

Fusobacterium nucleatum 1 gingivalis 2

Porphyromonas

I. (post - immune)

가 4,10).
1). - 가 IgG

2,3). T (immune deviation)

4 - 6),
T

가 helper T sub -
set ,
(episodic) 11 - 13). F. nucleatum
T 4). 11,14,15) biofilm¹⁶⁾
(phenotype) (coaggregation)

(opsonophagocytosis) 7,8,9).
20).
T

Fusobacterium nucleatum(F. nucleatum)

Porphyromonas gingivalis (P. gingivalis)
17 - 19).

Actinobacillus actinomyces -
comitans (A. actinomycescomitans)
가

F.
nucleatum P. gingivalis
P. gingivalis helper T
(polarization)

21).
F. nucleatum
P. gingivalis
P. gingivalis
valis 가
(opsonophago-
cytosis) P. gingivalis
F. nucleatum 가 T
(adoptive transfer)
P. gingivalis - helper T

II.

1.

F. nucleatum
ATCC 10953(American Type Culture Col-
lection, Manassas, VA) P. gingivalis
381(Dr. Schifferle, SUNY at Buffalo,
Buffalo, NY)
21)
, 10 Balb/c mice
PBS(phosphate - buffered saline)
(1) . 10
Balb/c mice F. nucleatum(5
× 10⁸ cells) 2
P. gingivalis(5 × 10⁸ cells)
2 (2). 10
Balb/c mice 2 P. gingi -
valis(5 × 10⁸ cells)
(3).

2. P. gingivalis- T line

21) P. gingivalis -
T line
, T (1 × 10⁶ cells/well)
12 well culture plate(Costar, Corning,
Corning, NY) P. gingivalis (1 × 10⁸
cells/well) irradiated
syngeneic splenocyte(2500 cGy)
. 2 , T
P. gingivalis - T line

T P. gingi -
valis 96 - well plate 24
replicate well 1, 3, 10 T
가 . 24 replicate
well
6 - well

T (culture
supernatants)
- 20

3. T

P. gingivalis - T FITC -
conjugated rabbit anti - mouse CD4
CD8 monoclonal antibody(PharMingen, San
Diego, CA) T
Epics Elite ESP(Coulter,
Hialeah, FL) flow cytometry

4. P. gingivalis- T

P. gingivalis

2 3 P. gingi -
 valis - helper T (1 × 10⁶
 cells/mouse) tail vein 10
 Balb/c . 10 Balb/c

, paired Student t - test

T
 T 3 ,
 P. gingivalis(1 × 10¹¹ cells)

P. gingivalis

T 72

ELISA

triplicate

, 96 - well plate(Corning, Corning, NY)

4 sodium carbonate buffer

rat anti - mouse IFN - , IL - 4

IL - 10(PharMingen, San Diego, CA, 4μg/ml)

coating . PBS/Tween 3

, well PBS + 10% fetal bovine

serum(PBS/FBS) 30

block PBS/Tween 3

PBS/FBS + 0.05% Tween 20(PBS/
 FBS/Tween) standard

recombinant mouse IFN - , IL - 4 IL -

10(PharMingen, San Diego, CA, 4 ng/ml)

가 3 ,

plate PBS/Tween 3

Biotinylated rat anti - mouse IFN - , IL - 4

IL - 10(PharMingen, San Diego, CA, 2μg
 /ml in PBS/FBS/Tween) well

가 1 .

