

# Conserved T CDR3 motif 2 T

가<sup>1</sup>, 가<sup>1</sup>, 가<sup>2</sup>,  
3  
1 . 1 . 3 . 2 . 2 . 1 . 1,2 \*

## Generation and maintenance of type II collagen-specific T-cell line expressing conserved TCR- CDR3 motifs among patients with rheumatoid arthritis

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= Abstract =

**Background:** To determine the molecular structure of type II collagen-specific T-cell receptors associated with rheumatoid arthritis (RA). **Methods:** We generated CII-specific T-cell lines of 8 RA patients by prolonged *in vitro* culture with bovine CII (bCII) and the immunogenic peptide (256-270) of human CII. The proliferation response towards CII stimulation was measured from the uptake of <sup>3</sup>H-thymidine. Changes in the secretion of Th1 and Th2 cytokines in the culture supernatant were measured by ELISA. The TCR clonotypes of these T-cells were examined by RT-PCR/SSCP analyses of all 22 V chains. **Results:** T-cells from patients' tissue exhibited strong proliferation index upon CII stimulation, which was maintained up to 6 months in the culture. The secretion of INF- from these T-cells increased along with the duration of culture time, while the amount of IL-4 production did not show significant changes. The SSCP band patterns of patients' T-cells appear as discrete bands unlike the smeary streak produced from normal samples. Some SSCP bands, each representing selected expansion of a TCR containing certain subtype of V peptides, appeared to be identical in more than one patients. Among these, the expansion of SSCP band representing the V 14 CDR3 region persisted after switching the antigen to the immunogenic human peptide (256-270). **Conclusion:** CII-reactive T-cells expressing distinct CDR3 motifs are selectively expanded in the peripheral blood and synovial fluid of RA patients, and their persistent proliferation upon CII stimulation, as well as the production Th1-type cytokines, may play pivotal roles in RA pathogenesis.

**Key Words:** rheumatoid arthritis, Type II collagen, T-cell receptor, INF- , CDR3

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가

가 가 T 가 Strand Conformation Polymorphism)  
CDR3 region SSCP  
band ,  
가 , 2 T  
aggrecan, cartilage glycoprotein gp39 <sup>12</sup> .  
T 가 *in vitro*  
T T 2  
T RT-PCR/SSCP  
2 cytokine T  
T 가  
(1), T  
가  
가 .  
T 20  
T 1.  
Southern Blotting RT-PCR (ACR) American College of Rheumatology  
T 8  
가 3 (Table 1).  
HLA-DR typing Han <sup>14</sup>  
primer  
RT-PCR/SSCP PCR  
T 2  
RT-PCR 22 V CDR3 SSCP (Single T

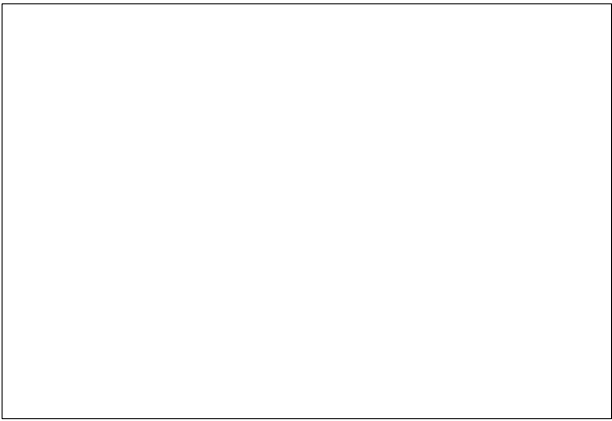
Table 1.

