Cell Research (2000),**10**, 39-49

Isolation and characteristics of autoreactive T cells specific to aggrecan G1 domain from rheumatoid arthritis patients

LI NING LI[1,*], DONG QING ZHANG[1], KUANG YAN ZHOU[1], ANNIE CARTMAN[2], JEAN YVES LEROUX[2], A ROBIN POOLE[2], YI PING ZHANG[2]

[1] *Shanghai Institute of Immunology, Shagnhai Second Medical University, Shanghai 200025, China*

[2] *Joint Diseases Laboratory, Shriners Hospital, Department of Surgery, McGill University, Montreal, Canada*

ABSTRACT

Our previous work showed that the cartilage proteoglycan aggrecan could induce an erosive polyarthritis and spondylitis in BALB/c mice and the G1 globular domain of the aggrecan (G1) contained the arthritogenic region. To elucidate whether autoreactive T cells to G1 are expressed in rheumatoid arthritis patients, we analyzed the frequency of human G1-specific T cells in the peripheral blood of five rheumatoid arthritis patients and tried to establish G1-reactive T cell lines from these rheumatoid arthritis patients. The results showed that the G1-specific T cells in PBL were detectable at the range of $4.97 \pm 0.5 \times 10^{-6}$ in peripheral blood lymphocytes. We have also generated 15 G1-specific T lymphocyte lines from these pateints with a standard split-well method. All these cells expressed fine specificity to human recombinant G1, but not to unrelated antigen. All the 15 lines expressed a pan-T cell marker and 13 of them selectively used the $\alpha\beta$ T cell receptor. Two of them used $\gamma\delta$ T cell receptor. The 13 of these T cell lines was CD4 positive. One line expressed

* Correspondence: Li Ningli, Shanghai Institute of Immunology, Shanghai Second Medical Unuiversity, Shanghai 200025, China

CD8. One line expressed both CD4 and CD8. Moreover, 14 out of 15 lines expressed the Th-1 cytokine profile, characterized by interferon- γ positivity and IL-4 negativity. No Th-2 type cell line was generated. These data provide strong evidence in favor of the presence of autoreactive T cells in the rheumatoid arthritis pateints. What is the mechanism(s) that these autoreactive T cells attack self-target and whether these G1-specific, Th-1 type T cell lines can induce arthritis in immune deficiency mice are currently under investigation.

Key words: *rG1-specific T cell lines, T cell receptor, cytokine profile, rheumatoid arthritis.*

INTRODUCTION

Rheumatoid arthritis (RA) is a common disease characterized by the chronic lesion of polyarthritis. The etiology and pathogenesis of RA remain unknown. Autoimmunity to cartilage antigens may play a significant role in the pathogenesis of chronic inflammatory polyarthritis. It is commonly accepted that cell mediated immune responses are involved in chronic inflammation since T and B lymphocytes and antigen presenting cells were observed to be enriched in the synovium fluid of RA patients[1]. In vivo studies showed that T cells infiltrating into the synovium expressed IL-2 receptor, IL-10 and IFN- γ[2] and activated CD4 T cells could be detected in the peripheral blood of RA patients. The candidate autoantigens may include type II, IX, and XI collagens, human cartilage glycoprotein-39 (HC-gp-39) and proteoglycan aggrecan (PG), among which special attention has been paid for the G1 globular domain and link protein (LP) of PG[3], [4]. Aggrecan is retained by binding to the glycosaminoglycan hyaluronan through its amino terminal, the G1 globular domain. This molecule is also present in other tissues containing hyaluronan-binding proteoglycans such as versican, media of the aorta, sclera of the eye and central nervous system[5].

The G1 globular domain consists of 374 amino acids, which, as reported, could induce polyarthritis and spondylitis in mice. The T and B cell epitopes at distinct regions in bovine aggrecan G1 domain has been identified[6]. Moreover, patients with RA exhibit CD4 T cell responses to human G1. However, the G1 reactivity can also be detected in peripheral blood of healthy controls in a very low prevalence. To see whether autoreactive T cells to G1 are expressed in immune repertoire of RA patients, in present study we analyzed the frequency of human G1-specific T cells in the peripheral blood and synovium

of arthritis patients using soluble recombinant G1 as antigen. Meanwhile the G1-reactive T cell lines from these patients were established. The characteristics in terms of their phenotypes, the profile of cytokine secreting and the skewed patterns of TCR repertoire of the cell lines were also determined.

