Establishment of a Mouse Model of Infection-Induced Atheroma Formation

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I. INTRODUCTION

Atherosclerosis is a common and insidious disease, which accounts for most of the morbidity seen in humans with coronary heart diseases and stroke. According to WHO report, cardiovascular diseases represent 20% of deaths worldwide (14 million annually), with 50% (leading cause) of all deaths in developed countries, and 16% (the third leading cause) of all deaths in developing countries. In Korea, 21% of death are due to complications associated with atherosclerotic disease[1].

Risk factors for atherosclerosis include: older age, male gender, smoking, body-mass index, levels of serum lipids, diabetes, hypertension, family history, and infection. Periodontal disease, as an infectious disease, has been associated with atherosclerosis in humans. Matilla has shown that dental infections are a major risk factor for adverse coronary events and overall mortality[2, 3]. DeStefano et al. has shown that men with severe periodontal disease have a 1.72 relative risk of death due to CHD as compared to men without periodontal disease[4]. It was concluded that an increased risk of coronary heart disease is associated with dental disease, particularly in young men under the age of 50. Recent evidence in the periodontal literature suggests that host inflammatory responses to bacteria, and not just the bacterial pathogens themselves, are responsible for the marked destruction found in severe periodontal disease. However, the molecular and biological mechanisms of this association are far from being understood. Several possibilities exist for the potential influence of chronic periodontal disease on the rate of progression of atherosclerosis. First, periodontal disease may alter host responses in such a way as to accelerate the atherosclerotic process. Second, an underlying alteration in immune function(for

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instance "hyperactive" monocyte/macrophage response[5, 6]) may increase the host susceptibility to atherosclerosis and periodontal disease, with no influence of the infection on the progression of atherosclerosis. Third, the above possibilities exist with the addition of bacterial infection of the atheromatous lesion (bacterial tropism to the atheroma) may be responsible for accelerating the progression of the atherosclerotic process with or without modulation of immune responses. Genetics is certain to play a significant role. Studies have shown various types of atherosclerotic and severe periodontal diseases are passed down through families. Another factor is the bacterial challenge itself[7]. Thus, it is proposed that these three major factors influence a hyperactive immune response: genetics, environment (diet and stress), and bacterial challenge.

Our researches have been focusing on developing an animal model to elucidate this mechanism of association between 2 disease entities. The subcutaneous chamber model of infection is a well-established and commonly used model of chronic infection with periodontal pathogens such as *Porphyromonas gingivalis* (*Pg*)[8, 9]. ApoE knockout $^{-/-}$ mice are highly susceptible to atherosclerosis when fed a high-fat diet[10]. Combined models can be used to evaluate the effects of periodontal infection on the development of atheroma lesions. By using the mouse as a model, these factors can be varied and outcomes assessed. The ApoE $^{+/-}$ is predisposed to atheroma lesions. This line will manifest atherosclerotic lesions in a relatively short period of time, as early as two months using high-fat diets, while C57B6 mice are predisposed to a much lesser degree to atherosclerosis and require six months of high-fat feeding to show mild atheroma formation. Previously, Gibbs et al[11] used ApoE heterozygous (ApoE$^{+/-}$) mice with a C57B6 background mouse line which were chronically infected with a non- disseminating strain of *Pg,* HG-405. This experiment failed to demonstrate an atherogenic response using *Pg* strain HG-405, in mice fed either a normal or a high-fat diet(Table 1).

This present study was aimed to examine the effects of chronic infection with a more toxic, disseminating strain of *P. gingivalis*, in the heterozygous ApoE+/- mice maintained on a normal diet, on local inflammation, and on short-term aortic atheroma formation. The hypothesis in this experiment was that disseminating strains of *P. gingivalis* (such as A7436) could enhance atheroma formation as compared to non-disseminating strains (HG-405) and infection-mediated cytokines could induce hyperlipidemia and thereby enhance atheroma formation.

Table 1. Atherogenic effects of normal and high-fat diet on wild type (C57B6J) and ApoE+/- mice, using a non-disseminating strain of *Pg* (HG-405)[11]

Mice	Diet	Challenge	Extent Lesion
C57B6J	normal	none	none
		HG-405	none
C57B6J	high-fat	none	+
		HG-405	+
ApoE+/-	normal	none	+
		HG-405	+
ApoE+/-	high-fat	none	+++
		HG-405	++

