シェーグレン症候群, 慢性関節リウマチ, 全身性エリテマトーデス 患者末梢血中の CD5⁺ B 細胞と活性化 B 細胞

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B-Cells expressing CD5 or activation antigens in the peripheral blood of Sjögren's syndrome, rheumatoid arthritis and systemic lupus erythematosus

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【Summary】

We determined CD5 antigen, interleukin-2 receptors (IL-2R) and transferrin receptors (TfR) on circulating B-cells in Sjögren's syndrome (SS), systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) by two-color flowcytometry.

The proportions of total B-cells were significantly higher in 15 SLE patients and were relatively higher in 17 patients with primary (I°)-SS, 20 patients with secondary (II°)-SS and 24 patients with RA as compared with 22 controls. The proportion of CD5⁺ B-cells was also higher in I°-SS and RA than controls, although the cells were significantly decreased in SLE patients. However, the proportions of CD5⁺ B-cells in total B-cells were significantly low in II°-SS, RA and SLE. Most of the patients with SLE were receiving prednisolone (PSL) treatment, and the dosage of PSL administered was negatively correlated with the CD5⁺ B-cells in II°-SS patients.

IL-2R⁺ B-cells were few and were within negligible range in any patients' groups, but the proportions of TfR⁺ B-cells were significantly high in the patients' groups, except for RA. TfR⁺ B-cells seemed to reflect the activity of diseases, since they positively correlated with the dosage of PSL in the patients.

Among the B-cells determined, the increase of total B-cells, CD5⁺ B-cells and TfR⁺ B-cells was proportional in only RA patients.

Our results, therefore, did not support the previous reports showing increased CD5⁺ B-cells in RA, SS or SLE patients. In addition, the results indicate that CD5 antigen might not be an activation antigen of B-cells, although the stimulations to B-cells might be different among patient's groups.

Key words : CD5⁺ B-cells,
activated B-cells,
Sjögren's syndrome,
rheumatoid arthritis,
systemic lupus erythematosus

【概 要】

シェーグレン症候群 (SS), 全身性エリテマトーデス (SLE), および慢性関節リウマチ (RA) 患者の末梢血 B 細胞に発現する CD5 抗原や interleukin-2 receptor (IL-2 R), transferrin receptor (TfR) などの活性化抗原を 2-color flowcytometry により測定した。

全 B 細胞の百分率は健康人 22 例と比べて SLE 15 例では有意に高値で, primary (I⁺-) SS 17 例, secondary (II⁺-) SS 20 例, RA 24 例でも高値を示した。CD5⁺ B 細胞百分率は I⁺-SS と RA で高値を示したが, SLE では有意に低率であった。一方, 全 B 細胞中に占める CD5⁺ B 細胞の百分率は II⁺-SS, RA, および SLE で有意の低値を示した。II⁺-SS では prednisolone (PSL) 投与量と CD5⁺ B 細胞百分率との間に負の相関を認めた。

IL-2 R⁺ B 細胞はきわめて少数で, いずれの疾患群でも測定感度以下であった。しかし, TfR⁺ B 細胞の百分率は RA を除くすべての患者群で有意に高率であった。さらに, TfR⁺ B 細胞百分率は PSL 投与量と正の相関を示すことから, TfR⁺ B 細胞の増加は疾患活動性を反映すると考えられた。

全 B 細胞, CD5⁺ B 細胞, TfR⁺ B 細胞のいずれも RA 患者でのみ比例的に増加傾向を示した。

今回の成績は RA, SS, あるいは SLE 患者では全 B 細胞中に占める CD5⁺ B 細胞の百分率が高値であるという従来の報告を支持しなかった。また, 私たちの成績は B 細胞に対する活性化刺激は個々の疾患によって異なる可能性があり, 少なくとも SS や SLE では CD5 抗原は B 細胞の活性化抗原ではないことを示唆している。

I . Introduction

Sjögren's syndrome (SS) is considered as an autoimmune disease characterized by chronic inflammation of lacrimal and/or salivary glands : a frequent association with other autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) is one of the clinical features of SS. In addition, hypergammaglobulinemia and production of a variety of autoantibodies are serological features commonly observed in these diseases.

Recent evidences have shown that an unique subset of B-cells, CD5⁺ B-cells, produce autoantibodies, including rheumatoid factors (RF) and anti-DNA antibodies, which have different characteristics from those produced by CD5⁻ B-cells¹⁾. Previous studies by others on the CD5⁺ B-cell subset in several autoimmune diseases demonstrated that the cells are increased in the peripheral blood (PB)

of patients with RA, SS or SLE²⁻⁵⁾.

The two B-cell subsets, CD5⁺ and CD5⁻ B-cells, have also been suggested to belong to different cell-lineages. Other evidences, however, indicated that the CD5 antigen can be expressed on B-cells as one of the activation antigens^{6,7)}.