MATERIALS AND METHODS

Patients

Six Caucasoid patients with RA, at an age range from 37 to 69 years (median 53.0) were studied. Each of them was diagnosed according to the criteria from American College of Rheumatology. Blood samples from 4 patients were provided by Rheumatoid Diseases Department of Montreal General Hospital. Blood and synovium fluid samples from 2 patients were collected at Surgeon Department of Toronto University. All patients informed consent for this study.

Isolation of human peripheral blood lymphocytes and synovium fluid cells

The peripheral blood leucocytes (PBL) of patients were obtained by gradient centrifugation on Ficoll-Hypaque (Pharmacia Biotech AB, Sweden) for 30 min, washed twice in Hank's balance salt solution, and counted and subjected to determine the specificity to the rG1 (for setting up T cell lines, described as below). Synovium fluid cells were isolated by centrifugation mixed with Hank's balance salt solution.

Production of the recombinant human rG1 domain

The recombinant G1 domain of human aggrecan was produced in the Baculovirus expression system. The construct was made by PCR using coding primers that included the signal peptide of aggrecan and non-conding primer that ended at the end of exon 6 of the molecule using cDNA template (generous gift of Dr. P. Roughley). A 6 histidine TAG was also added at the C-terminal end of the molecule. The PCR product was introduced into the pFASTBAC1 vector (GIBCO/BRL) by Bamhead1 and HindIII cohesive end ligation. The cloning and selection of the recombinant baculoviurs was done by using the GIBCO/BRL BACMID expression kit. The recombinant protein was produced by infection of the SF21 insect cell line for 3 to 4 d. Medium and cell lysate were pruified by using the Qiagen Nickel-agarose purification kit. Pure protein were analyzed on SDS-PAGE and Western blot and for its ability to bind HA beads. Concentration of protein were determined and the pure recombinant G1 domain was filtered-sterilized and used in assay.

Synthetic peptides

Twenty peptides covering the full length of human aggrecan G1 domain (residues 1-374) were synthesized at a 0.25 mM scale using standard Fmoc (9-fluorenylmethoxycarbonyl) chemistry on a model 431A solid-phase peptide synthesizer (Applied Biosystems, Inc., Foster City, CA). The peptides were up to 20-28 amino acids long and were overlapped by at least five amino acids. The crude peptides were purified by reverse-phase chromatography (Prep-10 Aquapore C8 column; Applied Biosystems Inc.) using an acetonitrile gradient in 0.1% trifluoroacetic acid.

rG1 specific T cell lines

rG1 specific T cell lines were established and characterized as described elsewhere[7],[8]. In brief, for primary culture, PBL and synovium fluid were collected from the patients with RA. The lymphocytes were adjusted to 10×10^6/ml and 100 μl of the cell suspension were added into each well in round-bottomed microtiter plate and co-cultured in RPMI 1640 medium supplemented with 5% fresh heat-inactivated autologous serum in presence of rG1 (20 μg/ml). After 3 d in culture, the 100 μl of supernatants were collected per well and stored at -80 ^{o}C for determining

the profile of cytokines, and 100 μl of fresh medium with human AB serum and rIL-2 (20 ng/ml) was replaced per well. Fresh medium with AB serum and IL-2 was changed every 3 d until frequency assay was done.

Assay estimating the rG1-specific T cell frequency

Two weeks after primed culture, venous blood was drawn from same patients and the PBLs were isolated again. The PBLs were irradiated with 4000 rads as a source of antigen presenting cells (APC). The primary cultured T cells (0.4×10^6 per well) were mixed with APC (1×10^5 per well) and co-cultured with rG1 at the same final concentration as in primary culture. After 48 h, the supernatant was collected for identifying profile of cytokines as done in primary culture and the cells were labeled with tritiated thymidine (NEN Boston, MA, 0.2 μCi/well) and harvested 16 h later with a Skatron Multichannel Cellular Harvester (Norway, PO, Box-3401 LIER). Radioactivity was quantified in a β-scintillation counter. The proliferation was expressed by cpm $\pm$ SD. Proliferation of rG1 specific T cells were expressed by stimulation index (SI, mean cpm with antigen was divided by mean cpm with medium alone). The rG1 positive T cells were picked up upon the SI > 3 and transfer to a new microtiter plates. The selected cells were incubated with fresh 5% AB serum and IL-2. Mediums were changed every 3 d until 2 w. The frequency of rG1 positive T cells was assessed by positive wells multiplying cells per well (1×10^5/well) versus total wells (60×10^5).

