showed significant increase of collagen deposition compared with wild-type mice [11], suggesting an immunoregulatory role for γ/δ T cells. Although initially beneficial, tissue repair processes become pathogenic when they are not regulated properly, resulting in substantial deposition of extracellular matrix (ECM) components. In chronic fibrotic diseases, aberrant healing may lead to fatal organ failure [12].

Because the precise role of γ/δ T lymphocytes in fibrosis is not well understood, the purpose of the present study was to examine phenotypic and functional abnormalities of γ/δ T cells in Japanese SSc patients. In addition, we assessed whether γ/δ T cells influence fibroblasts activation in SSc patients.

2. Materials and methods

2.1. Patients

Blood samples were obtained from 52 Japanese SSc patients (4 males and 48 females). The age (mean ± SD) of these patients was 56 ± 10 years. All patients fulfilled the criteria proposed by the ACR [13]. SSc patients were grouped according to the classification system proposed by LeRoy et al. [14]: 22 patients (19 females and 3 males, age 55 ± 13 years) had diffuse cutaneous SSc (dcSSc) and 30 patients (29 females and 1 male, age 57 ± 7 years) had limited cutaneous SSc (lcSSc). The disease duration of dcSSc patients and lcSSc patients was 2.6 ± 2.4 and 7.1 ± 10.1 years, respectively.

As disease controls, 13 patients with systemic lupus erythematosus (SLE; 1 male and 12 females; age 34 ± 9 years) who fulfilled the ACR criteria [15] and 6 patients with dermatomyositis (DM; 2 males and 4 females; age 42 ± 12 years) who fulfilled Bohan and Peter criteria [16,17] were included in this study. Control samples were obtained from 17 age- and gender-matched healthy Japanese volunteers (12 females and 5 males, age 44 ± 14 years). Blood samples and clinical and laboratory data were obtained at the same time. Patients underwent clinical assessments, and involvements of their organ systems were investigated. Skin score was measured by scoring technique of the modified Rodnan total skin thickness score (modified Rodnan TSS) [18]. Organ system involvements were defined as described previously [19,20]. The protocol was approved by the Kanazawa University Graduate School of Medical Science and informed consent was obtained from all patients.

2.2. Flow cytometric analysis

Peripheral blood mononuclear cells (PBMCs) were separated using Ficoll–Paque (Pharmacia Biotech, Uppsala, Sweden) after centrifugation and washed in phosphate-buffered saline (PBS). Erythrocytes were depleted by ammonium chloride lysis before use. Cells were stained in ice-cold PBS supplemented with 5% bovine calf serum immediately with a combination of a fluorescein isothiocyanate (FITC)-conjugated Ab, a phycoerythrin (PE)-conjugated Ab, and peridinin chlorophyll protein (PerCP)-conjugated Ab or 7-amino-actinomycin D (7AAD) (Molecular Probes, Eugene, OR) to examine the number and frequency of γ/δ T cells and surface markers on γ/δ T cells. Abs used in this study included FITC-conjugated anti-TCRγδ (B1; BD Pharmingen), FITC-conjugated anti-CD45RO (UCHL1; Beckman Coulter, Fullerton, CA), FITC-conjugated anti-CD4 (13B8.2; Beckman Coulter), FITC-conjugated anti-CD8 (B9.11; Beckman Coulter), FITC-conjugated anti-CD16 (3G8; Beckman Coulter), PE-conjugated anti-CD3 (UCHT1; Beckman Coulter), PE-conjugated anti-CD62L (DREG56; Beckman Coulter), PE-conjugated anti-CD69 (TP1.55.3; Beckman Coulter), PE-conjugated anti-CD56 (C5.9; Exalpha Biologicals, Shirley, MA), PerCP-conjugated anti-CD3 (SK7; BD Pharmingen), Biotin-conjugated anti-TCRγδ (B1; BD Pharmingen). A secondary staining of PE streptavidin (BD Pharmingen) was performed in combination with biotinylated antibody. Dead and damaged cells were labeled with 7AAD (Invitrogen Life Technologies, Carlsbad, CA), and were eliminated from the analysis. Cells were analyzed on a FACScan flow cytometer (BD Biosciences, San Diego, CA). Absolute number and frequency of γ/δ T cells in $CD3^+$ cells were calculated from the absolute lymphocyte counts.

2.3. γ/δ T cell isolation

γ/δ T cells were isolated from PBMC using a magnetic cell sorting $TCR\gamma/\delta^+$ T cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany), according to the manufacturer's recommendations. Briefly, non-γ/δ T cells (α/β T cells, NK cells, B cells, dendritic cells, granulocytes, monocytes, stem cells, and erythroid cells) were labeled using a cocktail of biotin-conjugated Abs and anti-biotin MicroBeads. The magnetically labeled cells were depleted by retaining on a magnetic column. After isolation, >80% of cells were $TCR\gamma/\delta^+$ by flow cytometric analysis. Non-γ/δ T cell fraction was used as a control.

