

Conserved T

CDR3 motif

2

T

가¹, 가^{1,2}, 가³, 가², 가², 가¹, 가^{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 ³H-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 T 가 Strand Conformation Polymorphism)
 CDR3 region SSCP
 band ,
 T
 2
 .
 12.
 aggrecan, cartilage glycoprotein gp39
 in vitro
 T
 T T 2
 RT-PCR/SSCP
 T
 2 cytokine T
 T 가
 (1), T
 가
 가 .
 T T 20
 1.
 Southern Blotting RT-PCR (ACR) American College of Rheumatology
 T T 8
 가 HLA-DR typing Han ¹⁴
 primer
 RT-PCR/SSCP PCR
 T T 2
 RT-PCR 22 V CDR3 SSCP (Single

Table 1.

	()	RF*	HLA-DR	SI (4)		
1	62	6.4	DR04/04	2.1	§	
2	59	1.2	DR01/15	§	3.1	
3	32	1.2	DR02/04	1.02	2.9	
4	45	2.5	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	§

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

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

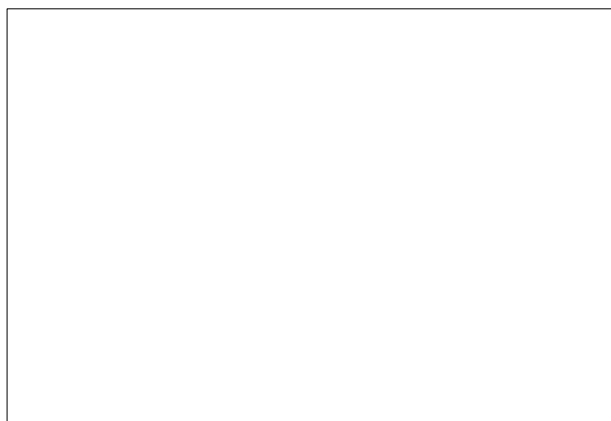


Fig. 1. < 1> 2
 T-cell line. S.I. (=stimulation index)
 immunodominant human peptide (256-270)
³H-thymidine uptake control
 antigen, chicken ovalbumine human peptide
 (271-285) ³H-thymidine uptake

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

INF-? 가 (Table 2,
 1). T

Table 2.

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

Conserved T CDR3 motif 2 T

3. RT - PCR - SSCP 2 T V CDR3

T

2 T , 가 30

(Fig. 3).

mRNA cDNA 4.