Restimulation and rG1 specificity assay

PBL were isolated from same patients and irradiated as APC as described above. rG1 specificity was assessed by co-culturing rG1 positive T cells (0.4×10^6 per well), APC (1×10^5 per well) and whole antigen (rG1) or peptide, WT (the supernatant of Baculovirus culture, 20 μg/ml) and PPD (purified protein derivative, 5 μg/ml) as nonspecific control in optimal concentrations. After 48 h, the supernatant of culture was collected for identifying profile of cytokines and cultured cells were labeled with ^{3}H-TdR and harvested 16 h later. Radioactivity was quantified in a β-scintillation counter as described above. The proliferation was expressed by cpm $\pm$ SD. rG1 and peptides specificity were expressed by stimulation index (SI > 3). rG1 or peptides positive T cells were stimulated and expanded. The restimulation and expansion cycles were repeated every 14-20 d. These cells were used for further studies.

The cytokine profile of rG1-specific T cell lines

The cytokine profile was analyzed quantitatively using Quantikine ELISA Kits (QuantilineTM, Minneapolis, MN). In brief, the supernatant of T cells collected at different culture times were diluted at ratio of 1:20 with carbonate buffer and 200 μl of the samples were added to the individual wells of flat-bottomed microtiter plates precoated with anti-IFN-γ or anti-IL-4 antibodies. The plates were aspirated and washed three times with PBS containing 0.1% (v/v) Tween 20 after incubating at room temperature for 2 h. 200 μl of polyclonal antibody against horse radish peroxidase (HRP) were added to the well and incubated for another 2 h. The plates were washed again with wash buffer three times. 200 μl of substrate (DAB) were added to the wells and incubated for 20 min at room temperature and then 50 μl of stop solution were added to each well. The concentration of cytokines were determined by reading at 450 nm within 30 min automatically with ELISA reader (Elx-808; Bio-Tek Instruments, Inc., Winooski, VT).

Phenotype and T cell receptor analysis of rG1-specific T cell lines

5×10^5 T cells were labeled by a mixture of monoclonal antibodies against human CD3 (FITC-CD3) and CD4 (PE-CD4) and CD8 (PE-CD8),$\alpha\beta$(PE-$\alpha\beta$), $\gamma\delta$ (PE-$\gamma\delta$) and Ig isotype matched FITC or PE-conjugated antibodies as negative controls. The 2 color fluorescence were determined by a Coulter Cytometer (EPICS XL 4 color) and the data were analyzed by System II software. The results are expressed as percentage of positive cells.

PCR analysis of the expressed TCR Vβ repertoire

T cell receptor restricted usage of rG1-specific T cell lines: The T cell receptor usage was assesed by RT-PCR[12]. In brief, total RNA was prepared using Trizol (GIBCO). 1 μg of total RNA was used to synthesize the first-strand cDNA by adding 15 pM of random primer (oligodt15) and 5 u of Moloney leukemia virus reverse transcriptase (RT) (GIBCO). The RT reaction was performed in the presence of RNase inhibitor, 10 pM dNTPs (Pharmacia, LKB Biotechnology AB) in a buffer containing 10 mM Tris-HCl (pH 8.3), 6 mM KCl at 42 °C for 60 min and stoped by heating the mixture at 95 °C for 5 min. The cDNA preparation was used for PCR Vb analysis. The different Vβ were amplified using 24 of 5' Vb-specific primers and a common 3' Cb primer. PCR amplification was carried out using 10 pM dNTPs, 2 u Taq polymerase (GIBCO) in 1 × Taq buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl 1.5 mM $MgCl_2$, and 0.01% [wt/vol] gelatin) in a final volume of 50 ml. Amplification was performed for 30 s at 94 °C, 45 s at 55 °C, and 60 s at 72 °C for 25 cycles. 10 μl of each PCR reaction was loaded on 1% agarose gel with 0.5 μg/ml ethidium bromide and electrophrosed at 70 voltage for 40 min. The Vβs were determined by the bands appeared on the agarose gel.