2.4. Coculture of γ/δ T cells and human fibroblasts

γ/δ T cells isolated freshly from SSc patients and normal controls were seeded at density of 1×10^4 cells/ml on 96 well plates. The cells were stimulated with 10 ng/ml soluble anti-CD3 Ab (eBioscience) and 10 ng/ml IL-2 (BD Pharmingen) for 2 days in PRMI1640 (Invitrogen Life Technologies) containing 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin (Invitrogen Life Technologies), and 100 μg/ml streptomycin (Invitrogen Life Technologies), and cultured at 37 °C in a 5% CO_2 humidified atmosphere. The normal human dermal fibroblasts from neonatal foreskins (Lifeline Cell Technology, Frederik, MD) were used in the current study. Human fibroblasts were seeded in 12-well plates matched for use with Cell Culture Insert (BD Falcon, NJ) at a density of 5×10^4 cells/well in Dulbecco's modified Eagle medium (DMEM)/10% heat inactivated FBS for 48 h to 80% confluency. The cells were studied between passages 4 and 8. γ/δ and non-γ/δ T cells after stimulation and plated fibroblasts were washed three times with PBS. γ/δ T cells were plated onto Cell Culture Inserts with 0.4 μm pore size (high pore density), and the inserts were fit into the 12-well plates containing fibroblasts cultures to initiate fibroblasts and γ/δ T cells coculture in fresh serum-free DMEM. After 3-day coculture, only fibroblasts were harvested and analyzed.

2.5. RNA isolation and real-time PCR

Total RNA was isolated from cultured fibroblasts using Qiagen RNeasy spin columns (Qiagen, Hilden, Germany) and digested with DNaseI (Qiagen) to remove chromosomal DNA in accordance with manufacturer's protocols. RNA was reverse transcribed into cDNA using the Reverse Transcription System (Promega, Madison, WI). Transcript levels were quantified using a real-time PCR method, according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). Sequence-specific primers and probes for COL1A1, COL1A2 were designed by Pre-Developed TaqMan Assay Reagents or TaqMan Gene Expression Assays (Applied Biosystems). Real-time PCR (1 cycle of 50 °C for 2 min, 95 °C for 10 min; 40 cycles of 92 °C for 15 s, 60 °C for 60 s) was performed on an ABI Prism 7000 Sequence Detector (Applied Biosystems). GAPDH transcript levels were used as controls to normalize mRNA levels. The relative expression of real-time PCR products was determined by using the $\Delta\Delta$Ct method [21] to compare target gene and housekeeping gene (GAPDH) mRNA expression.

2.6. Immunohistochemical staining for γ/δ T cells

γ/δ T cell infiltrations in the skin (5 μm in thickness) were examined by immunohistochemical staining in patients with SSc ($n = 20$; 12 dcSSc and 8 lcSSc patients, 6 males and 14 females) and 5 normal controls. Frozen skin tissues were acetone fixed and then incubated with 10% normal rat serum for 10 min at 37 °C to block nonspecific staining. Sections were stained with biotinylated mouse monoclonal IgG antibody specific for human γδTCR (BD Pharmingen), then with horseradish peroxidase-conjugated avidin–biotin complexes (Vectastatin avidin–biotin Complex Methods, Vector Laboratories, Burlingame, CA). Sections were finally developed with DAB (Histofine SAB-PO (M), Nichirei Bioscience, Tokyo, Japan) and counterstained with methyl green. Forty sections were examined for each patient. Two observers independently quantitated γδTCR positive cells.

2.7. Statistical analysis

Statistical analysis was performed using the Mann–Whitney *U* test for determining the level of significance of differences between sample means. Fisher's exact probability test for comparison of frequencies, and Bonferroni's test for multiple comparisons. Spearman's rank correlation coefficient was used to examine the relationship between two continuous variables. A p value less than 0.05 was considered statistically significant.

3. Results

3.1. The percentage and absolute number of γ/δ T cells were similar in SSc, other connective tissue diseases, and normal controls

Clinical and serological features in SSc patients are shown in Table 1. The percentage and absolute number of γ/δ T cells in blood from SSc patients and normal individuals were examined by flow cytometry (Fig. 1). The proportion of γ/δ T cell in $CD3^+$ cells tended to be increased in lcSSc patients, although no significant difference was observed between lcSSc and healthy control or dcSSc (Fig. 1A). There was no significant difference in the number of γ/δ T cells between SSc patients and healthy controls (Fig. 1B). The percentage and absolute number of γ/δ T cells were not different between the patients treated with steroid or other medications and those without. There was no association between the frequency or number of γ/δ T cells in the circulation and clinical features in our patients. Two patients with high proportion of γ/δ T cells are both positive for ACA, and did not have cutaneous ulcers, pulmonary hypertension, and interstitial pneumonia. As a disease control, the proportion and absolute number of γ/δ T cells from SLE and DM patients were also examined. The proportion and number of γ/δ T cells were comparable among patients with SLE, DM, SSc, and normal control.

