

I. T cell subsets (9-11), (8,11-13)가 INF- γ , IL-2, IL-4, IL-5, IL-6, IL-10 T cell subsets (subsets) 12,13)가 A. actinomycetemcomitans - helper T cell subset adoptive transfer model 14-16) helper T type 2(Th2) helper T type 1(Th1) P. gingi - valis - Th1 Th2 2,4), 17), Gemmell 4) 60%가 CD4 memory T cell subset 8), P. gingivalis - T cell line subset

(dichotomy) plate phosphate
(episodic) T buffered saline(PBS) 3 T
1). 가 T 2.
Fusobacterium nucleatum(F. nucleatum) 3 Balb/c ½
cage . 10
Balb/c PBS Balb/c
20-22). (1). 10 Balb/c
biofilm²³⁾ F. nucleatum 5 × 10⁸ 2 ,
P. gingivalis 5 × 10⁸ 2
20,24,25) 26-28). (2). 10 Balb/c P. gingivalis 5
F. nucleatum A. actinomycetemcomitans 2 × 10⁸ 2 3
가 tail bleeding
29). (intermediate and -20 .
late colonizers) 3. P. gingivalis - T line
T
F. nucleatum 7 T
P. gingivalis enrichment column(R&D System, Minneapolis, MN) T
(1 × 10⁶ cell/well
in 24 - well plate) RPMI 1640(Life Technologies) 10% fetal bovine serum(FBS) Penicillin/Streptomycin(가
100 unit/ml 100 micrograms/ml) 가
(RPMI/FBS+PS)
II. P. gingivalis
1. F. nucleatum ATCC 10953(American type Culture Collection, Manassus, VA) P. gingivalis 381(Dr. Schifferle, SUNY at Buffalo, Buffalo, NY) hemin(5µg/Mℓ) 10⁶ cell/well) 가 . 2
menadion(0.5µg/Mℓ) 가 tryptic soy broth(Difco, Detroit, MI) blood agar T 가 1 가

가 Cytometry, Coulter, Hialeah, FL)

T

P. gingivalis - T

line (5 × 10⁶ cell/well) P. gingivalis(1 × 10⁷ cell/well) 가 96 - well plate

, well T line T

1,3,10 cells/well 가 2

. 24 replicates T

. 72 6 - well culture plate T

T

- 20

4. T

96 - well plate T (1 × 10⁵ cell/well) RPMI 1640/FBS+PS F. nucleatum(1 × 10⁵ - 1 × 10⁸ /well) P. gingivalis(1 × 10⁵ - 1 × 10⁸ /well) 가 , (2,500 rads, 1 × 10⁶ cell/well) 가 72 3H - thymidine(1 μCi/well) 가 24

5. T

P. gingivalis- T FITC - conjugated rabbit anti - mouse CD4 CD8 monoclonal antibody (Pharmingen, San Diego, CA) FITC - conjugated rabbit anti - mouse T cell receptor (TCR) - T cell receptor monoclonal antibody (Pharmingen, San Diego, CA) T flow cytometry (Coulter Epics Elite XL

6. ELISA IgG 가

IgG 가

51). 96 - well plate P. gingivalis F. nucleatum 100μℓ triplicate

4 0.05% Tween 20 PBS 3 . PBS/Tween 50μℓ 1:50 well

2 PBS/Tween 3 100μℓ per - oxidase - conjugated rabbit anti - mouse IgG(H+L)(Jackson ImmunoResearch Laboratories, West Grove, PA) well

가 2 PBS/Tween 3 100μℓ tetramethylbenzidine(1 mg/ml) well

가 15 100μℓ 0.18M H₂SO₄ 가 . 450nm (optical density)

ELISA unit 100 IgG 가

paired Student's t - test

7. T

T P. gingi - valis 72

ELISA . 96 - well

Table 1. Cytokine concentration of culture supernatants of *P. gingivalis*-specific T cell clones from Groups 2 and 3*

