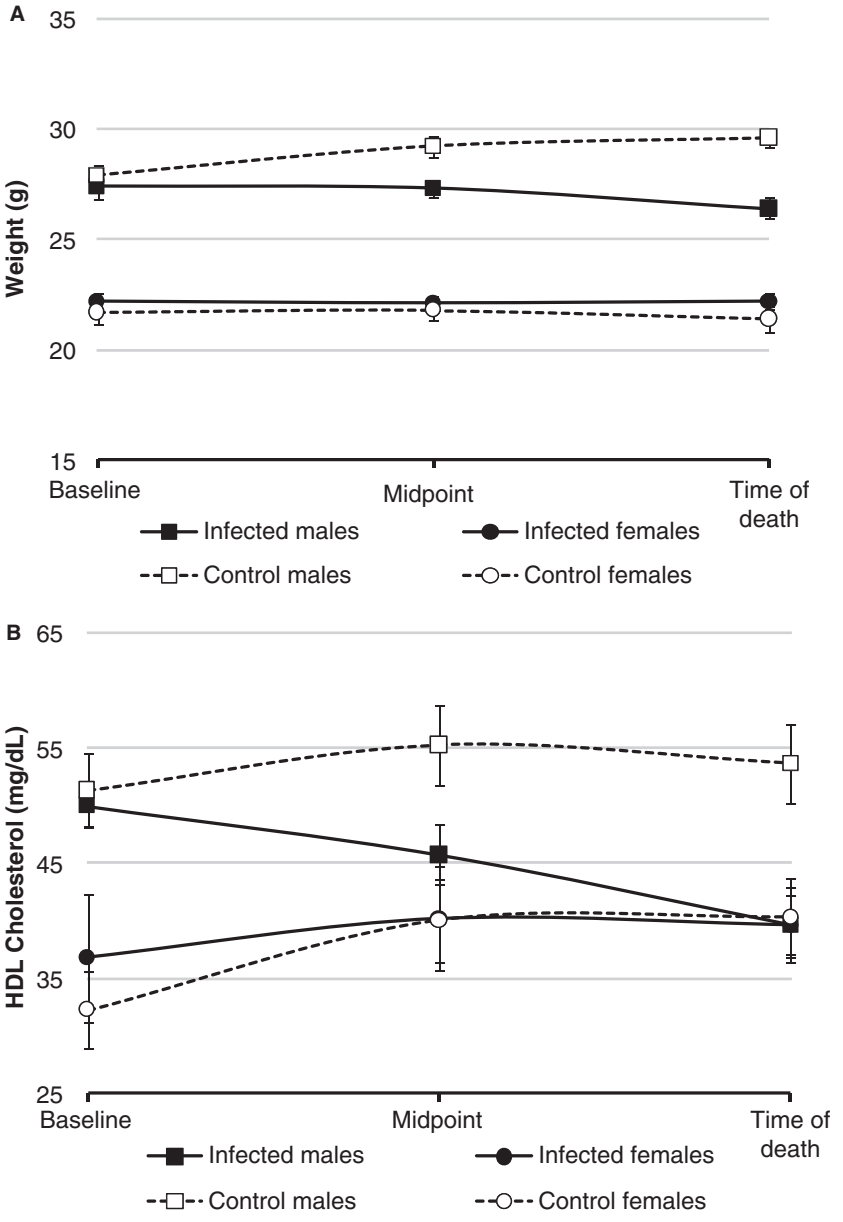

Fig. 2. Mean mouse weight (A) and mean serum HDL cholesterol level changes over time (B). Squares represent males and circles represent females. Open symbols and dotted lines represent control mice. Filled symbols and filled lines represent *P. gingivalis*-infected mice. Error bars represent SEM.

differed significantly between control and *P. gingivalis*-infected mice ($\beta_{\text{infection-by-time}} = 0.03 \pm 0.01, p < 0.01$). In control mice, log SAA remained fairly constant over time, while in *P. gingivalis*-infected mice log SAA increased by several-fold right after the first live infection and remained elevated until the time of death (Fig. 3). Moreover, on average, males had significantly higher log SAA values compared with females (Fig. 3; $\beta_{\text{sex}} = 0.44 \pm 0.16, p = 0.01$).