		T				
		( )	RF*	HLA-DR	SI ( 4 )	
1	62	6.4	475	DR04/04	2.1	§
2	59	1.2	211	DR01/15	§	3.1
3	32	1.2	179	DR02/04	1.02	2.9
4	45	2.5	136	DR09/09	1.83	3.5
5	47	5.3	§	DR09/09	2.7	3.7
6	38	5.7	580	n.d.	1.08	1.2
7	25	3.7	160	DR09/07	1.8	2.4
8	59	7.7	§	DR04/04	1.8	2.3
1	26	-	-	DR04/02	2.4	§
2	30	-	-	DR04/04	2.1	§
3	28	-	-	DR 15/16	1.2	§

Conserved T	CDR3 motif	2	T
	amino acids, 10% heat-inactivated fetal calf serum		
	1 ml RPMI-1640		
Ficoll-hypaque	non-T	B95-8	
Ficoll-Hypaque-RBC	Mini-MACS	EBV	1ml, 37, 5% CO <sub>2</sub>
CD3+	CD3-		
	CD3+	RPMI-1640	2 × 10 <sup>6</sup> cell/ml
culture	2 mM L-glutamine, 100 IU/ml		96-well round bottom plate
100 µg/ml	20 mM HEPES, 2 mg/ml	EBV transformed APCs	
sodium bicarbonate, 1mM	pyruvate, 50 µM	CD3+ T	RPMI-1640
(-mercapthoethanol, 1X nonessential amino acids,			96-well plate well
10% heat inactive fetal calf serum	가	가 2 × 10 <sup>5</sup> cells가	
2 × 10 <sup>5</sup>		APCs (EBV transformed non T )	
		2	T
2			40 µg/ml
	40 µg		chicken ovalbumin
chicken ovalbumin			
30	ovalbumin		human IL-2 (10unit)
control	30	immunogenic	7 well 1 × 10 <sup>5</sup>
human peptide (256-270)	non-immunogenic	control	40 µg/ml
peptide (271-285)	40 µg	(6).	10 resuspend
2	T		T
	<i>in vitro</i>	5	
	18	1µCi	180
<sup>3</sup> H-methylthymidine		<sup>3</sup> H-TdR	
multiple cell harvester (Cambridge Technology, Cam-			3. RT - PCR/SSCP T
bridge, MA)	liquid scintillation		T mRNA 1 unit (Gibco
counter (Model Tri-Carb 300C, Packard International)			BRL) 100pmol random hexamer (GibcoBRL)
		42	cDNA
	(SI [stimulation index] =		200 ng cDNA 25
cpm /	cpm ), 1,000 CPM	µl	100 nM dNTP, 0.3 IU Taq
	SI 2		polymerase (Takara, Korea) 20 pmol C(
	가		primer V 22가 V primer(7)
2. 2	specific T	T	CDR3 PCR
		PCR	94 45, 58 2,
MiniMACs Column	non-T-Cell(CD3-)	72 1	35 cycle
EBV transformation	B	DNA	가 PCR
	가	94	denaturation
. APC EBV	(APCs)		4% polyacrylamide 10% glycerol
CD3-	2 mM		non-denaturing
L-glutarnine, 100 IU/ml penicillin, 100 µg/ml	steptomycin,		nylon membrane
20 mM HEPES, 2 mg/ml sodium bicarbonate, 1mM		DNA	internal
pyruvate, 50 uM -mercapthoethanol, 1X nonessential		TCR C( probe (5'-AAC AAG CGT GTT CCC ACC	

CGA GGT CGC TGT GTT-3') Phototope  
star-detection kit (New England Biology, England)  
X

1. T  
2  
5 가 ,  
SI (Table 1).  
SI 가  
(not  
shown).  
가  
T  
. (Fig. 1)  
, 2  
SI 180



**Fig. 1.** < 1> 2  
T-cell line. S.I. (=stimulation index)  
immunodominant human peptide (256-270)  
<sup>3</sup>H-thymidine uptake control  
antigen, chicken ovalbumine human peptide  
(271-285) <sup>3</sup>H-thymidine uptake

12 가 .  
2. T  
2  
T Th1 Th2 type  
ELISA assay  
(Table 2).  
T 150  
INF- 가 가 IL-4  
2  
Th 1 type  
가 가  
T  
INF-  
IL-4  
40  
INF- 24pg/ml  
166pg/ml 가 (13).  
T-cell  
SI 2  
INF-? 가 (Table 2,  
1). T

