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Usage of T Cell Receptor Repertoire is Restricted in Synovial Lymphocytes in Rheumatoid Arthritis

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= Abstract =

Background: Rheumatoid arthritis is an autoimmune disease characterized by a chronic inflammatory process, primarily involving the synovial membrane of peripheral joints, where T cell activation is found. To address the superantigen stimulation in rheumatoid arthritis, T cell clonality and the expression of activation markers were analyzed. **Methods:** To detect *TCRBV* usage, inverse PCR and sequencing were done. Monoclonal antibodies were used for flow cytometric analysis of *TCRBV8* or *TCRBV5*. As results, a restricted usage of *TCRBV3* gene was detected in synovial lymphocytes from one rheumatoid arthritis patient. However, preferential usage for *TCRBV8*, which may be one indicator for stimulation by staphylococcal superantigen, was not obvious although general activation of T cells was found as high DR+ percentage in synovial T cells. These data show specific antigen rather than superantigen might involve the pathogenesis of rheumatoid arthritis.

Key Words: , . T Chain

, 가 (2).

T TCR (T cell
1% 가 receptor) 가 .
sublining layer
가 . 40-50%가 T
CD4+ 가 CD4:CD8 4:1 14:1
CD4+ 가

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* 1994 CMB-

lymphoid aggregate
lymphoid aggregate

가 CD4+ (3). 1990 T 가 TCR Vβ family V gene repertoire (4) T TCR 가 T 가 가 T 가 superantigen T 가 (6-8)가 (9,10) T 가 superantigen TCR repertoire T TCR repertoire가 (11). 가 가 Inverse PCR RNA RNeasy kit (Qiagen, Santa Claris, CA) First strand cDNA random primer (pd(N)₆, Pharmacia Fine Chemicals, Uppsala, Sweden) RNA 1μg random primer 0.1IU, dNTP 200 mM, DTT 800mM reverse transcriptase (Gibco BRL, Gaithersburg, MD) 400U 42 60 cDNA DNA polymerase 가 16 2 cDNA T4 DNA ligase 가 second stranded cDNA circularization PCR PCR degenerate primer forward primer 5'-GGG TCG ACC TGT GCA CCT CCT TCC CAT T-3' , inverse primer 5'-GCA TGC GGC CGC ATG GCC ATG GTC AAG AGA-3' primer 20pM, dNTP 200mM, MgCl₂ 2mM, KCl 50mM 0.001% gelatin 10mM Tris-HCl (pH; 8.3) Taq polymerase (Bioneer,) 2.5unit 가 PCR 94 1 , 55 1 , 72 2 30 . 30 72

10 elongation . PCR 1.5% agarose gel
 PCR 10µl . Inverse PCR
 Fig. 1

Gene cloning

50ng pT7Blue T-vector (Novagen, Madison, WI)
 PCR 0.2 pM DNA ligase (Gibco BRL,
 Gaithersburg, MD) 2U 가 16 2
 ligation . Competent cell (genotype : endA1
 hsdR17 (rk-, mk+) supE44 thi-1 gyrA96 relA1 lac [F',
 proAB, lacqZDM15, Tn10(tetr) recA] transformation
 50µg/MØ ampicillin, 15mg/MØ tetracycline, X-gal
 IPTG (Gibco BRL, Gaithersburg, MD)가 가
 LB agar plate clone
 . clones Sal I Not I
 insert (300 bp)가 clone automatic
 sequencer (; ALFexpress, Amersham Pharmacia
 Biotech AB, Uppsala, Sweden)

National Center for Biotechnology Information
 GenBank chain family

CD3, CD4, CD8 DR
 (Becton Dickinson, San Jose, CA) TCR β
 TCRBV5 TCRBV8 (T Cell Diagnostics, Cambridge,
 MA) . 5µ
 1 10µl 가 30 4
 0.1% bovine serum albumin PBS 2

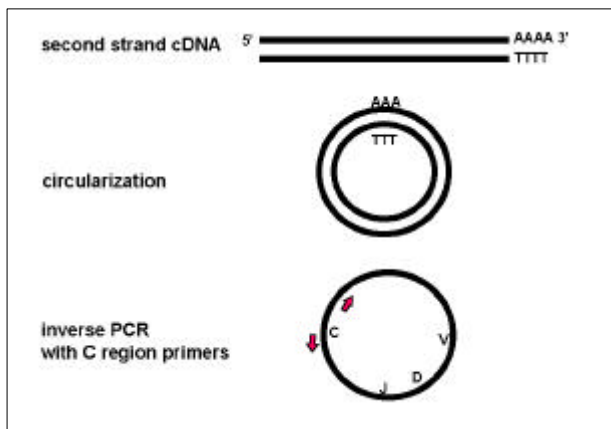


Fig. 1. Scheme of inverse PCR

(FACstar, Becton Dickinson, San
 Diego, CA)

1. TCRB V

(RA-1 RA-2)
 inverse PCR 300 bp
 PCR (Fig. 2). RA-1
 18
 TCRBV3
 10
 TCRBV3

T
 (Table 1).