Activated B-cells in the PB have also been studied in autoimmune diseases and suggested to be increased in SS, SLE or RA, although some controversial results have been reported⁸⁻¹¹⁾.

To elucidate the relationship of B-cell subsets and activated B-cells with immunologic conditions in these diseases, we determined both CD5 and activation antigens expressed on circulating B-cells by two-color flowcytometry.

II . Patients and methods

1. Patients

Thirty-seven female patients with SS were included in this study. SS was diagnosed by the

criteria based on the demonstration of objective evidences of lacrimal (ophthalmological methods using rose bengal and/or fluorescent dye) and/or salivary gland involvements (parotid sialography and/or salivary gland biopsy) as described elsewhere^{12,13}. Twenty of the patients were associated with systemic rheumatic diseases such as SLE (12 patients), RA (4 patients), mixed connective tissue disease (2 patients), polymyositis (one patient) and sarcoidosis (one patient). The remaining 17 patients did not have systemic diseases, but 9 of them were associated with organ-specific autoimmune diseases such as chronic thyroiditis (4 patients), idiopathic thrombocytopenic purpura (3 patients, one of them had Evans syndrome), primary biliary cirrhosis (one patient) and pernicious anemia (one patient). In this report, we classified the SS patients without systemic diseases as primary (I^+ -) SS and those with such diseases as secondary (II^+ -) SS.

Three of the 17 I^+ -SS patients (4.2 ± 1.4 mg/day, range 2.5~5) and 14 of the 20 II^+ -SS patients (13.0 ± 7.6 mg/day, range 3~25) were receiving prednisolone (PSL) at the time of this study. Two of the II^+ -SS patients with PSL treatment were further taking azathioprine (50 mg/day). Fourteen of the 17 patients with I^+ -SS were free from any systemic medications.

Fifteen patients with SLE (4 males and 11 females) and 24 patients with RA (4 males and 20 females) were also examined in this study. One of the SLE patients was associated with RA, and 4 of the RA patients were associated with autoimmune thyroid diseases. SLE and RA patients fulfilled the American College of Rheumatology criteria for each disease^{14,15}. The presence of SS in SLE and RA patients was excluded by clinical symptoms and screening tests for SS including measurements of lacrimal and salivary flow rates and parotid sialography, although detailed ophthalmological examinations and salivary gland biopsy were performed in selected patients. Fourteen of the 15 SLE patients (9.4 ± 16.9 mg/day, range 7.5~40) and 8 of the 24 RA patients (3.8 ± 6.6 mg/day, range 2.5~15) were

taking PSL at the time of this study. One SLE patient and one RA patient with PSL treatment were further on azathioprine (75 mg/day) or methotrexate (7.5 mg/week), respectively. Five of the 8 RA patients with PSL and 11 of the 16 RA patients without PSL were taking one of the slow-acting anti-rheumatic drugs in addition to non-steroidal anti-inflammatory drugs: auranofin 3~6 mg/day, D-penicillamine 100 mg/day, sulfasalazine 1.5~2 g/day, bucillamine 200 mg/day or lobenzarit disodium 240 mg/day.

Twenty-two healthy adults (11 males and 11 females) were used as controls. Their mean age (37.0 ± 13.5 years old, range 22~76) was not different from that of II^+ -SS (43.0 ± 15.4 years old, range 22~84), but was significantly younger than that of I^+ -SS (52.2 ± 16 years old, range 19~75, $p < 0.01$) or RA (50.4 ± 12.5 years old, range 18~68, $p < 0.01$). However, the controls were older than SLE patients (29.2 ± 11.3 years old, range 18~68, $p < 0.05$).

2. Monoclonal antibodies (MoAb)

The following combinations of fluorescein isothiocyanate (FITC)- and phycoerythrin (PE)-conjugated MoAb were used in this study: anti-Leu 1 (CD5) /anti-Leu 12 (CD19), anti-interleukin-2 receptors (IL-2 R, CD25) /anti-Leu 12, anti-transferrin receptors (TfR, CD 71) /anti-Leu 12, and anti-Leu 1/anti-OKT 3 (CD3). Leu-series antibodies and anti-TfR antibody were purchased from Becton-Dickinson Immunocytometry Systems (B/D). Anti-IL-2 R or OKT 3 antibody was purchased from T Cell Sciences, Inc. or Ortho Diagnostic System Inc., respectively. The mixture of FITC-murine IgG 1 and PE-IgG 2 (Simultest control®; B/D) was used as control reagents.

Twenty μ l of MoAb from B/D, 10 μ l of OKT 3 antibody and 5 μ l of anti-IL-2 R antibody were employed in each test.

CD5 MoAb reacts with all of the T-cells and a small number of B-cells in the PB. CD3 antibody is also reactive with mature T-cells. CD19 antibody recognizes all of the B-cells in the PB and precur-

sors of B-cells. IL-2 R and TfR are also reported to be expressed on activated B lymphocytes^{16,17}.

