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Frequency of gamma delta T cells in peripheral blood, synovial fluid, synovial membrane and lungs from patients with rheumatoid arthritis.

Takehide Mitogawa[*]   Koji Nishiya[†]

Zensuke Ota[‡]

[*]Okayama University,
[†]Okayama University,
[‡]Okayama Univresity,

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Abstract

The percentages and absolute numbers of gamma delta T cells per CD3 positive cells (T cells) in four different compartments, namely peripheral blood, synovial fluid, synovial membrane and lungs from patients with rheumatoid arthritis (RA) and in peripheral blood from healthy controls were studied by two color flow-cytometric analysis. The percentages (mean +/- SEM = 6.3 +/- 0.8%, n = 22) and absolute numbers (70 +/- 11/microliters, n = 22) of gamma delta T cells in peripheral blood from RA patients were not different from those of 22 age-matched healthy controls (7.5 +/- 0.9%, 81 +/- 17/microliters, respectively). The gamma delta T cells in peripheral blood from 50 RA patients were, however, significantly decreased in negative correlation with the value of CRP as a marker for inflammation, although they had no correlation with the titer of rheumatoid factor as an autoantibody. The percentages of gamma delta T cells in synovial fluid from 10 patients (3.3 +/- 0.5%, n = 10) or in synovial membrane from 5 patients (4.2 +/- 1.9%, n = 5) and in bronchoalveolar lavage fluid from 6 patients (3.6 +/- 0.8%, n = 6) were not different from those in peripheral blood from the same patients. Thus, gamma delta T cells are not the dominant infiltrating T cell subset in the inflammatory sites of RA patients.

KEYWORDS: rheumatoid arthritis, gamma delta T cells, synovial fluid, synovial membrane, bronchoalveolar lavage fluid

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Frequency of γ δ T Cells in Peripheral Blood, Synovial Fluid, Synovial Membrane and Lungs from Patients with Rheumatoid Arthritis

Takehide Mitogawa, Koji Nishiya* and Zensuke Ota

Third Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan

The percentages and absolute numbers of γ δ T cells per CD3 positive cells (T cells) in four different compartments, namely peripheral blood, synovial fluid, synovial membrane and lungs from patients with rheumatoid arthritis (RA) and in peripheral blood from healthy controls were studied by two color flow-cytometric analysis. The percentages (mean $\pm$ SEM $= 6.3 \pm 0.8\%$, n $= 22$) and absolute numbers ($70 \pm 11/\mu$l, n $=22$) of γ δ T cells in peripheral blood from RA patients were not different from those of 22 age-matched healthy controls ($7.5 \pm 0.9\%$, $81 \pm 17/\mu$l, respectively). The γ δ T cells in peripheral blood from 50 RA patients were, however, significantly decreased in negative correlation with the value of CRP as a marker for inflammation, although they had no correlation with the titer of rheumatoid factor as an autoantibody. The percentages of γ δ T cells in synovial fluid from 10 patients ($3.3 \pm 0.5\%$, n $= 10$) or in synovial membrane from 5 patients ($4.2 \pm 1.9\%$, n $= 5$) and in bronchoalveolar lavage fluid from 6 patients ($3.6 \pm 0.8\%$, n $= 6$) were not different from those in peripheral blood from the same patients. Thus, γ δ T cells are not the dominant infiltrating T cell subset in the inflammatory sites of RA patients.

Key words : rheumatoid arthritis, γ δ T cells, synovial fluid, synovial membrane, bronchoalveolar lavage fluid

Rheumatoid arthritis (RA) is known as one of the autoimmune disorders in which synovial tissue of joints is the major target organ involved (1). In addition to synovitis, the extra-articular manifestations such as subcutaneous nodules, lymphoadenopathy, pleuritis and pulmonary fibrosis are also noted in RA patients (2). Although the etiology of this unmanageable disease for joint destruction is not known yet, it is well recognized that the abberation in the immune system of these patients causes the perpetuation

of joint inflammation (1).