2. TCRB V8 TCRB V5

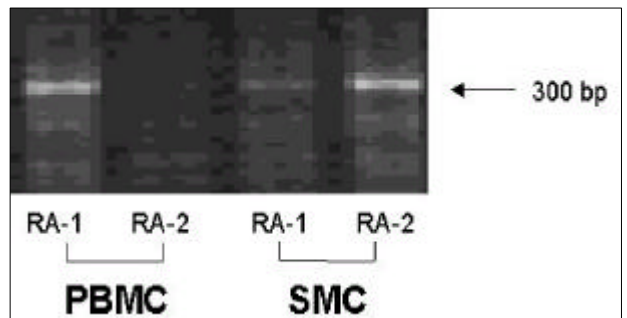


Fig. 2. Inverse PCR product. PCR was done with degenerate primers of TCRBC. PCR products were loaded on 1% agarose gel. PBMC; peripheral blood mononuclear cells. SMC; synovial mononuclear cells.

Table 1. Distribution of TCRBV family among T cell cDNA clones

	TCRB V	No. of positive clones
Patient (RA-1)	TCRB V1	1
	TCRB V3	10
	TCRB V5	4
	TCRB V7	1
	TCRB V21	2

Inverse PCR using C region primers was done to identify V region genes. Total 18 clones were tained and sequencing was done.

		T	T
Staphylococcal toxin superantigen		7.8%	1.9% 4.6%
	<i>TCRB V8</i> T	(Table 3).	CD3+ DR+
	<i>TCRB V8</i>	RA-1	가 RA-2
T (4.7 ± 1.7%)	T (2.3 ±	(Table 3).	가
1.8%)	(Table		
2).	(RA-2)	<i>TCRB V5</i>	
	6.0%		
	superantigen	<i>TCRB V5</i>	
	T (2.9 ± 2.4%)	TCR β chain family	α chain family β
	(Table 2).	chain family	가
3.		, α chain allelic exclusion	
		(17) allele	
2		α chain β chain	
50 U/ml	가	T	(18)가
	<i>TCRB V</i>	PCR	semi-quantitation RNA
CD8+	가	CD4+	
가	가	(Table 3).	TCR β
	anti- <i>TCRB V5</i>	anti- <i>TCRB V8</i>	17% (HLA-DQ2 가)
	RA-1	CD8+	20-40%가 <i>TCRA V12.1</i> 가
	<i>TCRB V5+</i> 가 0.8%, <i>TCRB V8+</i> 가 1.2%	가	가
	, <i>TCRB V5+</i> 가 1.8%, <i>TCRB V8+</i>		(19, 20)
가 1.0%	. RA-2 , <i>TCRB V5+</i>	<i>TCRA V12.1+</i> CD8+	
<i>TCRB V8+</i>	6.0%	<i>TCRA V12.1+</i> 가	

Table 2. *TCRB V5* or *TCRB V8* positive T cells in rheumatoid arthritis patients

Lymphocytes		<i>TCRB V5</i> positive %	<i>TCRB V8</i> positive %
Rheumatoid arthritis	PBMC	2.9 +2.4*	2.3 +1.8***
	SMC	4.1 +1.9**	4.7 +1.7****
Healthy control	PMBC	3.7 +1.0	3.7 +0.7

Cells were reacted with anti-*TCRB V5* or anti-*TCRB V8* monoclonal antibodies and a total of 10,000 to 20,000 cells were analyzed by FACstar. N= 21 for rheumatoid arthristis and 15 for healthy control. Data are expressed as mean + S.D.. PBMC; peripheral blood mononuclear cells. SMC; synovial mononuclear cells. Student's T test was done. *, **, ***, **** ; not significant at the p>0.01 level versus PBMC from healthy control group.

Table 3 Surface markers of synovial T cells of rheumatoid arthritis after *in vitro* culture

positive %	Fresh					3 months after culture				
	CD8	CD4	DR/CD3	<i>TCRB V5</i>	<i>TCRB V8</i>	CD8	CD4	DR/CD3	<i>TCRB V5</i>	<i>TCRB V8</i>
RA-1	50.3	27.9	14.3	0.8	1.2	11.3	64.5	29.8	1.8	1.0
RA-2	48.6	30.1	26.4	6.0	7.8	16.8	55.9	35.9	1.9	4.6

Cells were stained with each monoclonal antibodies and a total of 5,000 to 20,000 cells were analyzed by FACstar.

가 T
 inverse PCR T 2
TCRB V recombinant IL-2 가 3
TCRB V3 CDR3 CD4+ 가 가 DR+
 가 TCR
TCRBV3 T T
TCRB V3 T recombinant IL-2 가
 IL-2
*TCRB V3*가 가 가
 T chain
 family CD4+ T 가 (3)
*TCR VB3*가 superantigen CD8+
 가
 가 T , 가
 T 가 3 CD4+ T
 T (11). CD8+ T CD4+ T 가
 Superantigen staphylococcal toxin 가 IL-2
 superantigen CD4+ T 가 IL-2
TCRB V8 T 가
TCRB V8 T T DR 가
 가
 superantigen T 2.2 ± 1.0 (11)
TCRB V5 T DR+ T
 T 가 T
 가
 T 가 DR+ 가 CD4+ T CD8+ T
 T CD4+
 T 가
 (20) T TCR repertoire T
 TCR (20). 가
 T 40% IL-2
 TCR chain 가 T IL-15 T
 (21) cartilage proteoglycan 가
 (22, 23) type II collagen(24) 가 가 (25).
 T
 T
 가 가
 TCR repertoire

- 가 가 broad spectrum
가 T (26)
- T
- T
- 가 T T
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