RESULTS

The frequency of rG1-specific T cells separated from synovium and peripheral blood of paitients with RA

When the lymphocytes from synovium or PBL were co-cultured with rG1 for 3 d and restimulated with the same antigen after 2 w, the frequency of rG1 responsive cells were higher, compared with that of healthy individuals. The frequency of the rG1-specific T cells of PBL reached to 4.97 per 106 cells (4.97×10^{-6}) while that of synovium jumped to 9.85 per 106 cells (9.85×10^{-6}). This results indicated that there was high frequency of rG1-specific T cells indeed (Tab 1).

Tab 1. The frequency of G1-specific T cell in synovium fluid cells and PBL in RA patients

Case	Frequency of rG1 specific cells	
	SF	PBL
CK	9.7×10^{-6}	5.3×10^{-6}
MC	10.0×10^{-6}	6.3×10^{-6}
SL	NA	1.8×10^{-6}
RB	NA	5.0×10^{-6}
PV	NA	6.0×10^{-6}
MJM	NA	1.7×10^{-6}
Mean	9.85×10^{-6}	4.97×10^{-6}

NA: no sample available SF: synovium fluid

The rG1 specific T cell lines established from the patients with RA showed absolutely specific to rG1 or peptides

In this study, the T cell lines specific to rG1 or distinct region of G1 were generated from PBL. After 2 mon in culture with repeated stimulation using specific antigen and propagation in IL-2-conditioned medium, 15 lines were established. All these auto-reactive T cell lines-were found to be: (a) perfect specific rG1; (b) poorly reactive or non-reactive to purified protein derivative of tuberculin (PPD) and supernatant of Baculovirus

(WT). When cultured with twenty synthetic G1 peptides in the presence of APCs there were 5 lines (PV3, PV11, SL2, SL5, SL6) responding strongly to two peptides R15 and R16 which represent distinct regions, namely residues 328-353 and 349-374. (Tab 2).

Tab 2. The fine specificity of rG1-specific T cell lines

T cell line		Stimulation index (after antigen stimulation) rG1	WT	PPD	R15	R16
RB	9	6.3	2.7	0.59	0.3	0.1
	37	3.1	1.0	1.1	0.2	0.8
MJM	2	3.9	1.4	4.3	1.0	0.2
PV	3	2.3	0.3	1.5	10.5	19.1
	4	7.7	0.5	1.3	0.5	0.1
	6	28.0	10.7	2.4	0.4	0.3
	9	3.9	1.1	1.7	0.7	0.9
	10	17.2	1.0	4.3	0.9	0.1
	11	20.9	0.9	2.1	49.5	0.9
SL	1	7.9	0.21	3.7	0.5	0.8
	2	7.2	0.67	1.3	5.1	388.1
	4	4.5	0.14	0.9	0.3	0.7
	5	6.0	5.8	1.5	1.2	24.0
	6	1.1	5.2	2.1	1.1	48.8
	8	20.3	0.6	1.2	1.3	1.4

WT: culture supernatant of wild type insect cells Wt
PPD: purified protein derivative from tuberculin Sequence of peptide R15 and R16.
R15: ICYTGEDFVDIPENFFGVGGEEDITVG
R16: EDITVQTVTWPDMELPLPRNITEGEAG

Identification of phenotype of rG1-specific T cell lines

With direct immuno-fluorescence using a panel of anti-human monoclonal antibodies and FITC-labeled anti-IgG antibodies, we identified the phenotype of rG1-specific T cell lines. After 2 mon of restimulation and expansion cycles repeated every 14 to 20 d, the phenotypes of the 15 lines were analyzed by Coulter Cytometer and expressed as percentage of positive cell in the lines. CD3 was observed in all of the cell lines (95.6 $\pm$ 4.4%). 13 of the lines expressed CD4 (87.9 $\pm$ 7.5%) and poorly expressed CD8 (8.6 $\pm$ 6.3%). All the 13 lines used $\alpha\beta$ TCR (88.5 $\pm$ 10.5%) and poorly used $\gamma\delta$ TCR (5.2 $\pm$ 6.7%) (see Fig 1). Two lines used $\gamma\delta$ T cell receptor (89.3% and 68.4% respectively). Of the two lines, one expressed both CD4 (68.3%) and CD8 (36.8%). One line mainly expressed CD8 (50.8%).