There are two sets of immune cells involved in this abnormality. The B cells are producing an autoantibody, *i.e.,* rheumatoid factor, which makes the immune complexes with autologous IgG, activates the complement pathway and gives rise to the joint inflammation and systemic vasculitis (1). In the other set, the T cells with functions of cellular immunity are the predominant cells which infiltrate in synovia at the major site of RA inflammation (1). It has been described that helper/inducer T cells phenotypically with CD 4 positive were much more numerous in

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* To whom correspondence should be addressed.

372   Mitogawa *et al.*

RA synovial tissue (3). Moreover, the recent reports (4–6) revealed that "memory" T cells phenotypically with $CD4^+$ and $CD45RO^+$ (one protein molecule of $CD45$ isoforms) were the dominant T cell subset in joints rather than "naive" T cells phenotypically with $CD4^+$ and $CD45RA^+$.

The T cells recognized the antigen through T cell receptor (TCR) complexes consisting of the $CD3$ complex and the disulphide-linked heterodimers (α/β or γ/δ chains). The majority of T cells in peripheral blood expressed α/β chains, but a small proportion of T cells expressed γ/δ chains, called $\gamma\delta$ T cells (7). It has been recently clarified that γ/δ T cells have unique immune functions such as non MHC-restricted cytotoxicity, NK activity, reactivity to some bacterial infections and involvement in pathogenesis of autoimmune diseases (7).

The recent studies (8–10) revealed that the proportions of $\gamma\delta$ T cells in peripheral blood, synovial fluid or synovial membrane from RA patients were increased, indicating that these cells might be the major T cell subsets involved in RA synovitis. This observation was, however, not confirmed by the other investigators (11–13) who did not find any sigfnificant difference in the frequency of $\gamma\delta$ T cells between RA and controls.

In this study, we examined the proportion and absolute numbers of $\gamma\delta$ T cells in four different compartments, *i.e.*, peripheral blood, synovial fluid, synovial membrane and lungs of RA patients. We have found no significant increase of $\gamma\delta$ T cells in these compartments from RA patients when compared with healthy controls, although the proportion of $\gamma\delta$ T cells in peripheral blood has negative correlation with the value of CRP in RA patients. Our results were compatible with the findings of Smith *et al.* (11), Pope *et al.* (12) and Gerli *et al.* (13), indicating that $\gamma\delta$ T cells were not the major T cell subset involved in the inflammation of RA.

Materials and Methods

Patients. We studied fifty patients (15 males and 35 females) who fulfilled the American Rheumatism Association 1987 criteria for the diagnosis of RA (14) ranging in age from 20 to 73 years (mean: 54.1). The disease duration of these patients was an average of 9.0 years, ranging from 3 to 20 years. All patients were taking a type of nonsteroidal anti-inflammatory drugs (NSAIDs). Twenty patients were under gold therapy of weekly intramuscular injections of gold salt thiomalate at the dose of 10 or 25 mg, 19 patients were treated with oral administration of D-penicillamine or bucillamine at the dose of 100–300 mg/day, and 19 patients were taking an amount of less than 5 mg/day of prednisolone. Two patients were taking the low dose (25 mg/day) of immunosuppressive agents such as cyclophosphamide. The control population consisted of 50 healthy individuals (18 males and 32 females), ranging in age from 4 to 90 years (mean:43.1).

Peripheral blood. The total volumes of 4 ml of peripheral blood samples from the RA patients and healthy donors were collected in the tubes containing Na_2 EDTA (disodium salt of ethylenediamine-tetraacetic acid) by venesection. Two milliliters of each blood sample were used to measure for leukocyte cell count and differentials. The remaining 2 ml of blood samples were supplied for the cytometric analysis.

Synovial fluid. The synovial fluid samples were obtained by intra-articular puncture. The heparin sodium (10 u/ml, Upjohn, Tokyo, Japan) and hyaluronidase (20 u/ml, Mochida, Tokyo, Japan) were immediately added to them. Then, the samples were passed through a layer of iron-mesh (pore size 210 μm) and washed twice with phosphate buffered saline (PBS) and suspended in PBS.