Groups	Clone No.	INF -	IL - 4	IL - 10
Group 2**	1	-	11.2	4.6
	2	-	3.5	2.4
	3	-	8.4	4.6
	4	-	10.7	4.0
	5	-	1.9	2.4
	6	-	9.3	3.1
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

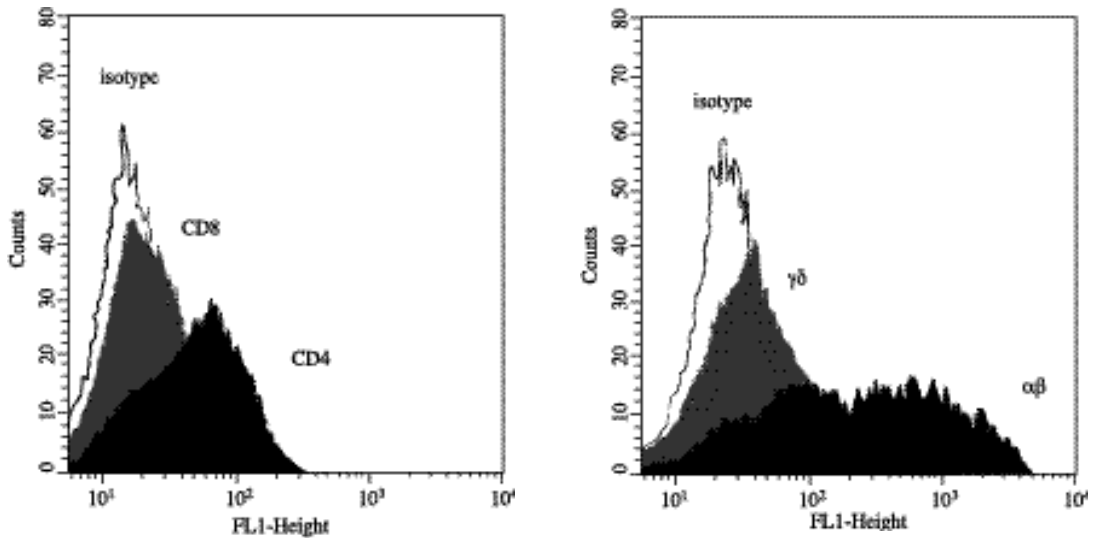


Figure 1. Flow cytometric analysis of *P. gingivalis*-specific T cell clones for CD4 or CD8 positivity(

plates 4	sodium car -	standard recombinant mouse IFN - ,
bonate buffer	rat anti - mouse	IL - 4 IL - 10 가
INF - , IL - 4 IL - 10	coating	3 PBS/Tween 3
. PBS/Tween 3		. biotinylated rat anti - mouse
PBS/FBS 30	blocking	INF - , IL - 4 IL - 10 가
PBS/Tween 3		1 PBS/Tween
PBS/FBS + 0.05% Tween 20		4 hydroperoxidase - conju -

Table 2. Phenotype of T cell clones and T cell receptors obtained from Groups 2 and 3

Groups	Th1/Th2	T - cell receptor
Group 2*	Th2	
	Th2	
	Th2	
	Th2	
	Th2	
	Th2	
Group 3*	Th1	
	Th1	
	Th1	
	Th1	

* Group 2 : immunized first with F. nucleatum and then with P. gingivalis
 Group 3 : immunized with P. gingivalis alone

gated streptavidin 가 37 30 F. nucleatum P. gingivalis
 . PBS/Tween 8 2 P. gingivalis - T
 o - phenylenediamine(1mg/Ml in 0.1M citrate 6 P. gingivalis 3
 buffer, pH 4.5) 가 20 P. gingivalis - 4
 4N 2 3 P. gingivalis - T
 H₂SO₄ well 490nm helper T (CD4)
 . Standard cytotoxic T (CD8)
 (Figure 1), - TCR
 , - TCR (Figure 1
). INF - , IL - 4 IL -
 10 Table 1 2
 III. T IL - 4 IL - 4
 IL - 10 , INF -
 1. T cloning , 3 INF -
 IL - 4 IL - 10