En face morphometric analysis of aortic atheroma lesion

Aortas from *P. gingivalis*-infected mice exhibited more red-stained, lipid-rich lesions than control mice (Fig. 4A). The lesions presented the aspect of early lesions, or fatty streaks, scattered along the aorta. The difference in mean atheroma lesion scores between control and *P. gingivalis*-infected mice was not influenced by sex ($p = 0.85$ for the interaction term sex-by-infection). There was no statistically significant difference in mean atheroma lesion score between males and females regardless of infection ($p = 0.97$). In contrast, the difference in mean atheroma lesion score between control and *P. gingivalis*-infected mice regardless of sex was significant ($p < 0.01$). Mean atheroma lesion scores were approximately threefold greater in *P. gingivalis*-infected mice compared with control mice, for both males and females (Table 1, Fig. 4B).

Bivariate associations between atheroma lesion scores, log-transformed SAA levels and serum HDL cholesterol levels

Increased serum levels of acute phase proteins and decreased serum levels of HDL cholesterol are risk factors for cardiovascular diseases and contribute to atherogenesis (9). Therefore, we investigated whether atheroma lesion scores were correlated to either log SAA values at time of death (day 41, log SAA_{41}) or HDL cholesterol values at experimental end-point ($HDL_{\text{end-point}}$).

The overall Spearman correlation coefficient (r_s) between atheroma lesion score and log SAA_{41} was positive ($r_s = 0.40$, $p = 0.01$), indicating that mice with high atheroma lesion scores also had elevated SAA levels at the time of death (Fig. 5). The correlation between atheroma lesion score and $HDL_{\text{end-point}}$ was not statistically significant ($r_s = -0.24$, $p = 0.11$), nor was the association between acute phase response (log SAA_{41}) and lipid metabolism ($HDL_{\text{end-point}}$; $r_s = -0.08$, $p = 0.60$).

Discussion

The biological pathways by which chronic periodontal infections may initiate and/or aggravate cardiovascular diseases remain unclear, particularly in how these pathways may differ between men and women. In this

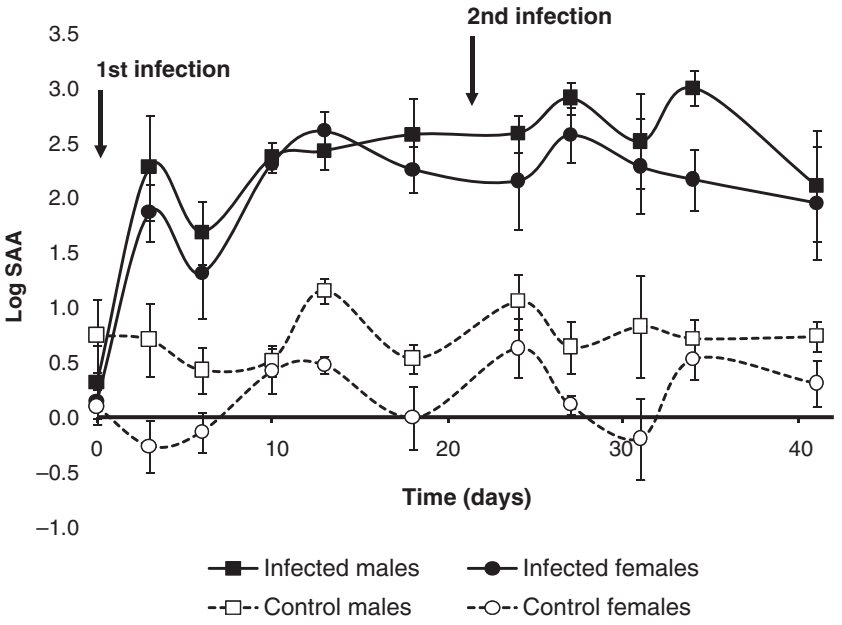

Fig. 3. Mean log-transformed serum SAA level changes over time. Squares represent males and circles represent females. Open symbols and dotted lines represent control mice. Filled symbols and filled lines represent *P. gingivalis*-infected mice. Error bars represent SEM.

study, we used a murine model of infection with the periodontal pathogen *P. gingivalis* to investigate whether male and female mice would respond differently to the infection in terms of systemic inflammation, lipid metabolism and atherosclerosis. Compared with humans, mice are less susceptible to develop atherosclerotic lesions, especially when they are fed a normal, controlled diet and their genetic background is minimally altered (26). Accordingly, in this study the control non-infected male and female heterozygous $ApoE^{+/-}$ mice fed a normal chow and killed at 4 months of age had low lesion scores. In comparison, the *P. gingivalis*-infected mice fed a normal chow presented with threefold higher lesion scores, suggesting that *P. gingivalis* infection contributed to initiate and/or exacerbate atherosclerosis. These results are consistent with previously published reports, using heterozygous $ApoE^{+/-}$ males challenged weekly by intravenous injections of *P. gingivalis* (20), or homozygous $ApoE^{-/-}$ males orally infected with *P. gingivalis* (21,22). Recurrent *P. gingivalis* bacteremia were further reported to induce atherosclerotic lesions in aorta and coronary arteries in pigs (33). Taken together, these results in animal models support the human epidemiological findings of an association between periodontal clinical presentation, as well as periodontal infection, with increased atherosclerosis as measured by increased carotid intima–media wall thickness (34,35).