PBS/Tween , hydroper -

oxidase - conjugated streptavidin

(PharMingen, San Diego, CA, 2μg/ml in
 PBS/FBS/ Tween) 가 37 30

. Plate PBS/Tween 8

o - phenylenediamine(1 mg/ml
 in 0.1 M citrate buffer, pH 4.5) 가

20

, 4 N H₂SO₄ well 가

, 490 nm

plotting

5. ELISA

IgG 가

IgG 가

22) . Phosphate

buffer P. gingivalis

(10μg/ml) 100μℓ 4

, 0.05% Tween 20

PBS(PBS/Tween) plate 3

PBS/Tween 50μℓ

well 가

. Plate

PBS/Tween 3 , well

100μℓ peroxidase - conjugated rabbit

anti - mouse IgG(H+L chain specific, Jack -

son ImmunoResearch Laboratories, West

Grove, PA) 가

. PBS/Tween well 100

μℓ tetramethylbenzidine(1mg/ml,

Kirkegaard and Perry Laboratories,

Gaithersburg, MD) 가 15

100μℓ

0.18 M H₂SO₄ 가 . 450 nm

100 ELISA unit

IgG 가

7. *P. gingivalis* (23,24) PMNL (% PMNL) blind examiner

37 15 propidium iodide cytospin cyanoacrylate

P. gingivalis PBS DAPI(4', 6 - diamidino - 2 - phenylindole dihydrochloride, 1 mg/ml) Polymorphonuclear leukocyte (PMNL) Hank's balanced salt solution (Life Technologies, Grand Island, NY)

8. Western blot analysis Laemmli가 1 mm - thick 10% running gel tall Minigel system (Hoeffer Scientific Company) SDS - PAGE

Table 1. Phenotypes of *P. gingivalis*- specific T cell clones from Groups 2 and 3 based on the flow cytometric analysis

Groups	Clone No.	CD4	CD8	Th/Tc subsets*
Group 2	1	+	-	Th2
	2	+	-	Th2
	3	+	-	Th2
	4	+	-	Th2
Group 3	1	+	-	Th1
	2	+	-	Th1
	3	+	-	Th1
	4	+	-	Th1

* based on the cytokine concentration in Table 2

Table 2. Cytokine concentration of culture supernatants of *P. gingivalis*- specific T cell clones from Groups 2 and 3*

Groups	Clones	INF -	IL - 4	IL - 10
Group 2**	1	-	6.8	-
	2	-	7.9	4.2
	3	-	4.3	3.7
	4	-	3.2	1.9
Group 3**	1	8.3	-	-
	2	1.5	-	-
	3	5.3	-	-
	4	4.0	-	-

* cytokine concentration (ng/ml)

** Group 2 : immunized first with *F. nucleatum* and then with *P. gingivalis*,

Group 3 : immunized with *P. gingivalis* alone

. 5 % - mercaptoethanol 100 buffer 5 6 , peroxidasec -
 10 (denaturing) onjugated goat anti - rabbit IgG 1
 80 10 .
 . SDS - PAGE 4 - chloro - 1 -
 , semi - dry blot system naphthol
 nitrocellulose ²⁶⁾ .
 nonfat dry milk 4.5%
 Block . block , III.
 Decaprobe(Hoeffer Scientific Compa -
 ny) 4 1. T (cloning)
 . 0.5 M NaCl 0.1% Tween 20(
) pH 7.5 20 mM Tris

Table 3. Pre - immune and post - immune serum IgG titer against *P. gingivalis* 381(ELISA units±s.d) and the mean % PMNL

	IgG titer to <i>P. gingivalis</i> 381	
	pre - immune	post - immune
Group 1(N=10) mean % PMNL	112.5 ± 12.3	117.4 ± 3.2 ND** 2.4 ±
Group 2(N=10) mean % PMNL	101.9 ± 18.2	1243.8 ± 131.5* ND 16.5 ±
Group 3(N=10) mean % PMNL	105.3 ± 10.0	1318.1 ± 138.9* ND 42.2 ±

* significant higher than pre - immune serum IgG titer(p < 0.01) or control group, and no statistical difference between Groups 2