Synovial membrane. The synovial membranes from 5 RA patients were obtained when total knee replacement surgery was performed. The elution of lymphocytes from RA synovial tissue was done according to the report by Abrahamsen *et al.* (15). The samples were finely minced with sterile scissors in Hanks' balanced salt solution without Ca^{++} and Mg^{++} (GIBCO Lab., Life Technologies, Inc., Grand Island, NY, USA) and digested with 1 mg/ml of collagenase (Worthington Biochemical Co., Freehold, NJ, USA) and 40 Kunitz units/ml of deoxyribonuclease I (Sigma Chemical Co., St. Louis, Mo, USA) for 60 min at 37 °C. The tissue cells obtained were passed through a layer of iron-mesh (pore size 210 μm; Ikemoto, Tokyo, Japan), washed twice with RPMI 1640 medium (GIBCO Lab.)

and incubated with the medium supplemented with 2 mM L-glutamine, penicillin G ($100\,\mu$g/ml), streptomycin ($100\,\mu$g/ml), 25 mM Hepes and 10 % fetal calf serum (FCS) on plastic dishes at 37°C in a 5 % CO_2 atmosphere overnight. After incubation, the nonadherent cells were obtained with prewarmed medium and again passed through a layer of iron-mesh. The cells were washed twice with PBS and resuspended in PBS.

Bronchoalveolar lavage fluid.   The bronchoalveolar lavage (BAL) was performed in 6 RA patients (2 males and 4 females) with the informed consent. These patients were not current smokers. All patients had slight roent-genographic evidence of interstitial lung disease with respiratory symptoms but no abnormal pulmonary function and were taking only NSAIDs. The blood samples were simultaneously obtained from all 6 patients in whom BAL was performed. After local anesthesia of the pharynx and airways with 2 % lidocaine spray, BAL was performed by the conventional method with a flexible fibreoptic bronchoscope (Olympus, BF 1 T 20) by instillation of boluses of 50 ml of 0.9 % saline pre-warmed at 37°C in the total volume of 100–150 ml. The fluid was filtered through gauze to remove gross mucus. The total volume of recovered fluid was noted. The cells were separated from the lavage fluid by low speed centrifugation (800 g for 10 min at 4°C) and the supernatant was discarded. The cells were resuspended in PBS and evaluated for total number of cells and differential cell count by May-Giemsa staining.

Monoclonal antibodies used.   The fluorescein isoth-iocyanate (FITC)-conjugated monoclonal antibodies (MoAbs) {anti-CD 3 (Leu 4) and anti-CD 8 (Leu 2 a)} and phycoerythrin (PE)-conjugated MoAbs{anti-CD 3 (Leu 4) and anti-CD 4 (Leu 3 a) } were purchased from Becton Dickinson, Mountain View, CA, USA. The T cells expressing the γ/δ gene products were identified with FITC-conjugated δ TCS 1 and TCR δ 1 (T Cell Sciences, Cambrige, MA, USA).

Flow cytometric analysis.   Each monoclonal antibody was diluted 5 times with PBS containing 0.1 % NaN_3 (PBS, pH 7.2). Twenty μl of each FITC-or PE-conjugated antibodies were added together to $100\,\mu$l of the whole blood samples, and then incubated in the dark for 60 min at 4°C. Two ml of FACS lysing solution (dilution 1:10, Becton Dickinson, Mountain View, CA) were added to the samples for hemolysis, following incubation for 5 min at 37°C. The cells were washed 3 times with PBS and resuspended in PBS. Similarly, the resuspended cells from synovial fluid, synovial membrane or BALF were stained with the monoclonal antibodies and analyzed by FACScan. For the two-color flow cytometric analysis, FACScan (Becton-Dickinson) flow cytometer was used. The analysis was performed by gating the lymphocyte population based on forward angle and 90° light scatter. Before counting the specific staining in each analysis, the Calibrite beads, leucogate and control antisera were used to avoid the non-specific staining. The minimum of 10^4 events were analyzed for each sample. A representative cytofluorogram for γ δ T cells was shown in Fig. 1.
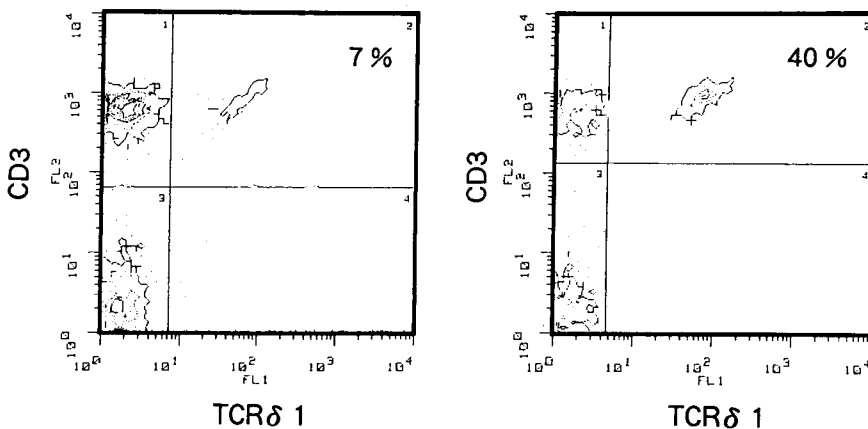