One possible mechanism by which *P. gingivalis* infection may impact the development of atherosclerotic lesions is via the induction of a systemic inflammatory response, as measured by increased serum levels of inflammatory cytokines and acute phase proteins. We report here that each live *P. gingivalis* challenge was followed by a significant and rapid increase in serum levels of the acute phase protein SAA that persisted for the duration of the experiment until the time of death. Moreover, log SAA levels measured at the time of death were positively correlated with atheroma lesion scores. Significantly increased interleukin 1 (IL-1) and SAA levels have also been reported in the murine bacteremia model of *P. gingivalis*-enhanced atherosclerosis (20). The crucial contribution of the systemic inflammatory response to the development of atherosclerotic lesions was further confirmed using cross-over knock-out mice for the *ApoE* and the IL-1 receptor 1 (*IL-1R1*) genes (36), where ablation of the *IL-1R* gene resulted in decreased atherosclerosis. Interestingly, in these different knock-outs, the mice that exhibited the largest aorta atheroma lesions also had the highest SAA levels. In the oral infection model, a positive significant correlation was reported between serum interleukin-6 (IL-6) levels and aortic atheroma lesion size (21). Since IL-6 is one of the major inducers of hepatic synthesis of acute phase proteins, including SAA (31), these results are consistent with our findings.

In a murine model of induced atherosclerosis of non-infectious origin, a statistically significant correlation between log plasma SAA and extent of atherosclerosis was reported (37). Moreover, in these female LDL receptor-deficient ($LDLR^{-/-}$) mice, SAA levels measured at 5 weeks of age were correlated with lesions measured at 10 weeks, suggesting both a predictive role of SAA levels and a direct effect of SAA on the development of atherosclerosis. The latter effect was supported by the demonstrated binding property of SAA to proteoglycans, leading to the retention of SAA-enriched HDL particles to vascular proteoglycans, thereby contributing to lipid accumulation in the vessel wall (37). Serum amyloid A accumulation in atheroma lesions was further confirmed recently, in both $ApoE^{-/-}$ and $LDLR^{-/-}$ mice fed a regular chow (38). Therefore, there is evidence in the literature to support our findings that SAA levels might be a biomarker of ongoing atherosclerosis in mice, and to suggest a direct contribution of SAA to atherosclerosis in mice.

To our knowledge, none of the previous studies of *P. gingivalis* infection in mice investigated differences between males and females. Baseline serum HDL cholesterol levels were significantly higher in males than in females, as is usually the case in healthy mice maintained on a regular diet [Mouse Phenome Database (accessed January 24, 2008, at http://www.jax.org/phenome)]. Elevated serum HDL cholesterol levels are considered to be protective for cardiovascular diseases,