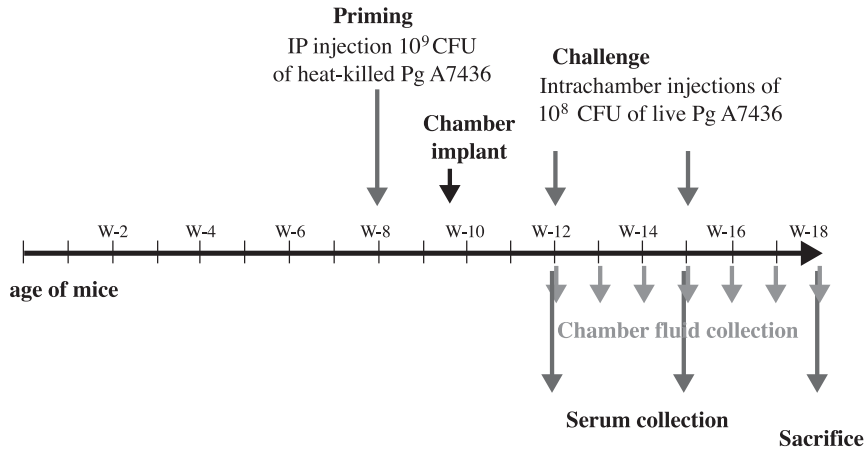


Figure 1. Animal protocol timeline.

II. MATERIALs and METHODs

C57B6J Mice were purchased from Jackson Labs (Barharbor, ME) at 6 weeks of age and ApoE$^{-/-}$ mice were supplied from Dr. Maeda's lab in UNC Medical School. Heterozygous mice ApoE$^{+/-}$ were bred using female ApoE$^{-/-}$ and male C57B6J reproductive pairs. Approximately nine weeks was needed to produce the ApoE$^{+/-}$ mice ready for the experiment. Female ApoE$^{+/-}$ mice were maintained on normal chow diet(from Purina) after weaning at 4 weeks of age. Genotype was confirmed by PCR using specific primers and genomic DNA extracts prepared from tail clippings.

Mice were immunized at week 8 with an intraperitoneal injection of either broth (bacterial culture medium) or a preparation of heat-killed *P.g.* strain A7436 (10^9 CFU/100㎕). Two subcutaneous chambers (0.4 X 1cm, 12 stainless steel coil) were implanted bilaterally in the flanks of each mouse at week 10 under general anesthesia by intraperitoneal injection of 0.10~0.15 ml/mouse of mixture of Ketaset (0.062 ml) and Rompum (0.22 ml) in saline (3.16 ml). Animals were randomized for the four groups: 1) live challenge with *Pg* A7436 and high fat diet (western type); 2) live challenge with *Pg* A7436 and low fat diet; 3) chamber injection with medium alone and high fat diet; 4) chamber injection with medium alone and low fat diet. The high fat diet was a mixture of 1 part of Thomas Hartroft diet (Teklad (TD88051) Test Diets, Madison, WI.) and 3 parts of Purina Breeder chow (resulting in the composition of total fat 15%, cholesterol 1.25%, and cholic acid 0.5%). At weeks 12 and 15, mice were challenged by injecting into chamber with either 100㎕ broth or a suspension of live *P.g.* (10^8 CFU/100㎕).

Animals were monitored weekly until sacrifice for clinical signs of cachexia and weight loss and the clinical response of chamber and surrounding tissue. The sloughing of coil chamber was monitored as result of inflammation and the day of sloughing documented. Chamber fluid samples were collected at days 0, 7, and 14 after each challenge and processed for evaluation of inflammatory mediator levels. Blood samples were collected by retro-orbital bleeding at weeks 12, 15, and 18, and further processed for evaluation of lipid levels and inflammatory mediator levels. At weeks 18, the mice were fasted overnight prior to being overdosed with Ketaset /Rompum mixture. The chest was opened

and the heart and vascular trees perfused with fresh 4%(wt/vol) phosphate-buffered (0.12 mol/L) paraformaldehyde (ph 7.4). The heart and attached aorta and other organ tissues were removed and placed in fresh paraformaldehyde until processed.

1. Evaluation of Chamber Fluid

The syringe was chamber fluid samples were collected by aspiration in a 1cc syringe containing loaded with 100㎕ of PBS buffer (to wet and prevent sticking of sample to the syringe) and weighed, and volume of collected fluid was estimated by weight (1 g = 1 ml). Collected fluid samples were stored frozen (-80℃) until analysis.

For chamber *Pg* verification, The 10 ㎕ aliquots of chamber fluid, already diluted into 500 ㎕ of RIA buffer, was plated on a Wilkins-Chalgren plate and grown in under anaerobic conditions at 37℃ for 4-5 days. *Pg* scores were determined by counting colonies as follows: *Pg*-score=0 for no colonies, *Pg*-score=1 for 1-10 colonies, *Pg*-score=2 for 10-100, *Pg*-score=3 for non-confluent but greater than 100 colonies and *Pg*-score=4 for confluent colonies[11].