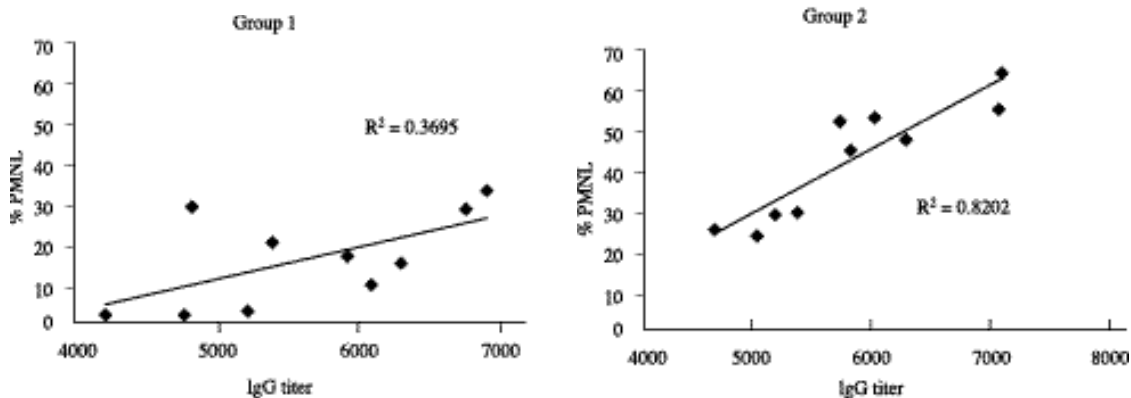


Figure 1. Plotting of linear relationship between anti -*P. gingivalis* IgG titers and opsonophagocytosis function of sera from groups 2 and 3

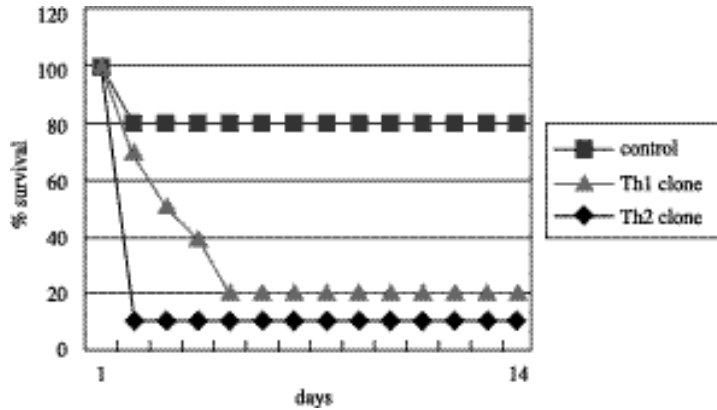


Figure 2. Survival rate of mice against the lethal dose of *P. gingivalis* which have been adoptively transferred with Th1 or Th2 clone derived group 2 or group 3, respectively.

2 3 *P. gingivalis* -
 helper T 4 T
 , CD4 ,
 (Tc) CD8 (Table
 1). *P. gingivalis* - T
 , 2 T IFN -

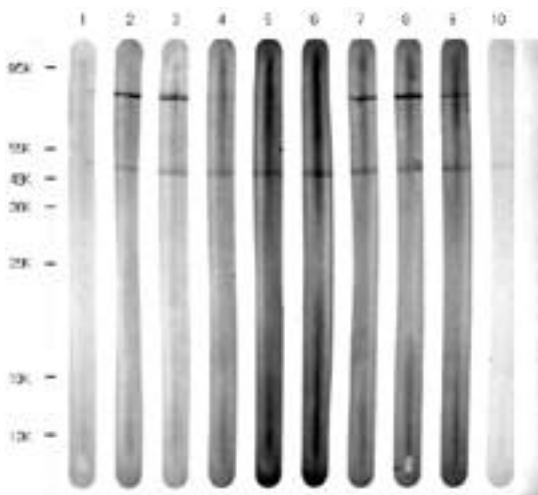


Figure 3. Western blot pattern of serum reaction with *P. gingivalis* fimbrial antigens. Upper band is 75 - kDa and lower band is 43 - kDa proteins, respectively. Lanes 1 - 3 and 7 - 9 denotes sera from group 3 and lanes 4 - 6 denotes from