Fig. 1   A representative cytofluorogram of γ δ T cells. Seven percent TCR δ 1 positive cells per CD3 positive T cells (on the left side) represent the average percentage of healthy controls. Forty percent TCR δ 1 positive cells per CD3 positive T cells (on the right side) represent the highest percentage in 50 RA patients studied. Twenty microliter of the monoclonal antibodies, PE-labelled anti-CD3 and FITC-labelled anti-δ chain (TCR δ 1) was added in 100 μ 1 of the whole blood sample and the cells were incubated for 60 min at room temperature. The red blood cells were lysed in the hypotonic solution and the white blood cells were washed twice with PBS and resuspended in 0.5 ml of PBS. Then, FACScan analysis was employed. See the Materials and Methods for details.

374　　　　　　　　　　　　　Mitogawa *et al.*

Statistical analysis. The statistical analysis between the values in groups was made by the Student's *t*-test.

Results

Lymphocyte subsets in peripheral blood from controls and RA patients. The white blood cell counts (WBC) in RA (mean ± SEM = 8800 ± 500/μl, n = 50) were significantly higher than controls (5700 ± 220/μl) in the relative neutrophilia since the percentages and absolute numbers of lymphocytes were decreased (Table 1). The T cells (CD3 positive cells) and the major T cell subsets, namely helper/inducer T cells (CD4$^+$) or suppressor/cytotoxic T cells (CD8$^+$) and 4/8 ratio were not significantly different from the controls. The percentages and absolute numbers of V δ 1$^+$ T cells stained by MoAb δ TCS1 (2.3 ± 0.5 %, 23 ± 7/μl, n = 24) were about one third of that of γ δ T cells stained by MoAb TCR δ 1 (7.1 ± 0.5 %, 81 ± 10/μl, n = 50) in the healty controls. The percentages and absolute numbers of V δ 1$^+$ (1.6 ± 0.3 %, 23 ± 5/μl, n = 19) and γ δ T cells (6.5 ± 0.9 %, 66 ± 8/μl, n = 50) in RA were not different from the controls (Table 1).

γ δ T cells in RA patients. We have shown in the previous report (16) that the percentages and absolute numbers of γ δ T cells in peripheral blood from 50 healthy controls (ranging in age from 4 to 90 years) were decreased with ageing. Therefore, the percentages and absolute numbers of γ δ T cells in peripheral blood from 22 age-matched controls (8 males and 14 females, mean of ages: 48.1 years, ranging from 22 to 76) and 22 RA patients (7 males and 15 females, mean of ages: 49.0 years, ranging from 20 to 72) were compared (Figs. 2 and 3). Those of γ δ T cells in RA (6.3 ± 0.8 %, 70 ± 11/μl, n = 22) were not different form that of the controls (7.5 ± 0.9 %, 81 ± 17/μl, n = 22). From these results, basing on the idea that γ δ T cells in peripheral blood might migrate into the

inflammatory sites of RA patients, we investigated those of γ δ T cells in synovial fluid (SF), synovial membrane (SM) and BALF. Unexpectedly, the mean of percentages and absolute numbers of γ δ T cells in these locations were lower (3.3 % in SF; 4.2 % in SM; 3.6 % in BALF) than that of peripheral blood (6.3 %, Figs. 2 and 3).