Levels of inflammatory mediators were measured by ELISA using commercially available kits (Cytoscreen mouse IL-1β , mouse IL-6, mouse TNF-α, BioSource International, Camarillo CA; PGE_2, Cayman Chemical Company, Ann Arbor, MI). Chamber fluids were diluted (1:10 for IL-1β, 1:50 for IL-6; 1:200 for PGE_2, and 1:50 for TNF-α) and measured quantities were expressed in pg/ml.

Finally, measured amounts of mediators were divided by chamber fluid weight collected and expressed in ㎍/ml.

2. Evaluation of Serum Lipid Levels

Blood samples were allowed to coagulate and plasma was collected after centrifugation and stored at -80℃ until analysis. Serum levels of total cholesterol and triglycerides were determined using diagnostic kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan, and SigmaDiagnostics, Inc., St. Louis, MO, respectively) by colorimetric enzymatic assay adapted to a microplate reader. Results were expressed as mean±SE of the average values obtained at weeks 15 and 18 for each animal and for each group of animals .

3. Evaluation of Atheroma Lesion

Mice were sacrificed by transcardial perfusion with 0.1M phosphate buffer containing 1 unit/ml Heparin sodium for 5 minutes, followed by 0.1M phosphate buffer containing 4 % paraformaldehyde for 10 minutes. The heart with the surrounding tissues was dissected and incubated in the same fixative formaldehyde solution for 48 hours at 4°C. Heart and aorta were cleaned from surrounding tissues, leaving the ascending aorta intact according to the method described by Paigen[12]. The upper part of the heart, containing the aorta was embedded in 25% gelatin, and sectioned on a line according to the tips of the atria, moving from the heart towards the aorta. This is at an angle corresponding to that which the aorta exits from the heart. Five mm-thick sections were mounted on slides and dried. The aortic sinus became apparent, as evidenced by the non-round shape of the aorta, with the aortic valves also being seen. When a round aorta is present again, and the valves are still clearly seen, sections are made every 10㎛ on a cryostat. Alternate 10㎛ sections were fixed on gel-coated slides. Histological sections were stained with Oil red O, and counterstained with hematoxylin.

For scoring of atherosclerotic lesions, five sections at 80㎛ intervals were evaluated for the cross-sec-

tional area of lesion, beginning where the aorta was rounded and valves appeared distinctly through to the endpoint where the valves disappeared, a distance of approximately 350㎛. These sections were evaluated on the basis of total foam cell deposit area divided by the aortic luminal area. Quantitative evaluation of the atherosclerotic lesion was performed by direct light microscopy and digital analysis of captured video images with the Scion Image software downloaded from the NIH. This software calculates the atheroma lesion score as the ratio of the outlined area of lesion by the area of lumen.

4. Statistical Analysis

Time and group effects on chamber cytokine levels and atheroma scores was determined by 1 way analysis of variance. Significant differences, if present, were compared using paired or non-paired T-test. Exploratory regression modeling for atheroma scores using cytokines for predictive variables may be considered.

III. RESULTs

1. Chamber Infection Outcome

When an infection has established in the chamber space, an immune response produced the cardinal signs of inflammation, erythema and edema. An established infection by intrachamber innoculation led to sloughing of the chamber. A striking difference was seen in chamber slough between high-fat and low-fat diet groups . The high-fat group rejected both chambers in 5 out of 8 animals (52.5%) and the low fat rejected 100 % out of 7 animals. The 1st chamber sloughed in 15 days in 6 among 8 animals(75%). and the second chamber in 10 days in 7 among 8 animals (87%) in high fat group and in low fat group in 12 days and 9 days in all animals(100%) after challenges. This slough of 1st chambers occurred earlier in low-fat fed group than high fat group.

As for *Pg*-scores, different patterns were seen between the various groups and strains. For both strains of mice the *Pg*-scores of the high-fat slough group were mostly in the 3 and 4 category indicating that the *Pg* levels remained very high until the chambers were sloughed. A different pattern was seen in the non-sloughing groups (high-fat and low-fat diets). The *Pg*-scores for both high-fat and low-fat animals with non-sloughing chamber were less than 2 or 0 categories. There was no difference in *Pg* score between high-fat and low-fat groups. Also, the $APOE^{+/-}$ strain tended to have more mice in the higher *P.g.*-Score categories as compared to the C57B6J strain.