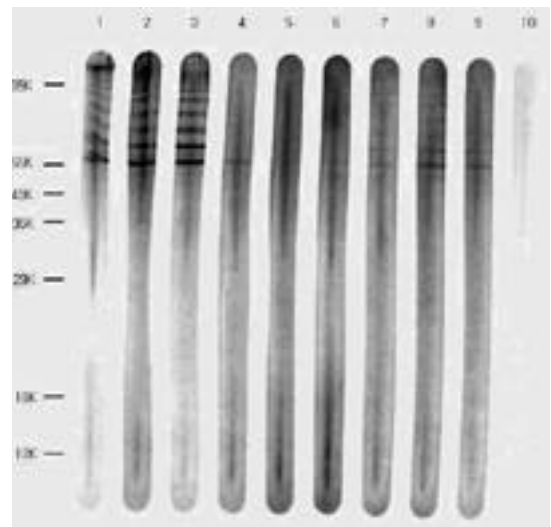


Figure 4. Western blot pattern of serum reaction with heat - dissociated *P. gingivalis* fimbrial antigens. Lanes 1 - 3 and 7 - 9 denotes sera from group 3 and lanes 4 - 6 denotes sera from group 2, respectively.

IL - 4 IL - 10
 helper T type 2 subset
 3 T IL - 4
 IL - 10 IFN -
 helper T type1 subset
 (Table 2).

2. native fimbrial oligomer (Figure 4, lane 4 - 6), 3 oligomer (Figure 4, lane 1 - 3, 7 - 9).

P. gingivalis 381 IgG 가 % PMNL 3

IgG 가 IgG 가 (p<0.01). P. T

gingivalis IgG 가 , 2 4,5,6). T

% PMNL 3 . P. P. gingivalis A. actinomycetemcomi -

gingivalis 381 IgG 가 % tans .

PMNL , 3 (r²=0.82) 2 P. gingivalis T

(r²=0.37) PBL 27,28,29) GMC 29,30,31,32,33,34)

(Figure 1). IFN - , IL - 2, IL - 4, IL - 5, IL - 6

IL - 10 T (polarization)

(heterogeneity)

3. T

30,31), PBL GMC

subsets .

P. gingivalis F. nucleatum P.

gingivalis T 21).

F. nucleatum P. gingivalis

17,18,19,35).

, F. nucleatum P. gingivalis

18).

4. Western blot analysis F. nucleatum P. gingivalis T

P. gingivalis F. nucleatum Th2 21)

2

P. gingivalis 43 - kDa fimbrial protein . F. nucleatum

(Figure 3, lane 4 - 6). (cross - reaction antigen)

, P. gingivalis 3 T P. gingivalis

43 - kDa 75 - kDa fimbrial P. gingivalis

protein (Figure 3, lane 1 - T 가 .

3 7 - 9). Fimbriae ,

, 2 Th1 Th2 subset

Th1 . P. gingivalis fimbrial protein P. gingi -
 valis
 . F. nucleatum
 5,36), P. gingivalis P. gingivalis
 37,38,39), P. gingivalis 75 - kDa fimbrial protein
 subset가 .
 F. nucleatum P. gingi - F. nucleatum
 valis . P. gin -
 givalis 75 - kDa fimbrial antigen
 10) .
 ,
 . P. gingivalis T P. gingivalis
 가 가 Th2 subset ,
 가 ,
 F. nucleatum
 .
 F. nucleatum V.
 immunodominant antigen
 P. gingivalis fimbrial protein
 10) 2
 . P. gingivalis F.
 nucleatum
 P. gingivalis 43 - kDa fimbrial 2
 protein . , P.
 gingivalis , T
 43 - kDa 75 - kDa fimbrial protein
 . Fimbriae
 , 75 - kDa protein VI.
 native
 fimbrial oligomer .
 , 3 oligomer
 . 75 - kDa fimbrial protein
 heat - dissociated oligomer
 . 3
 , 43 - kDa 75 - kDa