22 V primer CDR3

PCR , SSCP

V CDR3 가 RT-PCR/SSCP DNA

가 DNA . CDR3 DNA

T 가 , SSCP

22 V CDR3 가 smear 가 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

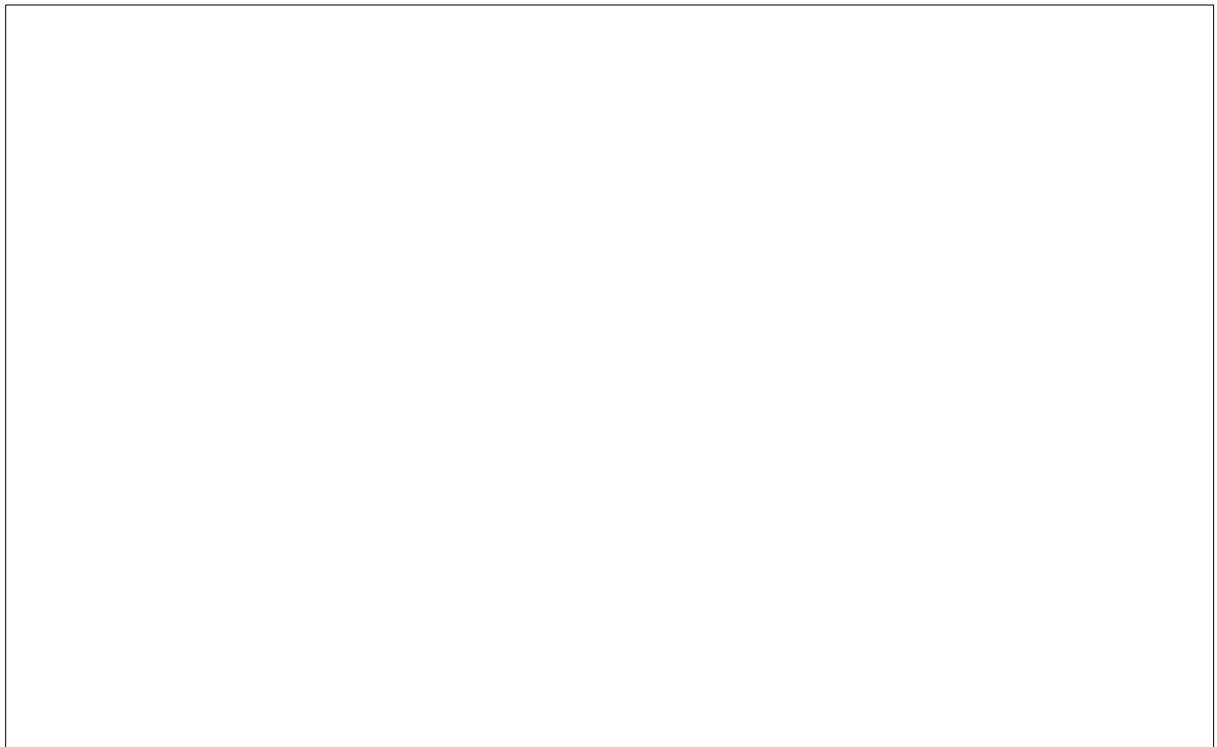


Fig. 2. T V CDR3 RT-PCR/SSCP T . lane PCR

22 V specific primer .

A. < 1 > B. < 6 >

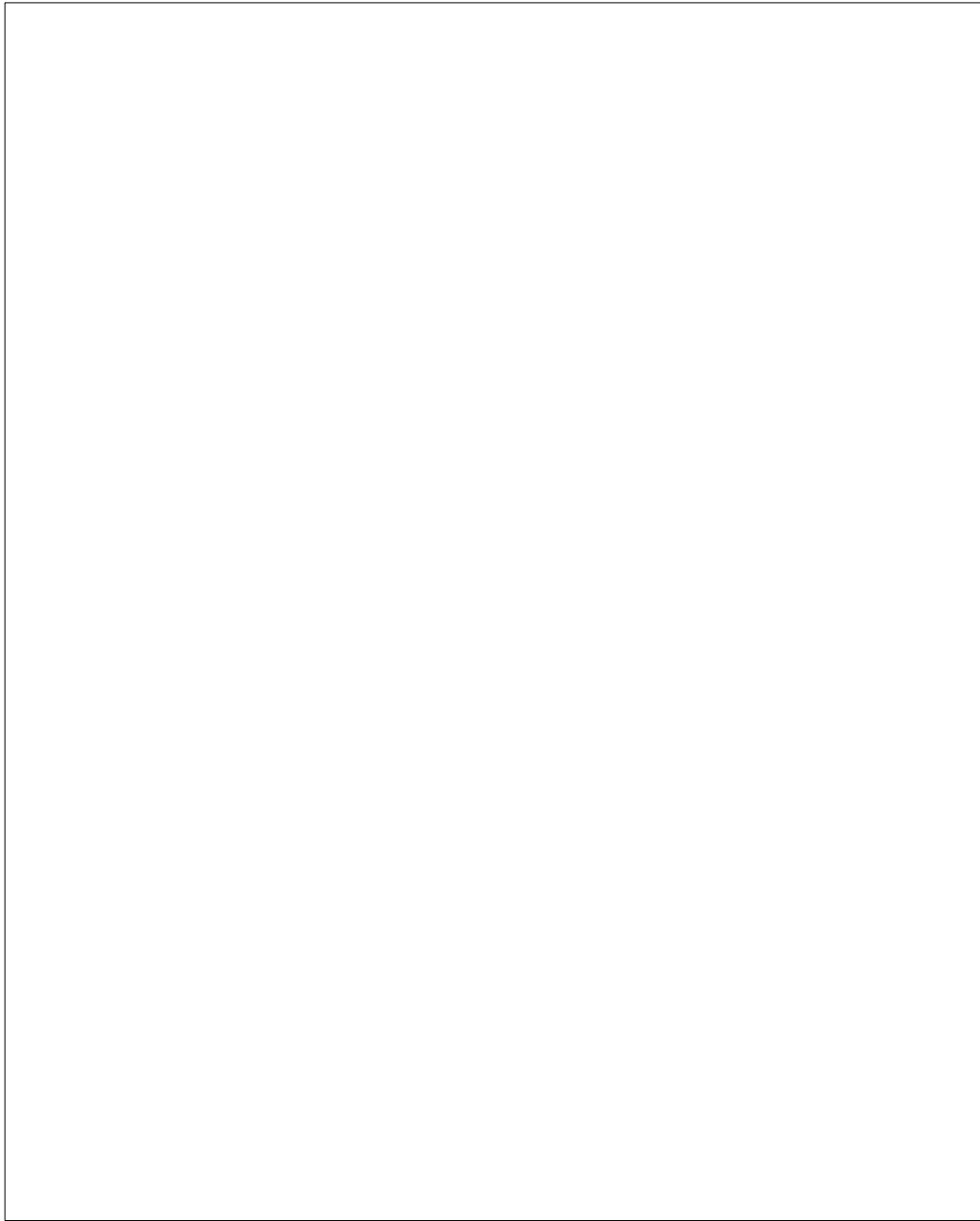


Fig. 3. RT-PCR/SSCP analysis of T cell receptor V_β CDR3 motifs. Lane A: PCR product from T cell line T-22 (30 cycles). Lane B: PCR product from T cell line T-2 (30 cycles). Lane C: PCR product from T cell line T-3 (30 cycles). Lane D: PCR product from T cell line T-30 (30 cycles). The RT-PCR/SSCP gel shows the CDR3 motifs for each lane.

peptide (256-270)

180

(Fig. 4B).

가

CDR3 motif