The cytokine profile of G1-specific T cell lines

The supernatants from primary culture, or culture supernatants from doing frequency and specificity assay were collected. The concentration of IFN-γ and IL-4 were determined with a standard ELISA assay. The results showed that there were no detectable cytokines in primary culture and low concentration of IFN-γin frequency assay (145.3 $\pm$ 95.2 pg/ml) (second re-stimulation). There were high concentration of IFN-γ (489.3 $\pm$ 452.1 pg/

ml) in culture supernatant in doing specificity assay. No IL-4 was detected in the cultures. These profile cytokines showed that most of the cell lines were Thead1 instead of Thead2 subset (Tab 3).

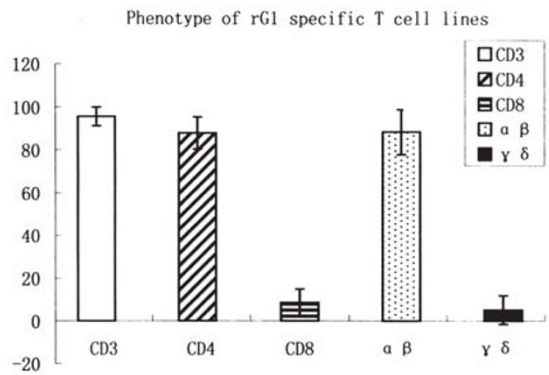

Fig 1.
Phenotype of G1 specific T cell Lines. The open bar represents the percentage average of CD3 expressed cell in the cell lines. The shaded bar represents the percentage of CD4 expressed cells in the cell lines. The black bar with white dot means the percentage of CD8 expressed cells in the cell lines. The dotted bar represents the percent of $\alpha\beta$ TCR expressed on the cell lines. The last black bar represents the percentage of $\gamma\delta$ TCR expressed cells in the cell lines.

Tab 3. The cytokine profile of G1-specific T cell lines

T cell line		Cytokine Concentration		T cell subset
		IFN-((*pg/ml*)	IL-4 (*pg/ml*)	
RB9		<15	<15.00	?
RB37		46.69 ± 2.41	<15.00	Th1
MJM2		ND	ND	NA
PV	3	102.56 ± 6.73	<15.00	Th1
	4	361.66 ± 4.35	<15.00	Th1
	6	ND	ND	NA
	9	15.65 ± 26.83	<15.00	Th1
	10	76.98 ± 3.07	<15.00	Th1
	11	289.55 ± 9.02	<15.00	Th1
SL	1	ND	ND	NA
	2	ND	ND	NA
	4	>1000.00	<15.00	Th1
	5	>1000.00	<15.00	Th1
	6	>1000.00	<15.00	Th1
	8	>1000.00	<15.00	Th1

* ND: no detected; NA: no sample available

TCR Vβ restricted usage

The restricted Vβ usage was analyzed by using RT-PCR method with 24 primers to amplify the Vβ. The results showed that the cell lines raised from patient PV preferentially used TCR Vβ17 or Vβ3 (Fig 2) although other Vb elements such as Vβ1, 18, 19 usage were also found fortuitously in some lines (data not shown). The preferential Vβ usage were not found in other cell lines.

Fig 2.
Agarose gel analysis of Vβ usage. Band 1: Marker; Band 3: Vβ3; Band 12: Vβ17 (around 400bp).

DISCUSSION

It is believed that autoimmunity to cartilage antigen is one of the most important mechanisms to induce rheumatoid arthritis[9]. Although there are many candidate autoantigens, the proteoglycan aggrecan was found to induce erosive polyarthritis and spondylitis in BALB/c mice. G1 domain of the proteoglycan aggrecan (G1) is the arthritogenic region and the T and B cell epitopes at distinct regions in bovine aggrecan G1 domain have been identified[6].

In our study, the results demonstrated that there are G1 autoreactive T cells in PBL and synovium of RA patients and the G1-specific T cell lines can be raised from these patients using rG1 in vitro. The frequency of G1-specific T cell lines can reach to a high level with 4.97×10^{-6} (from PBL) and 9.85×10^{-6} (from synovium). The T cell lines showed the fine specificity to rG1 with no cross reaction with WT and PPD. Moreover, all these cell lines expressed CD3 and most of them were CD4 positive. The cytokine profiles showed that these T cell lines polarized to Th1 with high level of IFN-γ instead of Th2. The results of Vβ usage indicated that some lines from PV selected Vβ17 or Vβ usage. But other lines do not show an obvious tendency of Vβ usage.