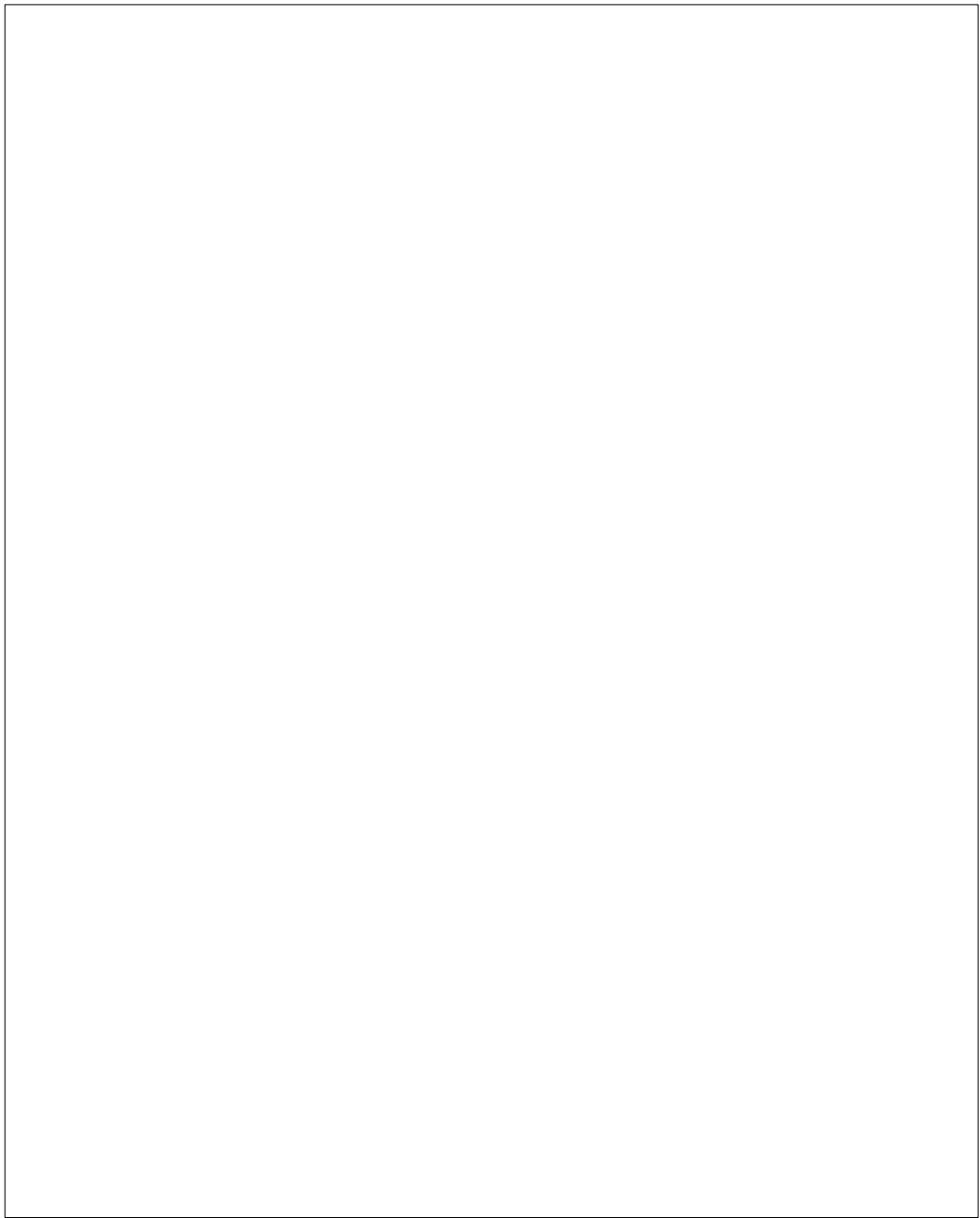
**Table 2.** T

Tissue		IFN- (pg/ml)	IL-4 (pg/ml)
< 1>	6	39	14
	20	88	16
	30	146	11
	150	387	4
< 1>	10	64	19
	20	146	17
	30	121	17
	150	387	4
< 1>	20	99	19
	30	171	21
	150	387	4
< 2>	20	78	8
	30	31	9