1. Caton, J. : Periodontal Diagnosis and Diagnostic Aids. In: Nevins M, Becker W, Kornman K, eds. Proceedings of The world Workshop in Clinical Periodontics. Chicago: American Academy of Periodontology I - I - I - 22, 1989.
2. Astemborski, J.A., J.A. Boughman,

- P.O. Myrick, S.B. Goodman, R.K. Woolen, S. Agarwal, J.W. Vincent, and J.B. Suzuki : Clinical and laboratory characterization of early onset peri - odontitis. *J. Periodontol.* 61: 557 - 563, 1989.
3. Ranney, R.R. : Diagnosis of peri - odontal diseases. *Adv. Dent. Res.* 5:21 - 36, 1994.
 4. Ebersole, J.I., and M.A. Taubman : The protective nature of host response in periodontal disease. *Periodontol.* 2000. 5: 112 - 141, 1994.
 5. Gemmell, E., and G.J. Seymour : Modulation of immune responses to periodontal bacteria. *Curr. Opin. Peri - odontol.* 2: 28 - 38, 1994.
 6. Taubman, M.A., J.W. Eastcott, H. Shimauchi, O. Takeichi, and D.J. Smith : Modulatory role of T Lymphocytes in Periodontal Inflammation In: Genco R, Hamada S, Lehner T, McGhee J, Mergenhagen S, ed. *Molecular Pathogenesis of periodontal Disease.* Washington, DC: American Society for Microbiology, 147 - 157, 1994.
 7. Baker, P.J., S. Carter, M. Dixon, R.T. Evans, and D.C. Roopenian : Serum antinody response to oral infection precedes but does not prevent Porphy - romonas gingivalis - induced alveolar bone loss in mice. *Oral. Microbiol. Immunol.* 14: 194 - 196, 1999.
 8. Sjostrom, K., R. Darveau, R. Page, C. Whitney, and D. Engel : Opsonic anti - body activity against *Actinobacillus actinomycetemcomitans* in patients with rapidly progressive periodontitis. *Infect. Immun.* 60: 4819 - 4825, 1992.
 9. Whitney, C.J. Ant, B. Mocla, B. Johnson, R.C. Page, and D. Enge : Serum immunoglobulin G antibody to Porphy - romonas gingivalis in rapidly progressive periodontitis: titer, avidity, and subclass distribution. *Infect. Immun.* 60: 2194 - 2200, 1992.
 10. Evans, R. T., B Clausen, H. Sojar, G. C. Bedi, C. Stintescu, N.S Rama - murthy, L.M. Golub, and R.J. Genco : Immunization with Porphyromonas gin - givalis fimbriae protects against peri - odontal destruction. *Infect. Immun.* 60: 2926 - 2935, 1992.
 11. Bolstad, A.I., H.B. Jensen, and V. Bakken : Taxonomy, biology, and peri - odontal aspects of *Fusobacterium nucleatum*. *Clin. Microbiol. Rev.* 9: 55 - 71, 1996.
 12. Moore, W.E. : Microbiology of periodontal disease. *J. Periodont. Res.* 22: 335 - 341, 1987.
 13. Moore, W.E., and L./V. Moore : The bacteria of periodontal diseases. *Periodontol.* 2000 5: 66 - 77, 1994.
 14. Bolstard, A.I., J. Tommassen, and H.B. Jensen : Sequence variability of the 40 - kDa outer membrane protein of *Fusobacterium nucleatum* and a model for topology of the proteins *Mol. Gen. Genet.* 244: 104 - 110, 1994.
 15. Kolenbrander, P.E., and J. London : Adhere today, here tomorrow: oral bacterial adherence. *J. Bacteriol.* 175: 3247 - 3252, 1993.
 16. Marsh, P.D., D.J. Bradshaw, G.K. Watson, and C. Allison : Role of *Fusobacterium nucleatum* in anaerobe survival in microbial communities. *J.*