Table 3. Serum IgG titer to P. gingivalis 381 or F. nucleatum 10953(ELISA units± s.d.) of preimmune and immune mice

Groups	Ig G to P. gingivalis 381		Ig G to F. nucleatum 10953	
	Pre - immune	Post - immune	Pre - immune	Post - immune
Group 1 (n=10)	104.5 ± 11.2	ND**	106.1 ± 10.8	ND**
Group 2 (n=10)	101.1 ± 14.7	975.0 ± 23.8***	114.0 ± 15.0	8 4 4 . 0 ±
Group 3 (n=10)	105.6 ± 15.6	1092.4 ± 27.1***	96.9 ± 11.5	9 4 1 . 3 ±

* mean IgG antibody titers s.d. from repeated experiments

** ND : not determined

Table 4. In vitro proliferating responses of P. gingivalis - specific T cell clones to P. gingivalis and F. nucleatum antigens*

Group	Clones	Antigens							
		P.gingivalis 381		F. nucleatum 10953		Control 1**		Control 2***	
Group 2	1	6,566	189	4,127	133	212	24	332	28
	2	13,451	576	9,112	327	420	31	449	58
	3	7,234	473	3,119	298	229	43	421	46
	4	7,161	329	8,328	457	267	92	816	79
Group 3	1	5,317	633	4,116	121	451	81	611	70
	2	9,481	535	4,192	182	539	87	539	87
	3	10,117	287	9,254	223	321	122	326	75

* unit expressed as mean counts per minute of triplicate measurements ± s.d.

** control 1 indicates only culture medium was used for stimulation of T cells

*** control 2 indicates T cells stimulated with Streptococcus mutans

(Table 1).

2 P. gingivalis - T
Th2 3

Th1 (Table 2).

2. P. gingivalis 381 F. nucleatum
10953 IgG 가

Table 3

2 3 IgG P. gingivalis
381 975.0 ± 23.8, 1092.4 ± 27.1
, F. nucleatum 10953
844.0 ± 28.3, 941.3 ± 23.3

(p < 0.01).

3. T

2 T P. gingivalis
F. nucleatum T 3
가 3,119 13,451 , 3
T 4,116 10,117

(Table 4).

2 3 T Table 4

IV.

F. nucleatum 10953 P. gingivalis 381

2 P. gin -
gingivalis - T 6 P. gingi -
valis 381 3 4

T

CD4 CD8

helper T
T IL - 4
IL - 10 type 2 subset , 3

IL - 4 IL - 10

INF - type 1 subset

P. gingivalis - T
type 2 helper T subset
rat

P. gingivalis P. gingivalis -
T Th1 30).

Gemmell 31) Balb/c

P. gingivalis ,
T 가 INF -

Th1 subset . Th2 subset
P. gingivalis (original anti -
P. gingivalis - T genic sin) ³²⁾
type 1 helper T T
P. gingivalis 381 F. nucleatum 10953 . 가 Klenerman
nucleatum 10953 type 2 Zinkernagel ³³⁾
helper T T
가
P. gingivalis - T , P. gingivalis - T
가 type 1 helper T type 2 helper T
F. nucleatum
IL - 4 IL - 10
INF - F. nucleatum -
T line F. nuclea -
tum - T clone . F. nucleatum
2 P. gingivalis -
Th subset F. nucleatum T
Th2 helper T cytotoxic T (sub -
. F. nucleatum 10953 P. gingi - set)
valis 381 2 (major histocompatibility complex)
Th2 subset 가 ³⁴⁾. T 가
nucleatum P. gingivalis F. nucleatum T
Th1 cell receptor가 homology sequence
Th2 subset P. gingivalis -
P. gingivalis F. nucleatum
. P. gingivalis - T F. sequence homology ^{35 - 37)}. Quarantino ³⁸⁾
nucleatum 3 T epiptope
2 T F. 가
nucleatum P. gingivalis P. gingivalis 381 F. nucleatum 10953
가 T T IgG 가 2 3
가
B T
memory T 2 . 3