Our previous work showed that there were rG1-specific T cell lines in healthy individuals[15]. The rG1-spcific T cell lines were isolated from the peripheral blood lymphocytes of healthy individuals but the frequency was very low in comparison with these of the patients[8]. This indicated that there were more autoreactive T cells in the RA patients. In the synovium of RA patients, the rG1 frequency was higher than that of PBL in patients. This means that there were more autoreactive T cells infiltrating in erosive region. In this study, for identification the specificity to pre-domain of rG1 in these T cell lines, the cell lines were co-cultured with 20 synthetic G1 peptides respectively. 5 lines

recognizing the peptides R15 (residues 328-353) and R16 (residues 349-374) were found. In our previous study in mice, there were two epitopes that are recognized by T cell lines specific to proteoglycan (PG) core protein or G1, located within residues 70-84 and 150-169 when natural G1 attached by glycosaminoglycan keratan sulfate (KS)[4] was used as antigen. Adoptive transfer of T cells specific for peptide 70-84 into BALB/c mice led to development of arthritis in knee joints. Moreover, two T cell hybridomas which recognized both human fetal and adult PG generated from BALB/c mice with severe inflammatory arthritis were also detected to respond exclusively to peptide 70-84[6]. Peptide 150-169 is a subdominant T cell epitope compared with epitope 70-84. However, in T cell lines that are produced against PG or G1, all consistently show a strong response to this peptide. Furthermore, peptide 150-169 is located within an absolutely conserved region of the aggrecan core protein in human, bovine, rat and mouse G1[6]. But, in this human lines study, no T cell lines responded to these two peptides were found. The interesting thing was the region of R15 and R16 is B cell epitope distributed near C terminal of G1 which only could be exposed after removing the KS chain from G1 molecule[6]. This results may demonstrate that the B cell may present the special epitope to the T cells and promoted T cells activating and proliferating. Also, it was possible that this structure protein could initiate the host immunological system and induce the auto-reactive T cells. Our results indicated that C terminal of G1 was same important as that of residues 70-84 and 150-169 in the pathogenesis of PG.

According to the cytokine profiles, T cells are divided into two subsets: Th1 and Th2. It is regarded that secreting IFN-γ is a marker of Th1 but IL-4 or IL-5 or IL-13 is an obvious marker of Th2[11]. In this study, most T cell lines were Th1 according to their cytokine profiles. They secreted high level of IFN-γ after they got second re-stimulation. This means that G1 activated the Thead1 cells instead of Th2. It was very interesting as the cell lines stimulated by rG1 were totally different from that stimulated by MBP (myelin basic protein) which induced high level of IL-13 (Dongqing Zhang et al, unpublished data). Our results consist with the previous report that the IFN-γ expression T cells were commonly found in the synovium of RA patients[2]. These results demonstrated that rG1 could contribute to cellular immunity in the patients with RA.

It was known that there are two types of TCR, $\alpha\beta$ TCR and $\gamma\delta$ TCR. In the healthy individuals, 95% of T cells express $\alpha\beta$ TCR and only 5 % of the T cells with $\gamma\delta$ TCR in the peripheral blood lymphocytes. Usually, most of the $\gamma\delta$ T cells distribute under mucosa of intestine and kill the bacteria lived in the cells. They bloom when individuals fall sick or are invaded by pathogenic organisms[12]. It is regarded that $\gamma\delta$ T cells play a very important role in the immune regulation. In this report, we showed that most rG1 specific T cell lines set up from patients with RA were $\alpha\beta$ T cells analyzed by monoclonal antibody. Only 2 lines gave gd T cell phenotype while no $\gamma\delta$ T cell line was established from the healthy donors[10]. The role of these $\alpha\beta$ and $\gamma\delta$ T cells in the pathoarthritis is still under investigation.