- Dent. Res. 76: 226(Abstr #1700), 1997.
17. Brook I., and I. Walker : The relationship between *Fusobacterium* species and other flora in mixed infection. J. Med. Microbiol. 21: 93 - 100, 1986.
 18. Feuille, F., J.L. Ebersole, L. Kesavalu, M.J. Steffen, and S.C. Holt : Mixed infection with *Porphyromonas gingivalis* and *Fusobacterium nucleatum* in a murine lesion model: potential effects on virulence. Infect. Immun. 64: 2094 - 2100, 1996.
 19. Gemmell, E., and G.J. Seymour : Interleukin 1, interleukin 6 and transforming growth factor - production by human gingival mononuclear cells following stimulation with *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. J. Periodont. Res. 28: 122 - 129, 1993.
 20. Tew, J.G., S.S. Thomas, and R.R. Ranney : *Fusobacterium nucleatum* - mediated immunomodulation of in vitro secondary antibody response to tetanus toxoid and *Actinobacillus actinomyces* *actinomycetemcomitans*. J. Periodont. Res. 22: 506 - 512, 1987.
 21. , , :
 22. Choi, J.I., R.E. Schifferle, F. Yoshimura, and B.W. Kim : Capsular polysaccharide - fimbrial protein conjugate vaccine protects against *Porphyromonas gingivalis* infection in SCID mice reconstituted with human peripheral blood lymphocytes. Infect. Immun. 66: 391 - 393, 1998.
 23. Cutler, C.W., R.R. Arnold, and H.A. Schenkein : Inhibition of C3 and IgG proteolysis enhances phagocytosis of *Porphyromonas gingivalis*. J. Immunol 151: 7016 - 7029, 1993.
 24. Genco, C.A., R.E. Schifferle, T. Njoroge, R.Y. Forng, and C.W. Cutler : Resistance of a Tn4351 - generated polysaccharide mutant of *Porphyromonas gingivalis* to polymorphonuclear leukocyte killing. Infect. Immun. 63: 393 - 401, 1995.

25. Laemmli, U.K. : Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*(London) 227: 680 - 685,1970.
26. Towbin, H., T. Staehelin, T., and J. Gordon : Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* 76: 4350 - 4354, 1979.
27. Gmmell, E., M. Kjeldsen, K. Yamazaki, T. Nakajima, M.J. Aldred, and G.J. Seymour : Cytokine profiles of *Porphyromonas gingivalis* - reactive T lymphocyte lines and clones derived from *P. gingivalis* - infected subjects. *Oral Dis.* 1: 139 - 146, 1995.
28. Mahanonda, R., S. Prachaney, and S. Tirawatanpong : Cytokine profiles of *Porphyromonas gingivalis* - reactive T cell lines from periodontitis patients. *J. Dent. Res.* 76: 230(Abstr #1732), 1997.
29. Yamazaki, Y, T. Nakajima, Y. Kubota, E. Gemmell, G.J. Seymour, and K. Hara : Cytokine messenger RNA expression in chronic inflammatory periodontal disease. *Oral Microbiol. Immunol.* 12: 281 - 287, 1997.
30. Fujihashi, K., K.W. Beagley, Y. Kono, W.K. Aicher, M. Yamamoto, S. DiFabio, J. Xu - Amano J, J.R. McGhee, and H. Kiyono : Gingival mononuclear cells from chronic inflammatory periodontal tissues produce IL - 5 and IL - 6, but not IL - 2 and IL - 4. *Am. J. Pathol.* 142: 1239 - 1250, 1993.
31. Fujihashi, K., M. Yamamoto, J.R. McGhee, and H. Kiyono : Type 1/type2 cytokine production by CD4⁺ T cells in adult periodontitis. *J. Dent. Res.* 73: 204(Abstr #818), 1994.
32. Gemmell, E., B. Feldner, and G.J. Seymour : CD45RA and CD45RO positive CD4 cells in human peripheral blood and periodontal disease tissue before and after stimulation with periodontopathic bacteria. *Oral Microbiol. Immunol.* 7: 84 - 88, 1992.
33. Manhart, S.S., R.A. Reinhardt, J.B. Payne, G.J. Seymour, E. Gemmell, J.K. Dyer, and T.M. Petro : Gingival cell IL - 2 and IL - 4 in early - onset periodontitis. *J. Periodontol.* 65: 807 - 813, 1994.
34. Yamazaki, Y, T. NakaJima, E. Gemmell, B. Polak, G.J. Seymour, and K. Hara : IL - 4 and IL - 6 producing cells in human periodontal disease tissue. *J. Oral. Pathol. Med.* 23: 347 - 353, 1994.
35. Ebersole, J.L., F. Feuille, L. Kesavalu, and S.C. Holt : Host modulation of tissue destruction caused by periodontopathogens: effect on a mixed microbial infection composed of *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. *Micro. Pathog.* 23: 23 - 32, 1997.
36. Gemmell, E., R.I. Marshall, and G.J. Seymour : Cytokines and prostaglandins in immune homeostasis and tissue destruction in periodontal disease. *Periodontol.* 2000 14: 112 - 143, 1997.
37. Kawai, T., M.E. Wilson, T. Nagasawa, H. Watanabe, J.W. Eastcott, D.J. Smith, and M.A. Taubman : Adoptive transfer of cloned antigen - specific Th1 lymphocytes produces