Table 1　Percentages and absolute numbers of lymphocyte subsets in peripheral blood from RA patients

Items		Controls	RA
WBC^a	(/μl)	5700 ± 220^b (50)^c	8800 ± 500 (50)*
Ly^d	(%)	30.7 ± 1.2 (50)	23.0 ± 1.4 (50)*
CD3$^+$	(%)	61.3 ± 1.4 (50)	58.4 ± 2.0 (50)
CD4$^+$	(%)	36.3 ± 1.2 (50)	36.9 ± 1.4 (50)
CD8$^+$	(%)	30.9 ± 1.3 (50)	25.4 ± 1.3 (50)
4/8 ratio		1.34 ± 0.1 (50)	1.67 ± 0.1 (50)
δ TCS1$^-$	(%)	2.3 ± 0.5 (24)	1.6 ± 0.3 (19)
TCR δ 1$^+$	(%)	7.1 ± 0.5 (50)	6.5 ± 0.9 (50)
Ly	(/μl)	1730 ± 90 (50)	1870 ± 110 (50)
CD3$^+$	(/μl)	1060 ± 60 (50)	1110 ± 90 (50)
CD4$^+$	(/μl)	390 ± 30 (50)	730 ± 60 (50)
CD8$^+$	(/μl)	340 ± 30 (50)	480 ± 40 (50)
δ TCS1$^+$	(/μl)	23 ± 7 (24)	23 ± 5 (19)
TCR δ 1$^+$	(/μl)	81 ± 10 (50)	66 ± 8 (50)

a: The white blood cell count stained by Türk solution was calculated in hemocytometer. *b:* Each value represents mean ± SEM. *c:* ( ) number of subjects. *d:* The leucocyte differentiation was done after May-Giemsa staining. * : The data were analyzed by Student's *t* test. p<0.001

Table 2　Negative correlation between percentages or absolute numbers of γ δ T cells in peripheral blood and CRP values

CRP^a (mg/dl)	γ δ T cells^b	
	Percentages(%)	Absolute numbers(/μl)
3.5 >	7.9 ± 1.4^c(29)	80 ± 12(29)
3.5 ≦	4.4 ± 0.6(21)	44 ± 7(21)

a: The concentration of CRP in sera was measured by laser nepherometry. (normal: ≦ 0.4 mg/dl) *b:* The γ δ T cells were detected by FACS analysis. (See Meterials and Methods for details) *c:* Each value represents mean ± SEM of values. ( ): number of patients * : p < 0.05

Negative correlation beween $\gamma \ \delta$ T cells in peripheral blood and CRP. In order to know whether the percentages and absolute numbers of $\gamma \ \delta$ T cells were correlated with the disease activity in RA, those of $\gamma \ \delta$ T cells were compared with the value of CRP which was the most reliable marker for the degree of synovitis (Table 2). The 50 RA patients were divided into two groups according to the CRP value, *i.e.*, tentative group I : 3.5 > mg/dl; group II: 3. 5 ≦ mg/dl. The percentages and absolute numbers of $\gamma \ \delta$ T cells in group II (4.4 ± 0.6 %, 44 ± 7/μl, n = 21) were significantly lower than that in group I (7.9 ± 1.4 %, 80 ± 12/μl, n = 29).

No correlation between $\gamma \ \delta$ T cells in peripheral blood and rheumatoid factor. Similarly, the percentages and absolute numbers of $\gamma \ \delta$ T cells in RA were compared with the titer of

rheumatoid factor (RF). There was no correlation between those of $\gamma \ \delta$ T cells and RF titer (Table 3).

Table 3 No correlation between percentages or absolute numbers of $\gamma \ \delta$ T cells in peripheral blood and RA test

RA test[a] (IU/ml)	$\gamma \ \delta$ T cells[b]	
	Percentages(%)	Absolute numbers(/μl)
≦ 18	6.1 ± 1.6[c] (8)	66 ± 18 (8)
18 ~ ≦ 100	6.5 ± 1.4 (29)	56 ± 11 (29)
100 <	6.5 ± 1.1 (13)	86 ± 16 (13)