	Conserved T	CDR3 motif	2	T
3. RT - PCR - SSCP	2		T	V CDR3
T			가	
2	T	,	30	(Fig. 3).
mRNA	cDNA	4.		
22 V primer	CDR3			
PCR	, SSCP			
V CDR3	가	RT-PCR/SSCP	DNA	
가 DNA		CDR3		
	T	가		, SSCP
22 V CDR3	가 smear	가		
(Fig. 2A),				(4, 9, 12).
V CDR3	가	T	V	CDR3
	(Fig. 2B).		8	SSCP
T	SSCP	V 14	가	(Fig. 4A).
SSCP	가	V 14		
가				
	(not shown).			immunogenic human



**Fig. 2.** T V CDR3 RT-PCR/SSCP lane PCR  
 22 V specific primer  
 A. < 1 > B. < 6>



**Fig. 3.** RT-PCR/SSCP analysis of T cell receptor V $\beta$  30 (0 )  
lane PCR 22 V specific primer  
A. < 2> T (0 ) B. < 2> T (30 )  
C. < 3> T (0 ) D. < 3> T (30 )

peptide (256-270) 180  
(Fig. 4B).  
가 CDR3 motif  
V 6 V 13.1 type (not  
shown). 가 MHC

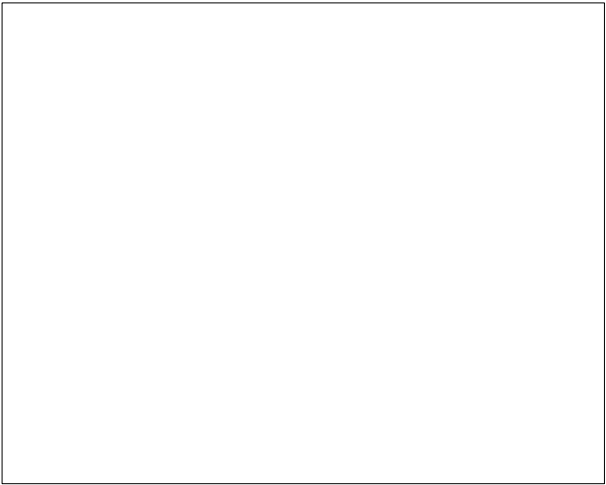


Fig. 4. 4

V 14 CDR3.  
A. T RT-PCR/ SSCP 4  
V 14 CDR3 ( )  
1: < 3> 2: < 4> 3: < 5> 4: < 6>.  
T

B. V 14 CDR3 SSCP ( )  
30 in vitro  
immunodominant human peptide (256-270)  
150 T  
1: < 1>  
T , 2: 9 T , 3:  
150 T .

T 가  
가 T

2 T 가

2

T  
T  
T 가 MHC  
T CDR3 ,

T V

가 T

T

T

(10).

2 T , in vitro

T

RT-PCR/SSCP ,

8 가 V 14 CDR3 가

.

2 T

.

RT-PCR/ SSCP

T clonality 가

(11), ( 2 ) T

2 T

.

6 T

2 가 T

(6).

2 가 4

T V 14

,

V 14 T 가

(2,5).

T

SSCP

smear ,

가

smear pattern

.

2 T 가

T SSCP

가 smear

(10).

2

T T 2

2

T

HLA-DR4가

(8, 11),

non-DR4 pattern

.

2

7

HLA-DR 1  
가

DR4 DR9, DR7

(6),  
HLA-DR  
T CDR3  
가

J

7 4  
V 14 CDR3

T

30  
human peptide (256-270)  
T 가

2 T  
immunodominant

(256-270)

T V

14 V 4, V 13.1

2 -reactive  
pathoge-

nesis T

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Conserved T	CDR3 motif	2	T
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