2. Chamber Fluid Cytokine level

Chamber fluid levels of inflammatory mediators IL-1β and PGE_2 were significantly increased in *P. gingivalis*-challenged animals as compared to the non-challenged animals at $p < 0.05$. IL-6 was detected in high level in *P. g* challenged mice, compared to non-dectable level in the control animals. TNF-α level showed large individual variations and no significant difference between the challenged and non-challenged groups.

On low fat diet, the post-challenge chamber levels of IL-1β and TNF α were significantly higher in *Pg* challenged animals compared to non-challenged animals ($p<0.05$), while IL-6 and PGE_2 levels were not. On high fat diet, the post-challenge chamber levels of PGE_2 were significantly higher in *Pg* challenged animals compared to non-challenged animals($p<0.05$), while the other three cytokines had only the trend to increase in *Pg* challenged animals($p>0.05$). IL-1β level in challenged animals was

Table 2. Effect of chronic infection with a disseminating strain of Pg (A7436) on chamber fluid levels of inflammatory mediators in ApoE+/- mice maintained on a normal (low fat) and high fat diet

		IL-1β	IL-6	PGE2	TNF-α
LFD	Non-Challenged (n=6)	1.3±0.6	0	309.9±10.0	0
	P.gingivalis-Challenged (n=7)	122.2±4.1	11.9±1.6	791.5±9.2	6.0±1.1
HFD	Non-Challenged (n=6)	8.87±1.42	19.49±1.53	111.15±3.14	6.10±1.29
	P.gingivalis-Challenged (n=6)	49.76±3.94	43.38±4.73	661.21±10.83	23.58±2.18

Values represents mean±SE(㎍/ml) for each group of mice.

Table 3. Effects of P. gingivalis infection on lipid profiles in mice

		cholesterol(mg/dl)	triglycerides(mg/dl)	HDL(mg/dl)	HDL/cholesterol
LFD	Non-Challenged (n=6)	50.88±0.68	55.91±0.61	23.82±1.11	48.0±1.60
	P.gingivalis-Challenged (n=7)	60.55±0.34	92.48±2.26	24.65±1.27	40.39±1.67
HFD	Non-Challenged (n=6)	223.42±2.01	38.46±1.19	14.11±0.82	5.31±0.55
	P.gingivalis-Challenged (n=6)	278.48±2.64	38.81±1.09	15.66±0.90	7.55±0.69

Values represents mean±SE(mg/dl) for each group of mice.

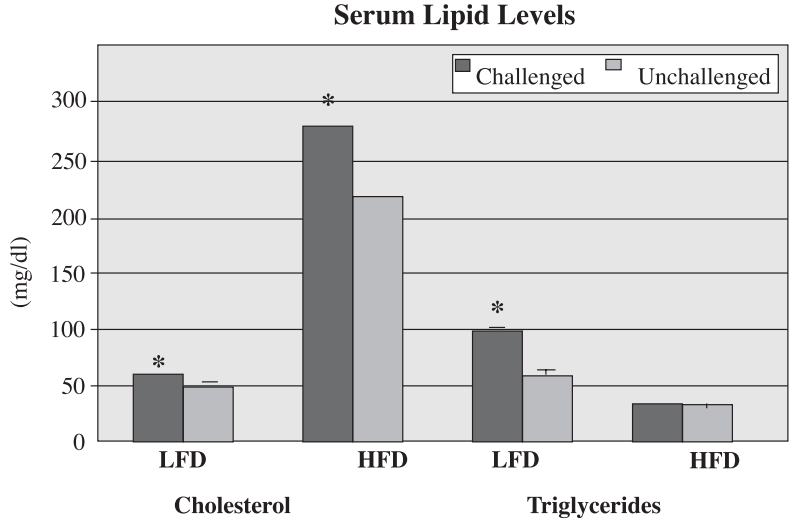
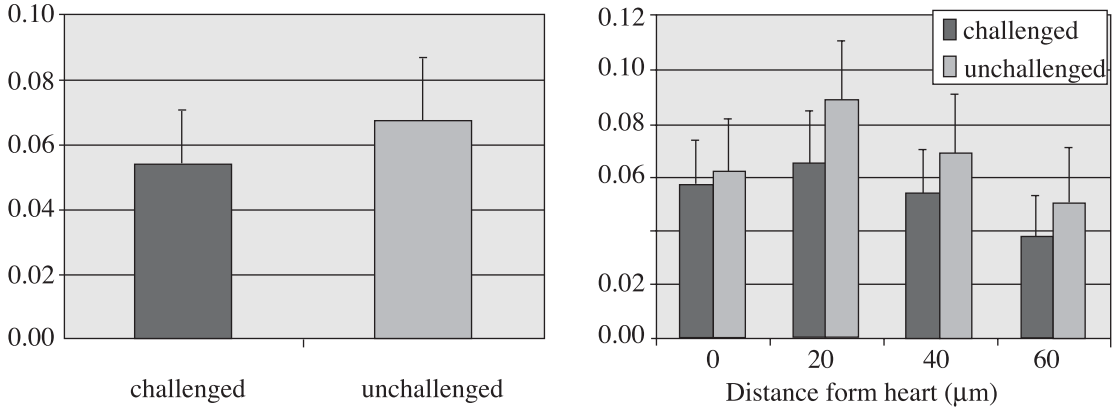