3.2. SSc γ/δ T cells showed phenotypic abnormalities

To assess γ/δ T cells phenotype in SSc patients (8 dcSSc and 12 lcSSc patients), the cell surface expressions of CD16, CD45RO, CD69, and CD62L were analyzed by flow cytometer (Fig. 2). Phenotypic abnormalities of γ/δ T cells were examined without stimulation. Representative histograms of each marker from $CD3^+TCR\gamma/\delta^+$ T cell (Fig. 2A) and $CD3^+TCR\gamma/\delta^-$ T cell (Fig. 2B) populations in SSc patient are shown. The geometric mean fluorescence intensity (GMFI) of CD16 on γ/δ T cells was higher in dcSSc patients than in controls (Fig. 3A). The expression levels of CD16 were not different between lcSSc and dcSSc or control (Fig. 3A). There was no significant difference in the expression levels of CD45RO molecules between SSc patients and normal controls (data not shown). CD69 expression was increased in γ/δ T cells from dcSSc compared to controls ($p < 0.05$), although there was no significant difference in CD69 expression between lcSSc and dcSSc or control (Fig. 3A). γ/δ T cells in dcSSc patients exhibited significantly lower CD62L expression levels than normal controls ($p < 0.05$, Fig. 3A). γ/δ T cells in lcSSc patients tended to have low GMFI of CD62L, however, no significant difference was observed in the expression of CD62L between lcSSc and control.

No significant difference of the positive frequencies of CD16, CD45RO, and CD62L between SSc patients and control (Fig. 3B). The frequency of cells expressing CD69, known as an early activation marker, was significantly increased in dcSSc and lcSSc patients compared to normal controls ($p < 0.05$ and $p < 0.05$, respectively; Fig. 3B).

3.3. Fibroblasts cocultured with SSc γ/δ T cells showed increased mRNA expression levels of COL1A2

To examine the effects of γ/δ T cells on fibroblasts, γ/δ T cells were cocultured with fibroblasts. Non-γ/δ T cell fraction was used as a control. The numbers of fibroblasts after cocultured with γ/δ T

Table 1
Demographics of SSc patients in this study.

	Total ($n = 52$)	dcSSc patients ($n = 22$)	lcSSc patients ($n = 30$)
Age (years)	56 ± 10	55 ± 13	57 ± 7
Sex, male:female	4:48	3:19	1:29
Disease duration		2.6 ± 2.4	7.1 ± 10.1
SSc specific antibody			
Topoisomerase I	13	13	0
Centromere	22	0	22
U1RNP	4	3	1
U3RNP	3	1	2
RNAP	7	5	2
Th/To	1	1	0
Not identified	3	1	2
Negative	1	0	1
Medications			
Prostaglandin analogs	45		
Prednisone	33		
Methotrexate	1		
Cyclosporine	1		
Bosentan	6		
Phosphodiesterase V inhibitor	1		

Clinical characteristics of the 52 SSc patients included in this study. SSc, systemic sclerosis; RNAP, RNA polymerase. Anti-U1RNP antibody was positive for 4 including 2 patients double positive with anti-topo I antibody.

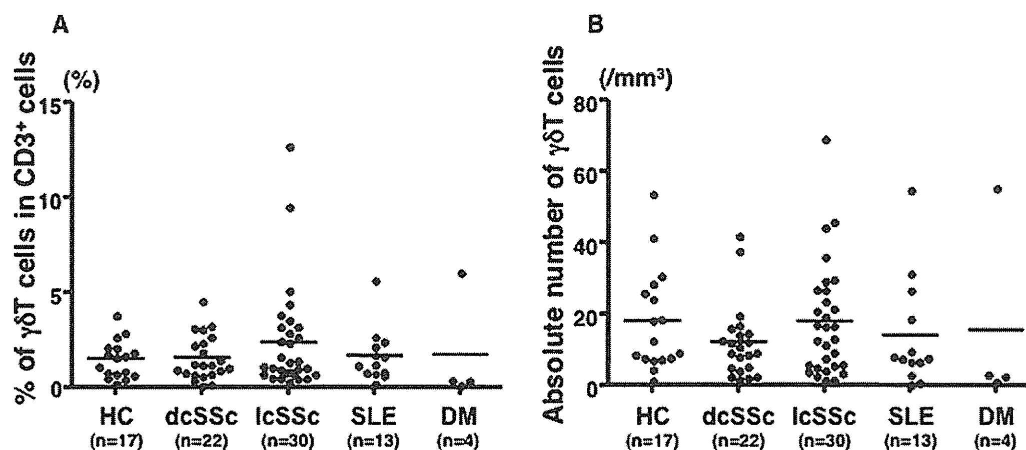

Fig. 1. Frequency and absolute number of peripheral blood γ/δ T cells in diffuse cutaneous systemic sclerosis (dcSSc) patients, limited cutaneous systemic sclerosis (lcSSc) patients, and healthy controls (HCs). The frequency of γ/δ T cells was determined by immunofluorescent staining with flow cytometric analysis (A). The absolute number of γ/δ T cells was calculated from the relative frequency of γ/δ T cells and the CD3$^+$ cells (B). The horizontal bars represent mean values. As a disease controls, the number and proportion of γ/δ T cells from patients with systemic lupus erythematosus (SLE) and dermatomyositis (DM).

cells or non-γ/δ T cells were similar between SSc and normal control (data not shown). mRNA was extracted from fibroblasts after cocultured with either γ/δ T cells or non-γ/δ T cells, and COL1A1 and COL1A2 mRNA expressions were examined by real-time RT-PCR ($n = 8$). COL1A1 mRNA expression level in fibroblasts after cocultured with γ/δ T cells was significantly higher than cocultured with non-γ/δ T cells in both SSc patients and healthy control subjects ($p < 0.05$; Fig. 4A). COL1A2 mRNA expression level in fibroblasts after cocultured with γ/δ T cells was also significantly higher than cocultured with non-γ/δ T cells in both SSc patients and healthy subjects ($p < 0.05$; Fig. 4B). Moreover, there was a significant increase of COL1A2 mRNA expression in fibroblasts cultured with γ/δ T cells from SSc patients than those from normal controls ($p < 0.05$; Fig. 4B). Even though we also measured mRNA expression of matrix metalloproteinase-1 in fibroblasts cultured with SSc were not significantly different from that cultured with control γ/δ T cells (data not shown).