F. nucleatum IgG 2
 IgG F. T Th2
 nucleatum 가 IgG P. IL - 10 F. nucleatum
 gingivalis Th2 P. gingivalis P. gingivalis
 IL - 10
 P. gingivalis IgG 가 P. gingi -
 valis F. nucleatum Th2 IL - 10 IL - 10
 INF - Th2 subset
 2 3 45). IL - 4 IL -
 P. gingivalis F. nucleatum 10 T
 opsonophagocytosis 가 46 - 48).
 P. gingivalis IL - 4 IL - 10
 P. gingivalis F. nucleatum 47,49,50)
 P. gingi - 47). Th2
 valis IgG 가 IL - 4 IL - 10
 40). P. gingivalis Th subset
 P. gingivalis F.
 nucleatum Th1 Th2
 adoptive T
 B T
 가
 F. nucleatum P. gingivalis
 T
 IL - 4 P. gingivalis - Th1 Th2
 F. nucleatum
 gingivalis A. actinomycetemcomitans rat P. gingi -
 valis - T F. nucleatum
 F. nucleatum T 30).
 IL - 4 Th2
 subset IL - 4가 Th1
 T Th2 가 ,
 41,42). F. 52 - 55), 56)
 nucleatum T IL - 4 Th2 57)
 subset
 43,44). (live)
 Th2 subset P. gingivalis A. actino -
 mycetemcomitans 가

T subset
epitope

V.

F. nucleatum ATCC 10953 P. gingi -
valis 381 T
10
F. nucleatum P. gingivalis ,
P. gingivalis
T in vitro
P. gingivalis - T
F. nucleatum P. gingivalis
F. nucleatum
P. gingivalis IgG 가
ELISA . F. nucleatum
P. gingivalis T
type 2 helper T subset
, P. gingivalis
type 1 helper T subset
T P.
gingivalis F. nucleatum
T
CD4, TCR
. F. nucleatum P. gingivalis
IgG 가
(p <0.01)
F.
nucleatum P.
P. gingivalis P.
gingivalis - helper T P.
gingivalis
helper T

VI.

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Cellular and Humoral Immune Responses to Sequential Periodontopathic Bacterial Immunization in Animal Model

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Antigen - specific T cell clones were obtained from mice immunized with *Fusobacterium nucleatum* ATCC 10953 (*F. nucleatum*) and/or *Porphyromonas gingivalis* 381 (*P. gingivalis*). 10 Balb/c mice per group were immunized with *F. nucleatum* followed by *P. gingivalis*, or with *P. gingivalis* alone by intraperitoneal injection of viable microorganisms. Spleen T cells were isolated and stimulated in vitro with viable *P. gingivalis* cells to establish *P. gingivalis* - specific T cell clones. T cell phenotypes and cytokine profiles were determined along with T cell responsiveness to *F. nucleatum* or *P. gingivalis*. Serum IgG antibody titers to *F. nucleatum* or *P. gingivalis* were also determined by ELISA.

All the T cell clones derived from mice

immunized with *F. nucleatum* followed by *P. gingivalis* demonstrated Th2 subsets, while those from mice immunized with *P. gingivalis* alone demonstrated Th1 subsets based on the flow cytometric analysis and cytokine profiles. All T cell clones from both groups were cross - reactive to both *P. gingivalis* and *F. nucleatum* antigens. Phenotypes of T cell clones were all positive for CD4. Mean post - immune serum IgG antibody levels to *F. nucleatum* or *P. gingivalis* were significantly higher than the pre - immune levels ($p < 0.01$, respectively). There were no significant differences in the antibody titers between the two groups. It was concluded that *P. gingivalis* - specific T cells initially primed by cross - reactive *F. nucleatum* antigens were polarized to Th2 subsets, while T cells stimulated with *P. gingivalis* alone maintained the profile of Th1 subset.