Figure 2. Effects of chronic P. gingivalis (A7436) infection on serum lipid levels in ApoE+/- mice maintained on a regular (low-fat) diet or high- fat diet. (* means the differences between 2 groups at P value 〈 0.05).

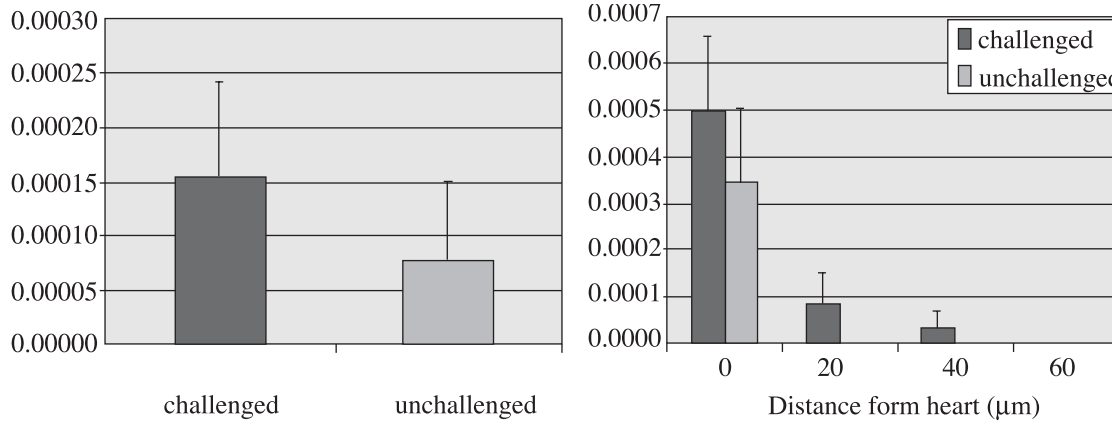
significantly higher in low fat group than in high fat group(p〈0.05).

3. Plasma lipid profile

Serum levels of both total cholesterol and triglycerides were increased in *P. gingivalis*-challenged animals compared to the non-challenged animals. In low- fat diet group, *P. g* challenge resulted in significant increase of cholesterol level and triglyceride both(P 〈 0.05, non-paired t test). Blood HDL level and HDL/total cholesterol ratio were significantly



A. ApoE$^{+/-}$ mice maintained on a high fat diet.



B. ApoE$^{+/-}$ mice maintained on a normal (low fat) diet

Figure 3. Effect of chronic infection with a disseminating strain of Pg (A7436) on atheroma lesion scores in ApoE+/- mice maintained on a high fat(A) and low fat normal diet(B).Plotted values represent calculated means, and error bars represent SEM, for each group of mice. Number of animals per group were: 8 challenged and 6 unchallenged high fat diet; 7 challenged and 6 unchallenged low fat diet. Comparisons between challenged and unchallenged mice were not statistically significant (P = 0.6 for high fat diet and 0.3 for low fat diet groups (t test).

higher in low- fat diet group compared to the high-fat group, and showed no signigicant difference between challenged and non-challenged groups.

4. Aorta Atheroma Score

The aorta atheroma formation was characterized as follows: non-challenged high-fat diet group(64775±89㎛²)〉 challenged, high-fat diet group (48104±35㎛²) 〉〉〉challenged, low-fat diet group 〉nonchallenged, low-fat diet group. Overall, lesion size was much greater for the APOE$^{+/-}$ high-fat group than the APOE$^{+/-}$ low-fat group. The mean total area and extent of atherosclerotic lesions were greater in *P. gingivalis*- challenged animals, as compared to non-challenged animals (162 ± 8 vs 89 ± 8㎛²), respectively in low- fat diet.