While not only SSc γ/δ T cells but also normal control γ/δ T cells have collagen synthesis effects on fibroblasts, γ/δ T cells from SSc have more potent effects on activating collagen synthesis.

3.4. *γ/δ T cell infiltrations in the skin were observed in SSc patients*

γ/δ T cell infiltrations in the skin were examined by using immunohistochemical staining in patients with SSc ($n = 20$; 11 dcSSc and 9 lcSSc patients), and healthy control subjects ($n = 5$). Representative pictures of TCRγ/δ$^+$ cells were shown in Fig. 5. We made 15 serial sections of skin specimens from 20 patients with SSc. A few γ/δ T cell infiltrations were observed 35% (7/20) of total SSc patients (5 dcSSc and 2 lcSSc patients), while it was absent in

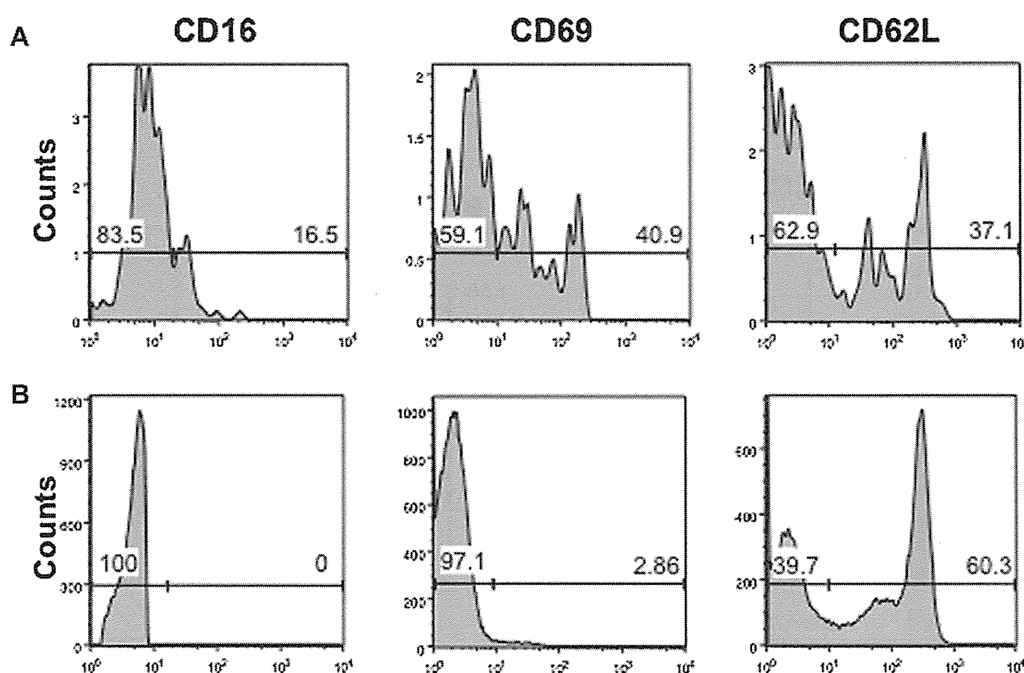

Fig. 2. Representative histograms of CD16, CD69, and CD62L expressions on CD3$^+$TCRγ/δ$^+$ T cell and CD3$^+$TCRγ/δ$^-$ T cell populations in SSc patient. Expressions of cell surface molecules were examined by flow cytometric analysis. Representative histograms of CD16, CD69, and CD62L expressions on CD3$^+$TCRγ/δ$^+$ T cells (A) and CD3$^+$TCRγ/δ$^-$ T cells (B) in SSc patient. The numbers represent the percentage of cells in total CD3$^+$TCRγ/δ$^+$ T cells (A) or CD3$^+$TCRγ/δ$^-$ T cells (B) within each gate.