periodontal bone loss. J. Dent. Res. 76: 231(Abstr #1738),1997.

38. Shimauchi, H., M.A. Taubman, J.W. Eastcott, and D.J. Smith : Migration of Th1 and Th2 Aa - specific clones into infected and noninfected gingival tissues. J. Dent. Res. 72: 243 (Abstr #1121), 1993.
39. Yamashita, K., J.W. Eastcott, M.A. Taubman, D.J. Smith, and D.S. Cox : Effect of adoptive transfer of cloned Actinobacillus actinomycetemcomitans - specific T helper cells on periodontal disease. Infect. Immun. 59: 1529 - 1534, 1991.

- Abstract -

Prior Exposure of Mice to Fusobacterium Nucleatum Modulates Host Response to Porphyromonas Gingivalis

Han - Yong Son, Sung - Jo Kim, Jeom - Il Choi

Department of Periodontology, College of Dentistry, Pusan National University

Multiple periodontal pathogens sequentially colonize the subgingival niche during the conversion from gingivitis to destructive periodontal disease. An animal model of sequential immunization with key periodontal pathogens has been developed to determine whether T and B lymphocyte effector functions are skewed and fail to protect the host from pathogenic challenge. The present study was performed to evaluate immunomodulatory effect of exposure to Fusobacterium nucleatum (F. nucleatum) prior to Porphyromonas gingivalis (P. gingivalis). Group 1 (control) mice were immunized with phosphate - buffered saline, Group 2 were immunized with F. nucleatum prior to P. gingivalis, while Group 3 were immunized P. gingivalis alone. All the T cell clones derived from Group 2 demonstrated type 2 helper T cell clone (Th2 subsets), while those from Group 3 mice demonstrated Th1 subsets. Exposure of mice to F. nucleatum prior to P. gingivalis interfered

with opsonophagocytosis function of sera against *P. gingivalis*. In adoptive T cell transfer experiments, in vivo protective capacity type 2 helper T cell clones (Th2) from Group 2 was significantly lower than type 1 helper T cell clones (Th1) from Group 3 against the lethal dose infection of *P. gingivalis*. Western blot analysis indicated the different pattern of recognition of *P. gingivalis* fimbrial proteins between sera from Group 2 and Group 3.

In conclusion, these study suggest that colonization of the subgingival niche by *F. nucleatum* prior to the periodontal pathogen, *P. gingivalis*, modulates the host immune responses to *P. gingivalis* at humoral, cellular and molecular levels.