Prior Immunization with *Fusobacterium Nucleatum* Interferes with Opsonophagocytosis Function of Sera against *Porphyromonas Gingivalis*

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I. Introduction

Fusobacterium nucleatum(Fn) is one of major periodontal pathogens which are most frequently recovered in subgingival areas of gingivitis patients[1,2]. The organism has been designated as an intermediate colonizer having receptors for both early colonizers and late colonizers, which enable them to behave as one of key pathogens in bacterial colonization process in the unique subgingival niche[3]. Clinically, conversion of gingivitis into destructive periodontitis has been reported to be closely associated with switching of the intermediate colonizers into the later colonizers[3,4]. The pivotal role of Fn in initiating destructive periodontal disease may be attributed to its immune modulating role[5]. Moore[6] stated that Porphyromonas gingivalis(Pg) may be important in some individuals, but it appears after much damage is already done, probably initiated by Fn. Further he claimed that Fn is the principal and most frequent cause of gingival inflammation that initiates periodontal disease[5].

Fn has a potent immune modulating activity for secondary immune response to Aa[7]. In a mixed infection with Pg, Fn has synergistic effects on virulence[8,9]. Fn - induced cultures produced more anti - Pg antibody than others[10]. It is highly probable that colonization of Fn subgingivally may modulate immune responses at B - or T - cell levels leading to synergistic or potentiating effects on Pg virulence. This phenomenon might result in immune deviation of serum antibody with inadequate functional capability of opsonophagocytosis of specific periodontal pathogens. Further, this events may skew antigen - specific T cell subset polarization resulting in deviation of antigen recognition11.

Elevated antibody response in patients with destructive periodontal disease has often been related to sub - optimal level of

protective antibody (opsonopahgocytosis)[12,13], while post-immune sera obtained with experimental immunization using a single periodontal pathogen demonstrated satisfactory level of protective function against the homologous bacterial challenge[14]. The reason is unclear why elevated IgG responses to periodontal pathogens do not necessarily reflect their functional adequacy. Such an immune deviation might be derived from the fact that destructive periodontal disease is a cumulative result of immunopathologic process responding to an array of colonizing microorganisms sequentially infecting in the subgingival environmental niche. Consequently, Fn may initially prime the immune cells and modify their responses to the successive organism, Pg. This could explain why one frequently observes non-protective serum antibodies to Pg in early onset periodontitis patients in a striking contrast to the results obtained from Pg-immunized experimental animals.

The aim of the present study was to compare the opsonophagocytosis function of serum antibody obtained from mice immunized with a single bacterial species, Fn, and those which were immunized with Fn prior to immunization with Pg.

II. Materials and Methods

1. Immunization of mice

Briefly, Fn ATCC 10953 and Pg 381(kindly provided by Dr. Schifferle, SUNY Buffalo, Buffalo, NY) were grown in anaerobic chamber. Whole cells were washed and resuspended in pre-reduced half-strength Ringer solution. 10 Balb/c mice(Group 1) were immunized by two intraperitoneal injections of 5×10^8 cells of Fn followed by two subsequent injections of 5×10^8 cells of Pg . Another 10 mice(Group 2) were immunized by two injections of 5×10^8 cells of Pg. Each immunization was made at two-week interval.

2. Determination of IgG titer

Sera were obtained at baseline and 10 days following the final immunization, and serum IgG levels against Pg 381 or Fn 10953 were determined by ELISA by the method described previously15. Briefly, formalin-fixed bacterial cells were diluted in phosphate buffer(10 ug/ml as determined by Lowry method) and coated on microtiter ELISA plate. After 24 hours of incubation at 4 ℃, plates were washed in phosphate buffered saline(PBS) containing 0.05 % Tween 20(PBS/Tween). Serially diluted mouse serum samples were added to each well and incubated for 2 hours at room temperature. Plates were washed three times with PBS/Tween and peroxidase-conjugated rabbit anti-mouse IgG(H+L, Jackson ImmunoResearch Laboratories, West Grove, PA) were added to each well and incubated for 2 hours at room temperature. After being wahsed three times with PBS/Tween, tetramethylbenzidine were added to each well and incubated for 15 minutes at room temperature followed by adding 0.18 M H_2SO_4 to stop the reaction. Optical densities read at 450 nm of wavelength were plotted as a function of serum dilution factor and regression analysis was

done. One of the pre-immune control serum was assigned an ELISA unit of 100 and serum IgG titer of other control and test sera were determined. For a statistical comparison of antibody levels between groups, a paired Student's t-test was performed.