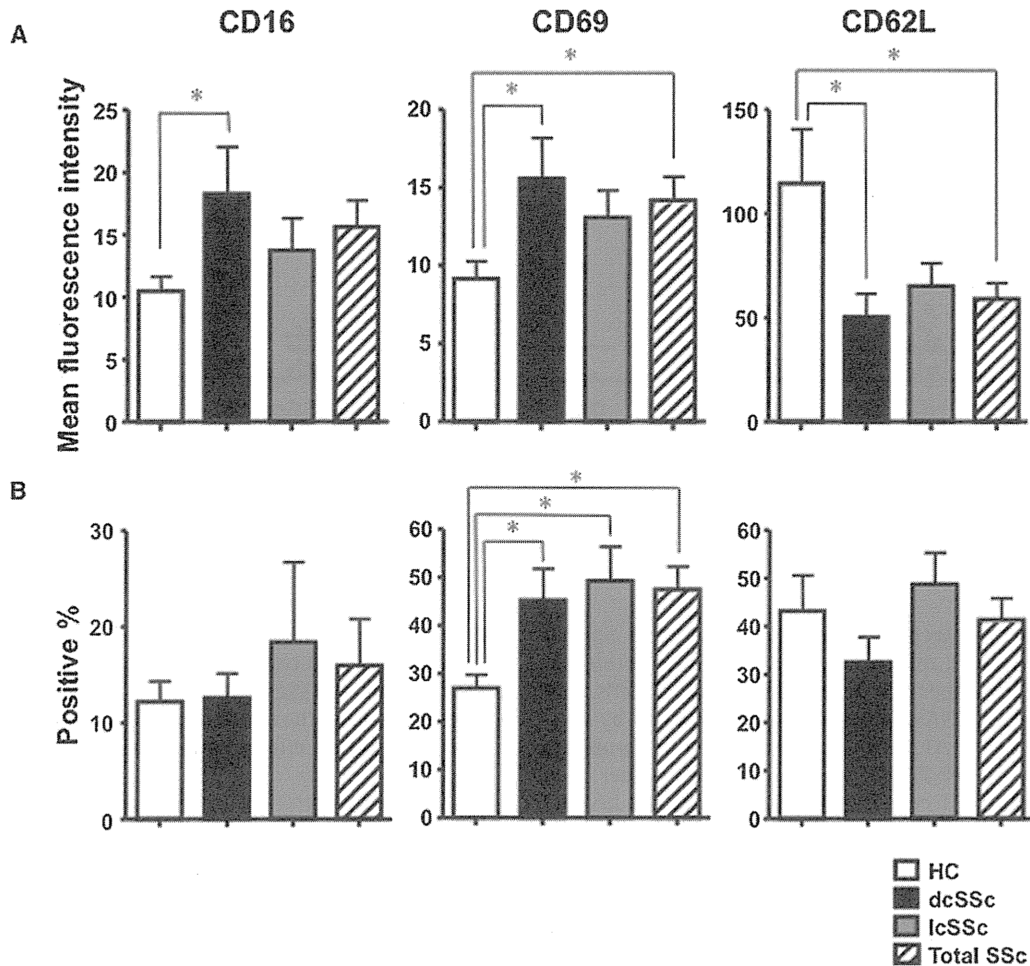

Fig. 3. Mean fluorescence intensity (MFI) of CD16, CD69, and CD62L on γ/δ T cells and the frequency of γ/δ T cells positive for CD16, CD69, and CD62L in peripheral blood from dcSSc patients, lcSSc patients, and HCs. Bar graph representation of the mean expression levels of CD16, CD69, and CD62L on γ/δ T cells (A) and the frequency of γ/δ T cells positive for CD16, CD69, and CD62L (B). All samples were analyzed by flow cytometry with identical instrument settings. Statistic significant differences between groups were indicated.

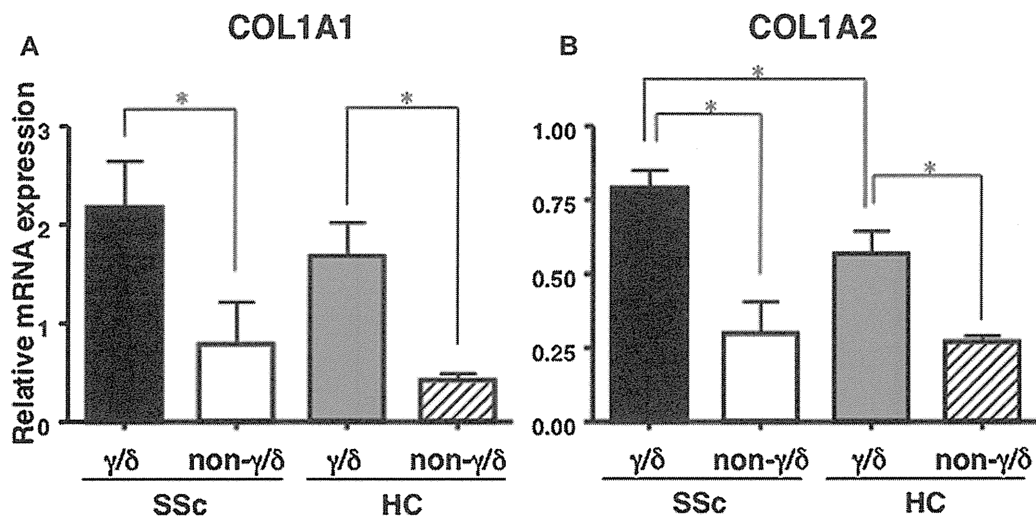

Fig. 4. mRNA expression levels of proalpha1(I) collagen (COL1A1) and proalpha2(I) collagen (COL1A2) in fibroblasts cocultured with γ/δ T cells from SSc patients or HCs. mRNA was extracted from fibroblasts after cocultured with either γ/δ T cells or non-γ/δ T cells and COL1A1 (A) and COL1A2 (B) mRNA expressions were examined by real-time RT-PCR. $^{*}p < 0.05$.