T cell receptor genes are assembled in progenitor lymphocytes by the process of V(D)

J recombination. Cells expressing different Vβ genes generally exhibit distinct functional characteristics. Also the diversity of TCR repertoire is produced by rearrangement and junctional variation of these gene fragments during differentiation of T cells. TCR Vβ restricted usage represents the T cells driven by antigen. There were a number of reports described the TCR Vb usage. The restricted Vβ usage had been identified by hybridization using Southern blot and Cβ probe. The anti-specific Vβ monoclonal antibodies also were used for detecting the BV usage. Analysis with RT-PCR are commonly used for study the Vβ usage recently. At least 24 fragments were found in the Vb repertoire[13]. Previous study has showed that there was selected Vβ 7 usage in the fresh T cells isolated from synovium of patients with RA but transferred to use Vβ6 after stimulated with IL-2[14]. We indicated that rG1 specific T cell lines established from BALB/c mice using restrictively the Vβ4 and Vβ8[6]. In this paper, we analyzed that G1 specific T cell lines from PBL of patients with RA using RT-PCR. We found that they tended to use Vβ3 and Vβ 17 in the cell lines from patients PV. This results was different from that in the cell lines from normal individuals which showed Vβ 2 TCR usage.

In conclusion, we reported that there were G1 auto-reactive T cells in the PBL and synovium of RA patients. G1-specific T cell lines can be raised from these patients using rG1 stimulation in vitro. The frequency of G1-specific T cell lines was much more higher than the frequency found in healthy donors. These T cell lines were characterized as Th1 expressing $\alpha\beta$ TCR mainly. No Th2 cell lines were generated in this study. Th1 cells are thought to be responsible for the induction of auto-immune response, because of their ability to secrete pro-inflammatory cytokines. Our data provide strong evidence in favor of the presence of auto-reactive T cells in the immune repertoire of RA patients.

REFERENCES

[1] Banerjee S, Webber C, Poole AR. The induction of arthritis in mice by the cartilage proteoglycan aggrecan: role of CD4+ and CD8+ cells. Cell Immunol 1992; **144**:347-57.

[2] Manicourt DH, Trili R, Fukuda K et al. Levels of circulating tumor necrosis factor and interleukine-6 in patients with rheumatoid arthritis. Arthritis Rheum 1993; **36**:490.

[3] Stuart JM, Watson WC, Kang AH. Collegen autoimmunity and Arthritis. FASEB J 1988; 2:2950-6.

[4] Glant TT, Mikeca K, Arzoumanian A, and Poole AR. Proteoglycan induced arthritis in BALB/C mice. Clinical fratures and histopathology. Arthritis Rheum 1987; **30**:201-12.

[5] Guerassimov A, Duffy C, Zhang YP, et al. Immunity to Cartilage link Protein in Patients with Juvenile Phermatoid Arthritis. J Pheumatol 1997; **24**:959-64.

[6] Zhang YP, Guerassinov A, Leroux JY, et al. Arthritis induced by proteoglycan aggrecan G1 domain in Balb/c mice: Evidence of T cell Involvement and the Immunosuppressive influence of keratan sulfate on recognition of T and B cell Epitopes. J Clin Invest 1998; **101**:1678-86.

[7] Zhang JW, Markovic S, Lacet B, Raus J, Weiner HL, Hafler DA: Increase frequency of interlukin-2 responsive T cells specific for myelin basic protein and proteolipid protein in peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. J Exp Med 1994; **179**:973.

[8] Li NL, Zhang DQ, Chou KY. Generation of auto-reactive T cells specific to aggrecan G1 domain from healthy individuals. Shanghai J Immuno in Chinese 2000; **16**:17-20.

[9] Poole AR. Cartilage in health and disease, In: Koopman WJ, editor, arthritis and allied conditions. 13th

ed. Baltimore: Williams and Wilkins; **1997**:255-308.
[10] Chen XH, Li NL, Liu BY, et al. The characteristics of G1 specific T cell lines of healthy individuals. Acta of SSMU, 2000. In press.
[11] Romagnain S. The Thead1/Thead2 paradigm. Immunol Today 1997; **18**:163.
[12] Hacher G, Wangner H. Proliferative and cytolytic responses of human gd T cells display a distinct specificlty pattern. Immunol 1994; **81**:564.
[13] Labrecque N, McGrath H, Subramanyam M, Huker BT, Sekaly RP. Human T cells Respond to mouse mammary tumor virus-encoded superantigen, Vb restriction and conserved evolutionary features. J Exp Med 1993; **177**:1735-43.
[14] Chrustioger M, Claudio L. Alex S. PCR-based analysis of the TCR repertoire in human autoimmune diseases. Immunol Today 1992; **13**:336.

Received Oct-14-1999. Revised Jan-31-2000. Accepted Feb-1-1999.