3. Opsonophagocytosis assay

Opsonophagocytosis assay of post-immune sera was done by the method previously described16,17. Briefly, Pg cells was washed in PBS and resuspended in DAPI(4',6-diamidino-2-phenylindole dihydrochloride, 1mg/ml). Polymorphonuclear leukocytes(PMNL) were separated from peripheral blood and were resuspended in Hank's balanced salt solution(Life Technologies, Grand Islands, NY) to be used for phagocytosis. Mixture of sera, stained bacterial cells, PMNL and propidium iodide were incubated at 37°C for 15 minutes. After cytospin, the specimen were fixed and covered with cyanoacrylate. Under the fluroscent microscope, % of

Table 1. Pre-immune and post-immune serum IgG titer against P. gingivalis 381 or F. nucleatum 10953(ELISA units + s.d) and the mean % PMNL

	IgG titer to P. gingivalis 381		IgG titer to F. nucleatum 10953	
	pre-immune	post-immune*	pre-immune	post-immune*
Group 1(N=10)	101.9 + 18.2	5657.8 + 913.0	108.1 + 10.9	2288.9 + 60.2
mean % PMNL	ND**	16.5 + 12.1	ND	ND
Group 2(N=10)	105.3 + 10.0	5808.2 + 829.5	99.2 + 11.1	2317.1 + 52.8
mean % PMNL	ND	42.2 + 14.6@	ND	ND

*significant higher than pre-immune serum IgG titer($p < 0.01$) by Student's t-test
**ND: not determined
@significantly higher than Group 1($p < 0.01$) by Student's t-test

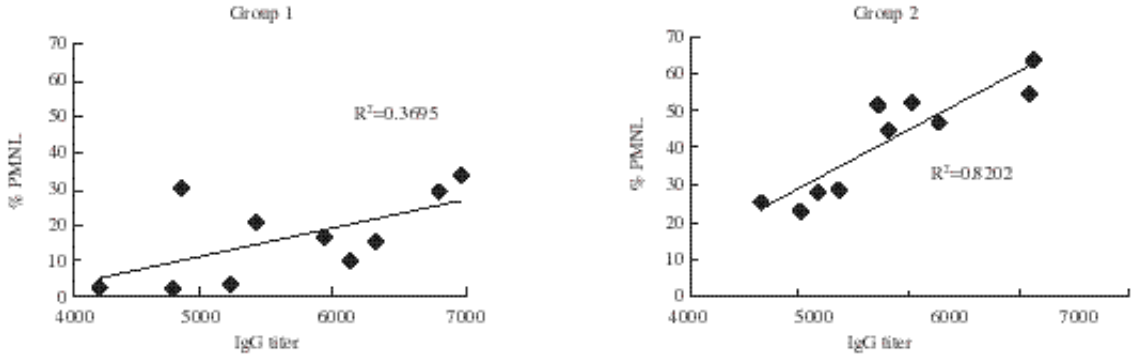

Figure 1. Diagrammatic representation of linear association between post-immune sera IgG titers and % of PMNL participating in phagocytosis(% PMNL) against P. gingivalis 381 in Groups 1 and 2. Correlation coefficients from regression analysis were denoted as R2 values.

PMNL participating in phagocytosis(% PMNL) were counted.

III. Results and Discussion

Pre - immune and post - immune IgG titer against Pg 381 or Fn 10953, and the mean % PMNL were depicted in Table 1. Both the two groups of immune mice showed a sub - stantial increase in IgG titers to both organisms when compared to pre - immune IgG titer($p < 0.01$). Though there was no statistically significant differences in IgG titers between the two groups, mean % PMNL was significantly higher in Group 2. When the linear relationships of post - immune IgG titers to Pg 381 were plotted against the % PMNL, it was much weaker in Group 1($r^2 = 0.37$) when compared with Group 2($r^2 = 0.82$)(Figure 1). It was highly probable that serum opsonophagocytosis functions were interfered by the prior immunization of another periodontopathic bacteria, which may, in part, explain the frequent observations of sub - optimal level of protective function of serum derived from multiple - pathogen - infected - peri - odontal patients, in contrast with those derived from single - pathogen - immunized animal. This may also demonstrate one of the immune deviating mechanisms of Fn in the process of converting gingivitis into destructive periodontal disease. In an effort to clarify this phenomenon at a molecular level, we are currenty investigating the dif - ferences in the antigenic recognition of sera from the two groups against Pg fimbrial protein which was widely known to be an immunodominant antigen in destructive periodontitis.

Group adoptively tranfered with Pg - spe - cific Th1 clone demonstrated a survival rate of 80 % during the observation period while those tranfered with Th2 clone showed a survival rate of 30 %. 90 % of the control group died of lethal dose(Figure 1)

IV. References

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국문초록

*Fusobacterium nucleatum*을 이용한 사전면역이 면역혈청의 *Porphyromonas gingivalis*에 대한 탐균작용의 장애에 대한 연구

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본 연구는 Balb/c mice를 이용하여 Porphyromonas gingivalis 381(Pg)로 면역하기 전에 Fusobacterium nucleatum ATCC 10953(Fn)로 면역한 Group 1(N=10)과 Pg로만 단독 면역을 시행한 Group 2(N=10)로부터 채취한 혈청의 Pg에 대한 식균능력을 비교하는 데 그 목적이 있다. 면역 후 혈청항체는 Pg에 대해 현저히 상승하였으나, 두 그룹간의 평균 항체역가는 통계적으로 차이가 없었다. 식균능력을 비교한 결과, Pg로만 단독 면역한 경우 식균능력이 Fn으로 먼저 면역한 Group 1의 경우보다 현저히 높았으며, 혈청항체역가와 식균지수와는 긴밀한 상관관계를 보였다. 결론적으로 치주세균의 사전 감염은 후속적인 세균감염에 대한 숙주 면역기능(식균능력)에 교란을 가져 올 수 있다.

Key Words: Opsonophagocytosis, Porphyromonas gingivalis, Fusobacterium nucleatum, immune modulation, antibody