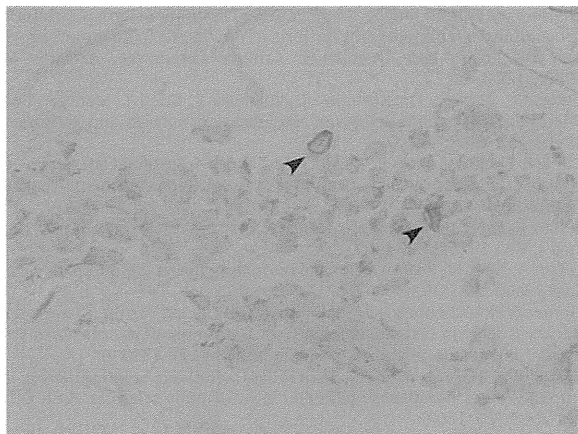

Fig. 5. Representative pictures of immunohistological analysis of TCRγδ⁺ cells in the skin of SSc patients. Cells positive for TCRγδ staining by immunohistochemical staining were indicated arrows (diaminobenzidine staining, original magnification 400×).

normal controls. Infiltrating $CD3^+$ T cells were counted using the same sample shown in Fig. 5, and the proportion of the γ/δ T cells in infiltrating $CD3^+$ T cells were 2/1260 (0.16%).

4. Discussion

In the current study, we showed the number of γ/δ T cells and the proportion of γ/δ T cells in $CD3^+$ cells were maintained in Japanese SSc patients (Fig. 1). These findings are in accord with the earlier report [22]. There was no association between the number or frequency of γ/δ T cells in the circulation and clinical features in our patients, although other investigators found decreased total γ/δ T cells in the PBMC of SSc patients with diffuse and late stage disease, pulmonary or muscle involvement and with anti-topo I Ab [23]. While there was a possibility that patients presenting digital ulcers particularly might have distinct T cell recirculation, we did not find any association between the number or frequency of γ/δ T cell and digital ulcers. To examine the phenotypic abnormalities of γ/δ T cells in SSc patients, we analyzed surface expressions of several markers on SSc γ/δ T cells (Figs. 2 and 3). Cell surface expression of CD69 was significantly elevated in dcSSc, and the percentage of CD69 positive cells was significantly increased in dcSSc and lcSSc patients compared to normal control. CD69 is an activation marker expressed not only many subsets of T cells but also on other cell types. CD69 expression has been used as the activation marker of γ/δ T cell and can be up-regulated after TCR cross-linking [24,25]. Previous studies identified oligoclonal $V\delta1^+$ T cells at the sites of inflammation, suggesting that antigenic selection may be triggering $V\delta1^+$ T cell expansion specifically [26]. Thus, these $CD69^+$ γ/δ T cells may be reacting with disease-related antigens. For further experiment, we may consider silencing experiments onto γ/δ T cells by using CD69 siRNA. CD16 expression was also significantly increased in dcSSc patients compared to controls. In a previous report, the percentage of $CD16^+$ cells in γ/δ T cells did not differ between SSc and normal control, however, the percentage of $CD16^+V\delta1^+$ T cells in γ/δ T cells was increased in SSc patients. It was suggested that these $CD16^+$ γ/δ T cells might exert cytotoxic activity in SSc patients [22]. Although γ/δ T cell repertoire was not determined, the increase of CD16 expression in our patients might be due to $CD16^+V\delta1^+$ T cell population. Flow cytometry of PBMC also revealed the decrease of CD62L expression in dcSSc patients compared to control subjects. As it was known that the lack of CD62L expression on γ/δ T cells represent tissue-homing cells with a variety of effector functions [27], decreased expression of CD62L in patients suggests that the possibility that these γ/δ T cells lacking CD62L expression may migrate to the skin or other affected organs and exert the effector functions. Taken together, despite the lack of correlation with specific clinical features of SSc, these results indicate that activated γ/δ T cells may influence disease pathogenesis. We suspect that not merely the phenotypic abnormality found in SSc γ/δ T cells, but also other changes due to in vivo chronic activation had influence in the collagen expression. Because these surface markers expressions on γ/δ T cells were not examined in other rheumatic diseases, it is unclear whether these aberrant expressions were specific for SSc. Moreover, although we did not find any association between γ/δ T cell abnormalities and treatments, there was a possibility that the therapies including immunosuppressant agents, glucocorticoids, prostaglandin analogs, bosentan, and phosphodiesterase V inhibitors had the potential to modify the frequency of these cells in the peripheral blood in SSc.

In the early stage of SSc, activated fibroblasts expressing high levels of mRNA for type I and III collagen are often seen near blood vessels in association with mononuclear cell infiltrations [28]. To evaluate the effect of γ/δ T cells on fibroblasts, we examined the COL1A1 and COL1A2 mRNA expressions of fibroblasts cocultured with γ/δ T cells. Purified γ/δ T cells were separated from fibroblasts by the cell culture insert in order to examine the interactions γ/δ T cells and fibroblasts without actual physical contact in our study. Because we expected that cytokines produced by γ/δ T cells have effects on fibroblasts, we performed the coculture experiment with T cells separated from fibroblasts. From the observation of COL1A1 and COL1A2 mRNA expressions in fibroblasts cocultured with γ/δ T cells, while γ/δ T cells not only from SSc but also normal control have the potential role to activate fibroblasts at some extent, SSc γ/δ T cells had more potent ability to induce activation of fibroblasts than normal control. In a previous report, the supernatant from activated SSc γ/δ T cells showed the proliferative and collagen synthesis activity on fibroblasts [29]. Together with our findings, γ/δ T cells have effects of enhance collagen synthesis in fibroblasts without direct cell contact. In contrast, recently, Bendersky et al. reported $V\gamma9^+$ T cells were equally and persistently represented in SSc patients at levels similar to healthy controls, and they preserved the ability to respond to the classical $V\gamma9^+$ T cell auto-antigen isopentenyl pyrophosphate and to execute disruption of fibroblast growth and induction of apoptotic death with contact-dependent manner [30]. While the reason for the discrepancy among the reports is unclear, it might be due to the physical contact between γ/δ T cells and fibroblasts, which may influence a different response pattern. Alternatively, different antigens (stimulations) could induce expansion of functionally different γ/δ T cell subpopulations. Further studies will be required to confirm our findings.