V_β 6 V_β 13.1 type shown).

(not

가

MHC

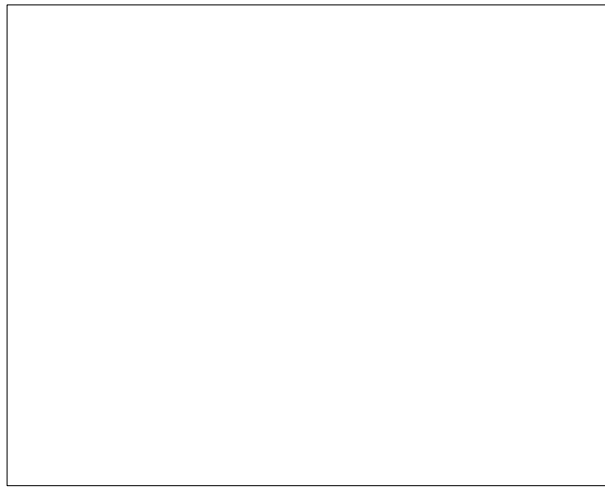


Fig. 4. 4

V 14 CDR3.
 A. T RT-PCR/ SSCP 4
 V 14 CDR3 ()
 1: < 3> 2: < 4> 3: < 5> 4: < 6>
 T
 9
 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 가
 2
 T V HLA-DR4가
 가 T
 T non-DR4 (8, 11),
 pattern
 T 가 2
 (10). 7

T
 2 , *in vitro*
 RT-PCR/SSCP
 8 가 V 14 CDR3 가
 2 T
 RT-PCR/ SSCP
 T clonality 가
 (11), (2) T
 T
 6
 2 가 T
 (6). 2 가 4
 T V 14 가
 V 14 T 가
 (2,5). T
 SSCP
 smear
 가 smear pattern
 T SSCP
 가 smear
 (10). 2
 T
 T 2
 T 가
 2
 T
 HLA-DR4가
 (8, 11),
 pattern
 가 2
 7

DR4 DR9, DR7 HLA-DR 1
 2 (6), HLA-DR 가
 J T CDR3
 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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