a: The titer of rheumatoid factor was measured by laser nephelometry, using particles coated with human γ-globulin.   *b:* The $\gamma \ \delta$ T cells were detected by FACS analysis. (See Meterials and Methods for details)   *c:* Each value represents mean ± SEM of values.
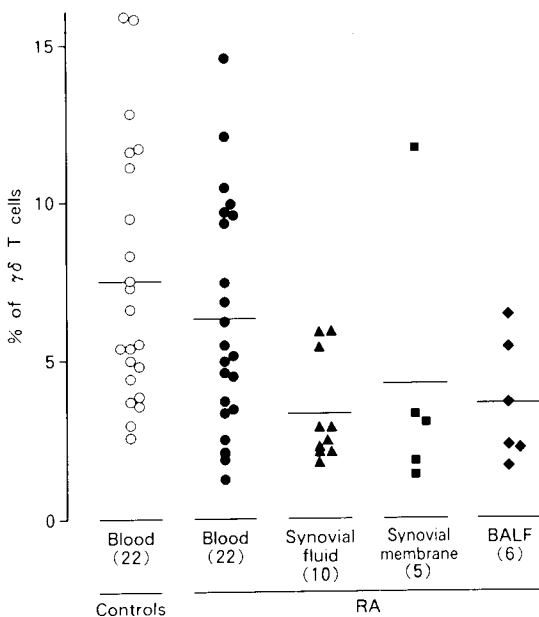(   ): number of patients

Fig. 2 The percentages of $\gamma \ \delta$ T cells per CD3 positive cells in peripheral blood from 22 age-matched controls (○) and RA patients (●) and synovial fluid (▲), synovial membrane (■) and bronchoalveolar lavage fluid (◆) from RA patients. (   ) : number of patients. The bar represents the mean of values.
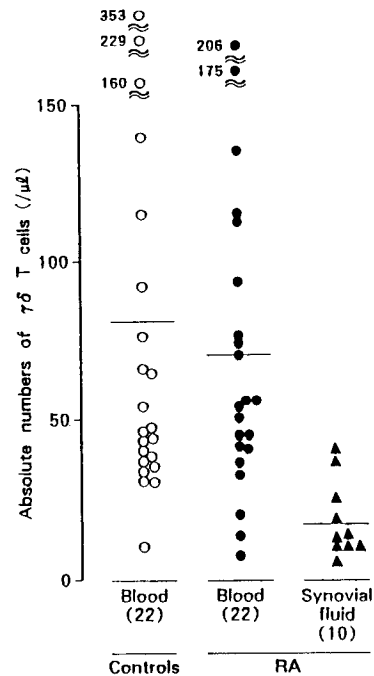
Fig. 3 The absolute numbers of $\gamma \ \delta$ T cells in peripheral blood from 22 age-matched controls (○) and RA patients (●) and synovial fluid (▲) from RA patients. (   ) : number of patients. The bar represents the mean of values.

Mitogawa *et al.*

Table 4 Percentages and absolute numbers of lymphocyte subsets in peripheral blood and synovial fluid obtained simultaneously from 10 RA patients

Items		Peripheral blood	Synovial fluid
WBC^a	(/μl)	8380 ± 580^b (10)^c	12000 ± 940 (10)*
Ly^d	(%)	20.9 ± 2.8 (10)	6.2 ± 0.6 (10)*
CD3$^+$	(%)	53.1 ± 2.9 (10)	72.9 ± 4.0 (10)*
CD4$^+$	(%)	31.7 ± 2.0 (10)	35.9 ± 4.8 (10)
CD8$^-$	(%)	22.7 ± 1.9 (10)	35.3 ± 2.3 (10)*
4/8 ratio		1.54 ± 0.2 (10)	1.1 ± 0.2 (10)
TCRδ1$^+$	(%)	5.1 ± 1.2 (10)	3.3 ± 0.5 (10)
Ly	(/μl)	1740 ± 260 (10)	720 ± 70 (10)*
CD3$^+$	(/μl)	905 ± 150 (10)	530 ± 60 (10)
CD4$^+$	(/μl)	285 ± 50 (10)	200 ± 40 (10)
CD8$^-$	(/μl)	210 ± 40 (10)	190 ± 30 (10)
TCRδ1$^+$	(/μl)	37 ± 5 (10)	17 ± 4 (10)*

a, b, c, d: See the footnotes in Table 1.
* : p<0.01

Table 5 Percentages and absolute numbers of lymphocyte subsets in peripheral blood and bronchoalveolar fluid (BALF) obtained simultaneously from 6 RA patients