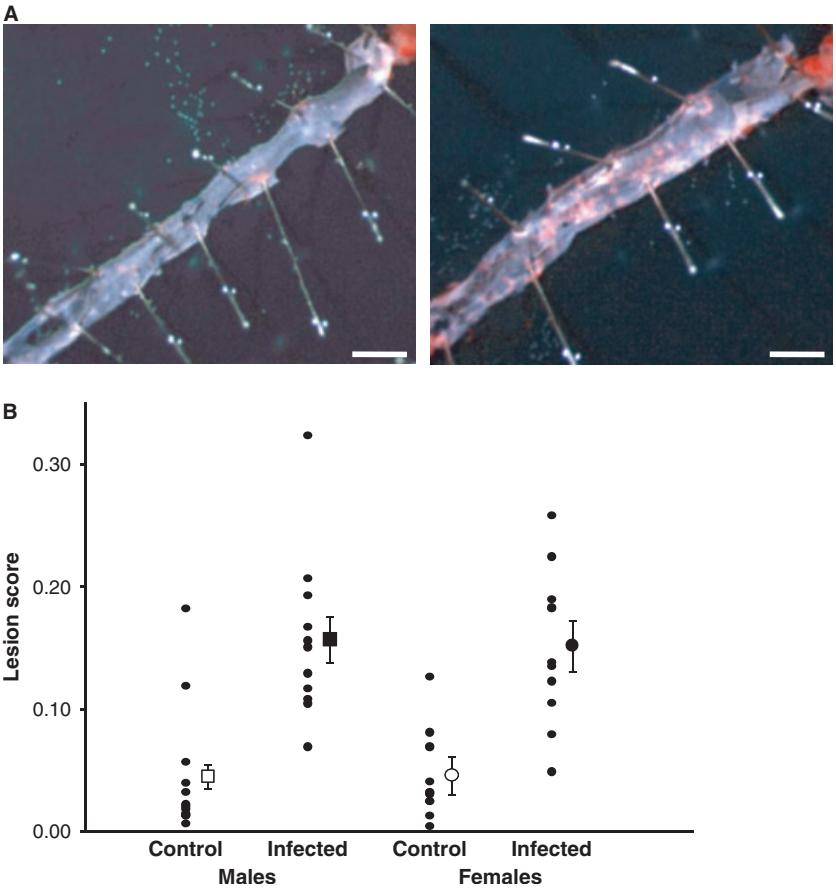

Fig. 4. (A) Representative *en face* histomorphometric analysis showing the absence of atheroma lesion in a dissected aorta from a control mouse (left panel) and the severity of atheroma lesion in a dissected aorta from a *P. gingivalis*-infected mouse (right panel). Sudan IV staining; magnification ×10. (B) Calculated percentages of aortic surface area covered by atheroma lesions (aortic atheroma lesion scores). Values for individual mice and means for each group are represented (open square for control males and filled square for *P. gingivalis*-infected males; open circle for control females and filled circle for *P. gingivalis*-infected females). Error bars represent SEM.

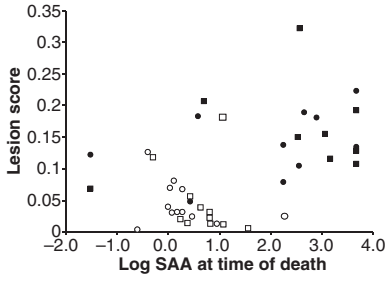

Fig. 5. Scatter plot representation of percentage of aortic surface covered by atheroma lesions (aortic atheroma lesion score) and log-transformed serum SAA levels (log SAA) at time of death. Values are plotted for each mouse ($n = 45$). Squares represent males and circles represent females. Open symbols represent control mice and filled symbols represent *P. gingivalis*-infected mice.

while elevated serum non-HDL cholesterol and elevated serum LDL cholesterol levels are considered to be risk factors for cardiovascular diseases and for the development of atherosclerotic lesions (39). Serum HDL cholesterol levels did not vary extensively over time in control males and females or in *P. gingivalis*-infected females. Serum HDL cholesterol levels decreased notably over time in *P. gingivalis*-infected males to the levels observed in the other groups. This is in agreement with findings from Hashimoto and colleagues that on average, serum HDL cholesterol levels decreased significantly in homozygous $ApoE^{-/-}$ males challenged weekly by intravenous injections of *P. gingivalis* (40). Overall, our findings suggest that in these young mice, *P. gingivalis* infection reduced the original difference between males and females, bringing the males to similar levels of risk for atherosclerosis as females.

In spite of the baseline differences observed in serum HDL cholesterol, we did not find any significant difference in aortic atheroma lesion scores between males and females. Mice were still relatively young at the time of killing (17 weeks of age), and atherosclerotic lesions take time to develop. In the intravenous infection model, heterozygous $ApoE^{+/-}$ male mice fed a normal low-fat diet, challenged weekly for 10 weeks with *P. gingivalis*, did not develop any significant atherosclerotic lesions (20). However, when challenged for at least 14 consecutive weeks with *P. gingivalis* a significant increase in atheroma lesion scores was observed (20,37). Moreover, the atherogenic effects of *P. gingivalis* and high-fat diet were shown to be additive in these mice, and SAA levels were significantly higher in infected mice fed a high-fat diet than in infected mice fed regular chow (37).

Our findings suggest that increased SAA levels could be considered as a biomarker of ongoing atherosclerosis in mice, although no causal effect of the chronic acute phase response on atherosclerosis was investigated; conversely, *P. gingivalis* may potentially act directly on the vessel walls and enhance atherosclerosis by interacting with the endothelium or smooth muscle cells. However, regardless of the mechanisms involved, these findings represent a proof-of-concept that chronic systemic inflammation and acute phase response secondary to infection with the major periodontal pathogen *P. gingivalis* may play a crucial role in the initiation and development of atherosclerotic lesions. Our findings suggest that differences between males and females in their response to *P. gingivalis* infection should be explored in murine models of atherogenesis, to help clarify the different associations observed in men and women between increased serum CRP levels, decreased serum HDL cholesterol levels, periodontal disease

severity and increased risk for cardiovascular events.

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