In ApoE$^{+/-}$ mice maintained on a high fat diet, a

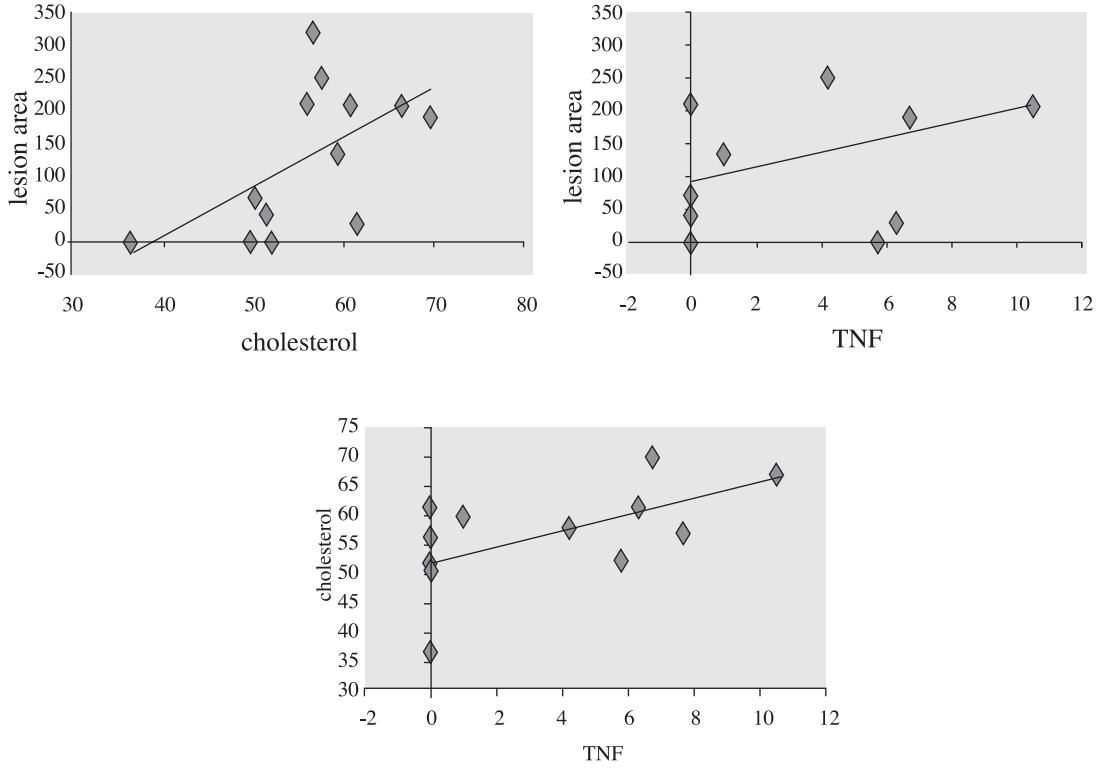


Figure 4. Associations between atheroma lesion, serum cholesterol levels, and chamber TNFa levels in ApoE+/- mice maintained on a low fat diet, infected or not with the disseminating strain of Pg A7436. The chamber fluid cytokine and blood lipid levels were plotted versus the aorta atheroma lesion to examine the data for a correlation between these outcomes. Atheroma lesion size was significantly correlated to serum cholesterol levels (R2=0.58), and chamber fluid levels of TNF-α (R2=0.38) and serum cholesterol levels were correlated to chamber fluid levels of TNF-α(R2=0.60).

chronic infection with the disseminating strain of *Pg* (A7436) did not increase the atheroma lesion size at any of the section levels examined (Figure 3A). However, in ApoE$^{+/-}$ mice maintained on a normal diet, a chronic infection with the disseminating strain of *Pg* (A7436) induced an increase in the size of atheroma lesion, especially at the section levels closest to the heart (Figure 3B).

When the chamber fluid cytokine and blood lipid levels were plotted versus the aorta atheroma lesion, atheroma lesion size was correlated to serum cholesterol levels (R^2=0.58, P=0.038), and chamber fluid levels of TNF-α (R^2=0.38, P=0.199) and serum cholesterol levels were correlated to chamber fluid levels of TNF-α (R^2=0.60, P=0.029)(Figure 4.)

IV. DISCUSSION

Growing evidence form case-control and population-based studies supports an association between periodontal disease and atherosclerosis[2-5]. but does no define whether the relation is casual or otherwise. Recent evidence in the periodontal literature suggests that host inflammatory responses to bacteria, and not just the bacterial pathogens themselves, are responsible for the marked destruction found in

severe periodontal disease. That is, the metabolic machinery used to destroy the periodontal tissues and bone is derived mostly from the host.[6] Vascular pathology caused by immunologic reaction to bacteria appears to be similar to that caused by atheroma formation. In addition to dietary and genetic factors, some authors suggest that bacterial infections of unknown origin may contribute to acceleration of cardiovascular disease[13-15].