Items		Peripheral blood	BALF
WBC^a	(/μl)	6500 ± 1100^b(6)^c	0.13 ± 0.14 (6)
Ly^d	(%)	36.2 ± 3.0 (6)	6.7 ± 2.8 (6)
CD3$^+$	(%)	55.1 ± 2.3 (6)	71.4 ± 5.4 (6)
CD4	(%)	34.1 ± 3.1 (6)	28.5 ± 5.6 (6)
CD8$^-$	(%)	25.6 ± 1.9 (6)	25.4 ± 4.3 (6)
4/8 ratio		1.4 ± 0.2 (6)	1.4 ± 0.3 (6)
TCRδ1$^+$	(%)	8.7 ± 2.4 (6)	3.6 ± 0.7 (6)

a, b, c, d: See the footnotes in Table 1.

Comparison between γ δ T cells in peripheral blood and in synovial fluid or BALF simultaneously obtained from the same patients. The percentages and absolute numbers of γ δ T cells in peripheral blood were compared with those in simultaneously obtained synovial fluid (SF) from the same RA patients or those in BALF. The mean of percentages and absolute numbers of γ δ T cells in peripheral blood and in SF was not different (Table 4). The mean value of γ δ T cells in BALF was relatively lower than in peripheral blood (Table 5).

Discussion

The majority of T cells for T cell receptor complexes express CD3 and a heterodimer molecule (α β chains) with either CD4$^+$ (helper T cells) or CD8$^+$ (killer T cells) on their cell surface to recognize both of the antigen and HLA molecules (17). It has been known recently, however, that there are a small proportion of T cells (1-15 %) which have CD3 molecule and γ δ chains with neither CD4 nor CD8 (17). Those double negative (CD3$^+$ CD4$^-$ CD8$^-$) T cells are called as γ δ T cells having the separate cell lineage from T cells with α/β chains (18). The γ δ T cells are distributed almost evenly throughout the lymphoid system in humans (19). The known functions of γ δ T cells are: 1. host defense against the invaders from exogeneous milieu (7), 2. specific immune responses to *Mycobacterium tuberculosis* (20) or *M. leprae* (21), 3. non-MHC restricted cytotoxicity or NK activity (22-24). In the clinical studies, the increase of γ δ T cells in peripheral blood has been previously reported in patients with allogeneic bone marrow transplantation (25), primary immunodeficiency syndrome (26), Epstein-Barr virus infection (27), HIV-1 infection (28) and pulmonary sarcoidosis (29), but the decrease of γ δ T cells in peripheral blood from patients with systemic lupus erythematosus has also been described (30).

In this study, the percentages of γ δ T cells (TCR δ 1$^+$) per CD3 positive cells in peripheral blood of 50 healthy controls (mean: 7.1 % ranged from 1.9 to 17.3 %, n = 50) were consistent with the values reported by the other investigators (19, 31, 32). The monoclonal antibody (MoAb), TCR δ 1, reacted with the common epitope of V δ gene products. Thus, the percentages of TCR δ 1$^+$ cells (γ δ T cells) represent the whole of δ chain expressed T cells. The MoAb, δ TCS 1, reacted with only the V δ 1 gene product on T cells, representing one third of γ δ T cells (Table 1). After birth, the percentages of γ δ T cells in peripheral blood were increased at the peak age of