γ/δ T cells produce a wide variety of cytokines, chemokines and growth factors. Ohtsuka reported that supernatant from γ/δ T cells stimulated by *Mycobacterium tuberculosis* lysates and IL-2 induced proliferation of fibroblast and synthesis of collagen, and the synthesis of collagen was inhibited by anti-TGF-β Ab [29]. We examined mRNA expressions of several cytokines including TGF-β, TNF-α, IFN-γ, IL-4 and IL-6 in γ/δ T cells after the stimulation in vitro (data not shown). We detected the expressions of TGF-β, IFN-γ and TNFα, although these expression levels were comparable between SSc patients and normal controls in γ/δ T cells. Cytokines are considered to play critical roles in SSc through various and complex mechanisms. While Th2 cytokines facilitate fibrosis, Th1 cytokines can augment autoimmune responses, which may influence on fibrosis in SSc. Alternatively, it is also possible that the detection of Th1-polarized cytokines from γ/δ T cells in the circulation might be a compensatory response to the progression of fibrosis. Recently, many reports suggested that γ/δ T cells are the

major source of IL-17 [10,31]. After exposure to bleomycin, but not *Schistosoma mansoni* eggs, IL-17A produced by $CD4^{+}$ and γ/δ T cells induced significant neutrophilia and pulmonary fibrosis, and cooperative roles for IL-17A and TGF-β in the development of fibrosis were suggested [32]. We also measured mRNA expression of IL-17A in γ/δ T cells after stimulation, and no expression of 1L-17A was detected. Although the precise mechanisms of the induction of fibrosis by γ/δ T cells need to be further examined, our data suggests several cytokines may be involved.

Finally, we confirmed γ/δ T cell infiltrations in SSc affected skin. Studies has described accumulation of activated T lymphocytes expressing a selective Vδ chain gene expansion of the γ/δ T cell receptor (TCR) in the lung [26] and in the skin [22] of the patients with SSc. In SSc patients, these cells display adhesion molecules [22], produce Th1-polarized cytokines [33], and damage endothelial cells [34]. These results suggest that their effector function in the development of the disease. Because the number of γ/δ T cell infiltration was very small, we could not show precise proportion of γ/δ T cells in infiltrating total T cells.

In conclusion, our findings suggest that γ/δ T cells that showed activated phenotype may contribute to tissue fibrotic processes by induction of collagen synthesis in SSc, and to the disease pathogenesis.

Acknowledgments

We would like to thank Ms. Masako Matsubara and Ms. Yuko Yamada for technical assistance and Dr. Shinji Shimada for helpful discussion.