The present study was designed to test whether innoculation of an established periodontal pathogen into subcutaneous chamber imitating periodontal infection contributes to the development and progression of atherosclerosis in a susceptible animal model. The $ApoE^{+/-}$ mice used in this study have on 1 functional ApoE gene and are more susceptible to atherosclerosis than are normal mice (C57BL/6)[-10], unlike $ApoE^{-/-}$ mice, in which atherosclerosis spontaneously develops. This decreased ability to produce Apo-E protein negatively alters serum lipid profiles when the animals are subjected to a high-fat diet. By using these mice as model, these factors can be varied and outcomes assessed. We utilized surgical stainless steel wire coil implanted subcutaneously[8,9].Using this chamber model, it's known that this sequestered space permits a minimal infectious dose that is reduced by more than 100x over the non- chamber subcutaneous challenge model. This line would manifest atherosclerotic lesions in a relatively short period of time, as early as two months using high-fat diets[11], while C57BL/6 mice are predisposed to a much lesser degree to atherosclerosis and require six months of high-fat feeding to show mild atheroma formation. Being challenged with noninvasive strain of *Porphyromonas gingivalis* (Pg HG405), the $ApoE^{+/-}$ mice produced more pronounced lesion and much higher PGE_2 in chamber fluid and compared to C57BL/6 mice regardless of diets. The chamber cytokine response to infection of increased PGE_2 secretion was severe in C57BL/6 mice on a high fat diet than mice on a low fat diet and similar in $ApoE^{+/-}$ mice between high and low fat diet. However, chronic exposure to noninvasive strain of *P. gingivalis* led to neither accelerated progression of atheroma formation in the aorta nor increased chamber PGE_2 production in susceptible mice. In this previous study, *Pg* challenge did not increase atheroma lesion in high-fat diet group, in contrast to increasing lesion in low-fat diet group. It was reported that lipoproteins bind and inactivate bacterial endotoxin and lipoprotein-bound endotoxin is less able to elicit TNF release by macrophages[16-18]. This protective effect of high serum lipoprotein in $ApoE^{+/-}$mice maintained high fat-diet could explain why bacterial challenge didn't show any significant effect on the lesional progression and PGE_2 production.

In this study, interval of challenge was determined as 2~3 weeks, based on the Genco et al[7,8] that viable isolates were recovered from the chamber fluid to 14 days in immunized Balb/c mice. We have confirmed this by taking culture of the chamber fluid aliquot Until chambers sloughed off 7~10 day after Pg inoculation, chamber fluid aliquots (10㎕) resulted in more than 100 colonies. This finding means presence of 50x100 viable cells in whole chamber fluid(total 500㎕ after being diluted in PBS) and compared to the original bacterial cells (10^9) inoculated, the recovery rate was above the level of 1/200000. A striking difference was seen in chamber slough between high-fat (52.5%) and low-fat diet groups (100 %) . This sloughing of 1st chambers occurred earlier in low-fat fed group than high fat group. This result may indicate that dietary factors modulate the inflammatory response (proinflammatory cytokine) to the Gram negative bacteria *P. gingivalis*. Especially increased blood fat level could neutralize the endotoxicity. Interestingly, no differ-

ence was detected between the high-fat and low-fat diet groups for the APOE+/- strain. Chamber fluid levels of inflammatory mediators IL-1β and PGE_2 were increased in *P. gingivalis*-challenged animals as compared to the non-challenged animals. On low- fat diet, the post-challenge levels of IL-1β and TNF α were significantly higher in *Pg* challenged animals compared to non-challenged animals, while IL-6 and PGE_2 levels were not. On high fat diet, the post-challenge chamber levels of PGE_2 were significantly higher in *Pg* challenged animals compared to non-challenged animals, while the other three cytokines had only the trend to increase in *Pg* challenged animals.. IL-1β level in challenged animals was significantly higher in low fat group than in high fat group

Blood levels of both total cholesterol and triglycerides were increased in *P. gingivalis*-challenged animals. In low- fat diet group, *Pg* challenge resulted in significant increase of cholesterol level. Blood HDL level and HDL/total cholesterol ratio were significantly higher in low-fat diet group compared to the high-fat group, and showed no significant difference between challenged and non-challenged groups. This result implicate that gram(-) bacterial infection could affect the lipid metabolism and result in hyperlipidemia depending on the fat contents in diet. These results provide evidence that this process could be mediated by cytokine production under the influence of bacterial infection and result in increase in the level of neutral fat and contribute to the atheroma formation. In this process, intraoral periodontal infection, as one very common entity of chronic localized infection, could be the etiologic or contributing factor. Therefore, further researches are need to elucidate the mechanism and the predisposing factors.