5 or 6 years, then tended to decrease in adults (31, 32). We have also shown in the previous report (16) that the percentages and absolute numbers of $\gamma\,\delta$ T cells in peripheral blood were decreased according to ageing. In contrast, the percentages of δ TCS 1^{+} cells in peripheral blood were, however, unchanged after birth (32). For these reasons, we compared the percentages and numbers of $\gamma\,\delta$ T cells in peripheral blood between 22 age-matched controls and RA patients. There was no significant difference in those of $\gamma\,\delta$ T cells between both groups (Figs. 2 and 3). The mean values of percentages and absolute numbers of $\gamma\,\delta$ T cells in peripheral blood from RA patients (6.3 %, 70 cells/μl) were lower than those from the controls (7.5 %, 81 cells/μl). These results were consistent with the reports by Smith et al. (11), Pope et al. (12) and Gerli et al. (13), but were different from those by Brennan et al. (8, 33) and Reme et al. (34) who showed the increase of $\gamma\,\delta$ T cells in RA peripheral blood. The reason for this discrepancy among the investigators is not known, although there were differences in the number of patients studied, the treatment engaged, the advance of the disease, or ethnic background. The number of RA patients examined in this study was fifty, which was greater than in the other reports which ranged from 7 to 29 patients, ref. 8–13, 33, 34 and, to our knowledge, this investigation is the first report for the Japanese RA patients. Brennan et al. (8) had insisted that RA patients with the increased $\gamma\,\delta$ T cells were in the early stage of disease duration because of those cells functioning for the first line defense in hosts. Recently, we had a chance to examine the percentages and absolute numbers of $\gamma\,\delta$ T cells in peripheral blood and synovial fluid from an RA patient (32 years-old, male), whose disease had started within the previous 2 months. The percentages of $\gamma\,\delta$ T cells in peripheral blood and synovial fluid from this patient were 6.5 % and 3.0 %, respectively, which were not increased in comparison with the value of controls. In conclusion, the

percentages and absolute numbers of $\gamma\,\delta$ T cells in peripheral blood from RA patients were not different from healthy controls in this study. The value of $\gamma\,\delta$ T cells in RA, however, had negative correlation with CRP (Table 2). From these findings, it is possible to speculate that $\gamma\,\delta$ T cells may migrate to the inflammatory sites such as synovial fluid or synovial membrane when RA patients had the high activity indicated by the increased CRP value. This possibility was denied by the evidence that no increase of $\gamma\,\delta$ T cells was found in synovial fluid or synovial membrane (Figs. 2 and 3). In an earlier study by Mortensen et al. (35), it was shown that CRP bound in vitro to the cell surface of human T cells and altered their functions as mixed lymphocyte culture reaction. This fact, furthermore, allowed us to speculate that CRP might bind to TCR molecules and decrease the percentages of $\gamma\,\delta$ T cells found in peripheral blood from RA patients.

It has been described by Brennan et al. (33) that there was a significant correlation between the levels of $\gamma\,\delta^{+}$ T cells and CD 5^{+}B cells in the patients with RA or Sjögren's syndrome and healthy persons. The percentages and absolute numbers of $\gamma\,\delta$ T cells in peripheral blood from RA patients had no correlation with the specific autoantibody produced in RA, RF titer (Table 3). We have, however, shown in the previous report (36) that the RF titer in RA had the positive correlation with the percentages of CD 5^{+}B cells, which are recognized as the producer cells for RF (37). In addition, there was no correlation between $\gamma\,\delta$ T cells and CD 5^{+}B cells in the 50 RA patients studied (36).

In the comparative study for T cell subsets in peripheral blood and synovial fluid from the same RA patients, CD 8^{+} T cells in synovial fluid were significantly increased in the percentages but not the numbers (Table 4), being compatible with the results by Fox et al. (38). Unexpectedly the $\gamma\,\delta$ T cells in synovial fluid were rather significantly decreased in numbers but not the percentages than that in peripheral blood from RA patients (Table 4). As far as we know, there has been no

Mitogawa *et al.*

report concerning $\gamma \delta$ T cells in BALF from RA patients. In this study, the mean percentages of $\gamma \delta$ T cells in BALF were also lower than that in peripheral blood from the same RA patients (Table 5). In addition, we could not find the increased numbers of $\gamma \delta$ T cells in synovial membrane as the major inflammatory lesion (Fig. 3). These results indicated that $\gamma \delta$ T cells might be not the major T cell subset infiltrated in the inflammatory sites of RA patients.

The $\gamma \delta$ T cells respond to mycobacterial 65 kd heat-shock protein (HSP) possessing the cross reactivity with autologous HSP (39). Therefore, the novel idea for understanding the involvement of $\gamma \delta$ T cells, playing a pivotal role in the immune response to HSP, in the autoimmune diseases such as RA, could be raised (40). This possibility is, however, not feasible because of the evidence that synovial fluid lymphocytes from patients with RA and other forms of inflammatory synovitis equally responded to HSP (41).

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