References

[1] White B. Immunopathogenesis of systemic sclerosis. Rheum Dis Clin North Am 1996;32:695–708.
[2] Furst DE. Rational therapy in the treatment of systemic sclerosis. Curr Opin Rheumatol 2000;12:540–4.
[3] Furst DE, Clements PJ. Hypothesis for the pathogenesis of systemic sclerosis. J Rheumatol 1997;24(Suppl. 48):53–7.
[4] Steen VD, Medsger Jr TA. Epidemiology and natural history of systemic sclerosis. Rheum Dis Clin North Am 1990;16:1–10.
[5] White B. Immunologic aspects of scleroderma. Curr Opin Rheumatol 1995;7: 541–5.
[6] Roumm AD, Whiteside TL, Medsger TA, Rodnan GP. Lymphocytes in the skin of patients with progressive systemic sclerosis. Quantification, subtyping, and clinical correlations. Arthritis Rheum 1984;27:645–53.
[7] Bhogal RK, Stoica CM, McGaha TL, Bona CA. Molecular aspects of regulation of collagen gene expression in fibrosis. J Clin Immunol 2005;25:592–603.
[8] Jameson J, Ugarte K, Chen N, Yachi P, Fuchs E, Boismenu R, et al. A role for skin gammadelta T cells in wound repair. Science 2002;296:747–9.
[9] Jameson J, Witherden D, Havran WL. T-cell effector mechanisms: gammadelta and CD1d-restricted subsets. Curr Opin Immunol 2003;15:349–53.
[10] Braun RK, Ferrick C, Neubauer P, Sjoding M, Sterner-Kock A, Kock M, et al. IL-17 producing gammadelta T cells are required for a controlled inflammatory response after bleomycin-induced lung injury. Inflammation 2008;31:167–79.
[11] Simonian PL, Roark CL, Diaz del Valle F, Palmer BE, Douglas IS, Ikuta K, et al. Regulatory role of gammadelta T cells in the recruitment of CD4+ and CD8+ T cells to lung and subsequent pulmonary fibrosis. J Immunol 2006;177:4436–43.
[12] Meltzer EB, Noble PW. Idiopathic pulmonary fibrosis. Orphanet J Rare Dis 2008;3:8.
[13] Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Arthritis Rheum 1980;23:581–90.
[14] LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol 1988;15:202–5.
[15] Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.
[16] Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). N Engl J Med 1975;292:344–7.
[17] Bohan A, Peter JB. Polymyositis and dermatomyositis (second of two parts). N Engl J Med 1975;292:403–7.
[18] Clements PJ, Lachenbruch PA, Seibold JR, Zee B, Steen VD, Brennan P, et al. Skin thickness score in systemic sclerosis: an assessment of interobserver variability in 3 independent studies. J Rheumatol 1993;20:1892–6.
[19] Hayakawa I, Hasegawa M, Takehara K, Sato S. Anti-DNA topoisomerase II alpha autoantibodies in Japanese patients with systemic sclerosis. Arch Dermatol Res 2005;297:180–3.
[20] Hayakawa I, Hasegawa M, Matsushita T, Yanaba K, Kodera M, Komura K, et al. Increased cutaneous T-cell-attracting chemokine levels in sera from patients with systemic sclerosis. Rheumatology (Oxford) 2005;44:873–8.
[21] Meijerink J, Mandigers C, van de Locht L, Tönnissen E, Goodsaid F, Raemaekers J. A novel method to compensate for different amplification efficiencies between patient DNA samples in quantitative real-time PCR. J Mol Diagn 2001;3:55–61.
[22] Giacomelli R, Matucci-Cerinic M, Cipriani P, Ghersetich I, Lattanzio R, Pavan A, et al. Circulating $Vdelta1^{+}$ T cells are activated and accumulate in the skin of systemic sclerosis patients. Arthritis Rheum 1998;41:327–34.
[23] Holcombe RF, Baethge BA, Wolf RE, Betzing KW, Stewart RM. Natural killer cells and gamma delta T cells in scleroderma: relationship to disease duration and anti-Scl-70 antibodies. Ann Rheum Dis 1995;54:69–72.
[24] Prado-Drayer A, Teppa J, Sánchez P, Camejo MI. Immunophenotype of peripheral T lymphocytes. NK cells and expression of CD69 activation marker in patients with recurrent spontaneous abortions, during the mid-luteal phase. Am J Reprod Immunol 2008;60:66–74.
[25] Wesch D, Beetz S, Oberg HH, Marget M, Krengel K, Kabelitz D. Direct costimulatory effect of TLR3 ligand poly(I:C) on human gamma delta T lymphocytes. J Immunol 2006;176:1348–54.
[26] Yurovsky VV, Sutton PA, Schulze DH, Wigley FM, Wise RA, Howard RF, et al. Expansion of selected V delta 1+ gamma delta T cells in systemic sclerosis patients. J Immunol 1994;153:881–91.
[27] Eberl M, Engel R, Beck E, Jomaa H. Differentiation of human gamma-delta T cells towards distinct memory phenotypes. Cell Immunol 2002;218:1–6.
[28] Scharffetter K, Lankat-Buttgereit B, Krieg T. Localization of collagen mRNA in normal and scleroderma skin by in-situ hybridization. Eur J Clin Invest 1988;18:9–17.
[29] Ohtsuka T. Effect of gammadelta T cell supernatant on human skin fibroblast proliferation and collagen production—possible role of transforming growth factor-beta and basic fibroblast growth factor. Int J Dermatol 2008;47: 1135–40.
[30] Bendersky A, Markovits N, Bank I. $Vgamma9^{+}$ gammadelta T cells in systemic sclerosis patients are numerically and functionally preserved and induce fibroblast apoptosis. Immunobiology 2010;215:380–94.
[31] Sutton CE, Lalor SJ, Sweeney CM, Brereton CF, Lavelle EC, Mills KH. Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. Immunity 2009;31: 331–41.
[32] Wilson MS, Madala SK, Ramalingam TR, Gochuico BR, Rosas IO, Cheever AW, et al. Bleomycin and IL-1beta-mediated pulmonary fibrosis is IL-17A dependent. J Exp Med 2010;207:535–52.
[33] Giacomelli R, Cipriani P, Fulminis A, Barattelli G, Matucci-Cerinic M, D'Alò S, et al. Circulating gamma/delta T lymphocytes from systemic sclerosis (SSc) patients display a T helper (Th) 1 polarization. Clin Exp Immunol 2001;125: 310–5.
[34] Kahaleh MB, Fan PS, Otsuka T. Gammadelta receptor bearing T cells in scleroderma: enhanced interaction with vascular endothelial cells in vitro. Clin Immunol 1999;91:188–95.

厚生労働科学研究費補助金
難治性疾患等克服研究事業（難治性疾患克服研究事業）

強皮症における病因解明と根治的治療法の開発

平成23年度～平成25年度　総合研究報告書
発　行　平成26年3月
発行所　強皮症調査研究班事務局
東京大学医学部附属病院皮膚科内
〒113-8655
TEL03-5800-8661，FAX 03-3814-1503