The aorta atheroma formation was different according to the diet fed and Pg challenge, as follows: non-challenged high-fat diet group〉challenged, high-fat diet group 〉〉〉challenged, low-fat diet group 〉nonchallenged, low-fat diet group. Overall, lesion size was much greater in high-fat group than low-fat group. The lesion was confined to aorta area adjacent to heart in low-fat diet group whereas it was located extensively in high-fat diet group. In low-fat diet, the mean total area and extent of atherosclerotic lesions were increased in *Pg* challenged animals, compared to non-challenged animals. These data suggest that *P. gingivalis* infectious challenge enhances atheroma lesion formation ion low-fat diet group, associated with an increase in local inflammatory mediator levels. However, the protective effect of high serum lipoprotein in $ApoE^{+/-}$mice maintained high fat-diet could explain why bacterial challenge didn't show any significant effect on the Il-1β and TNF-α level and lesional progression.

The atheromatous lesion in low-fat diet group was so small even though the bacteria was challenged. $ApoE^{+/-}$ mice which were frequently challenged with disseminating *Pg* strain for long periods (longer than 3 months) would be expected to develop atherosclerotic plaques earlier and to a greater degree than non-challenged mice. Li et al[19] investigated the effect of repeated systemic inoculations with *Pg*, a putative periodontal pathogen, on the progression of atherosclerosis in $ApoE^{+/-}$ mice. In their experiment, 10-week-old, male $ApoE^{+/-}$ mice fed either a high-fat diet or regular chow were inoculated intravenously with live *Pg* (10^7 CFU) or vehicle once per week for 10, 14, or 24 consecutive weeks(relative long period). Atherosclerotic lesions of the proximal aortas and aortic trees were more advanced in *Pg*-challenged animals than in vehicle control animals and occurred earlier (at 14 weeks) when no lesions were apparent in control animals. At 24 weeks after inoculation, proximal aortic lesion

size quantified by histomorphometry was 9-fold greater in chow-fed mice inoculated with *Pg* than in noninoculated mice and was 2-fold greater in *Pg*-inoculated versus noninoculated high-fat diet fed mice; all atherosclerotic lesions were macrophage-rich. *Pg* ribosomal DNA was found in the aortas, livers, and hearts 24 weeks after inoculation. These results provide evidence that long-term systemic challenge with *Porphyromonas gingivalis*, an oral pathogen, can accelerate atherogenic plaque progression. Li et al's study did not prime the mice and used less viable bacteria cells.

As for the relation between bacterial infection and atherosclerosis, other lines of evidence exist that implicate non-dental local infections as risk factors for heart disease[20-22]. Controlled clinical studies have suggested that "milder bacterial infections" like respiratory and dental infections are risk factors for CHD. Valtonen's review of the literature on this subject concludes that a growing amount of evidence is present to link infection and atherosclerosis.[15] Chronic Chlamydia pneumonia infections have been well known to be associated with coronary heart disease[26, 21]. Certainly, more severe infections are seen to be major risk factors for acute myocardiac infarction and stroke. In the more acute infections, thrombosis formation may be mostly related to hypercoagulable states. Lipid and prostaglandin metabolisms are significantly altered by acute infections[15].

Multiple metabolic effects can be seen in response to LPS or infection. Triglyceride clearance may be reduced in response to LPS challenge due to reductions in lipoprotein lipase activity. Increases in VLDL levels may be seen due to increased de novo synthesis of fatty acids or re-esterification of fatty acids[23, 24]. Bacterial challenge induces the production of several inflammatory mediators known to change the metabolism of fatty acids. In the rat model, TNF is thought to mediate lipogenesis through stimulation of interleukin-6 production which stimulates hepatic lipogenesis by increasing hepatic citrate concentrations[25]. Thus, inflammatory mediators may have a role in the induction of hyperlipidemic states, especially in individuals which overproduce these cytokines. It appears that inflammation can lead to hyperlipidemic states, which may then increase inflammatory responses, presenting a possible vicious cycle[26].

In conclusion, the disseminating strain of *Pg* A7436 appears to be atherogenic, although mild, in $ApoE^{+/-}$ mice maintained on low fat diet.

Chronic and repetitive infection with the disseminating strain of *Pg* A7436 triggers a local inflammatory response that is associated with an elevation of serum cholesterol levels and with the magnitude of the atheroma score.

V. CONCLUSION

In this particular mouse model of the effect of disseminating strain of *Pg* A7436 in $ApoE^{+/-}$ mice maintained on low fat diet *Pg* infection leads to increased local inflammatory mediator levels, some of which were significantly associated with enhanced atheroma formation.

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