3. Two-color flowcytometry

The method was already described elsewhere¹⁸. Briefly, PB-mononuclear cells (PBMC) were separated by the standard Ficoll density gradient centrifugation. Erythrocytes in the PBMC fraction were eliminated by using hypotonic solution. After washing with Dulbecco's phosphate buffered saline (PBS), PBMC were suspended in PBS containing 5% heart-inactivated fetal calf serum and 0.1% sodium azide. 100 μ l of the PBMC (1×10^7 cells/ml) was mixed with FITC-MoAb and then corresponding PE-MoAb. After incubation at 4°C for 30 minutes in the dark, the PBMC were washed with PBS containing 0.1% sodium azide, and were suspended in 0.5 ml of 1.0% paraformaldehyde in PBS. The PBMC were stored at 4°C for 16 hours under the condition avoiding light. After passing the PBMC through a nylon-mesh filter, five thousand cells were measured by a flowcytometer (FACScan®; B/D) and lymphocytes surrounded by appropriate settings of forward and 90° light scatter gates were analyzed. Before measurements, the conditions of the flowcytometer were automatically set by a FACS can Auto Compensation Program using three Cali BRITE™ flowcytometer beads (B/D), unlabelled, FITC-, and PE- labelled beads. The percentage of positively or negatively stained cells with FITC- and/or PE-MoAb was determined by the thresholds carefully set by using corresponding control cells which were incubated with Simultest control®. The percentage of the cells which were negative for both FITC- and PE-murine IgG was always higher than 99.9% in the control samples (Figure 1).

4. Serological parameters (Table 1)

The levels of serum gammaglobulins, IgG, IgA, IgM and complement component (C3), titers of RF (hemagglutination test) or anti-DNA antibodies (Crithidia method), and erythrocyte sedimentation rates (ESR) were measured at the time of blood

sampling.

Wilcoxon test and linear regression analysis were employed for statistical evaluations in the present study.

III. Results

1. Lymphocyte counts and percentages of total B-cells and T-cells (Table 2)

Lymphocyte counts in the PB were significantly decreased in patients with II°-SS, SLE or RA as compared with controls. The percentages of total B-cells determined as CD19⁺ cells were significantly higher in SLE patients, and were relatively higher in patients with SS or RA than those in controls. Little differences were identified between the percentages of T-cells determined as CD19⁻ CD5⁺ T-cells and those as CD3⁺ T-cells in any groups, although T-cells were significantly decreased in RA patients.

2. CD5⁺ B-cells (Table 3)

The percentages of CD5⁺ CD19⁺ B-cells in the PB were higher in patients with I°-SS or RA than those in controls, although no significant difference was found. On the other hand, the cells were significantly decreased in SLE patients. When the percentages of CD5⁺ CD19⁺ B-cells in total B-cells were calculated, the values were significantly lower in patients with II°-SS, SLE or RA than those in controls.

3. B-cells expressed activation antigens (Table 4)

Activated B-cells in the PB were determined as IL-2 R (CD25)⁺ CD19⁺ cells or TfR (CD71)⁺ CD19⁺ cells. The percentages of IL-2 R⁺ B-cells were lower than detectable range by the flowcytometric method employed in any groups, and no statistically significant difference was found in the cells. The proportions of TfR⁺ B-cells were also very low, but were significantly higher in patients with I°-SS, II°-SS or SLE than those in controls. When the percentages of TfR⁺ B-cells in total B-cells were considered, the values were also significantly higher in

Figure 1 Examples of FACS analysis of peripheral blood lymphocytes

(A) 99.95% of lymphocytes (left lower quadrant) from a patient with rheumatoid arthritis associated with Sjögren's syndrome was not stained with FITC-murine IgG 1 and PE-murine IgG 2. (B) Her lymphocytes were stained with FITC-CD 5 MoAb and PE-CD 19 MoAb. The left or right upper quadrant indicates CD 5⁻CD 19⁺ B-cells (18.64%) or CD 5⁺CD 19⁺ B-cells (6.88%), respectively. The right lower quadrant indicates T-cells (56.80%). (C) Another example from a patient with systemic lupus erythematosus is shown. 100.00% of the lymphocytes was not stained with FITC- and PE- murine IgG. (D) In the patient, the percentage of CD 5⁺CD 19⁺ B-cells (1.97%) was lower than that of previous patient. The percentage of CD 5⁻CD 19⁺ B-cells or T-cells was 16.59% or 71.60%, respectively.

Table 1 Serological features in the patients

	I ⁺ -SS	II ⁺ -SS	SLE	RA
ESR (mm/h)	34.9±25.1 (6-86, n=13)	41.6±25.7 (4-105, n=12)	29.3±33.7 (1-117, n=11)	43.0±28.8 (6-130, n=24)
γ -gl (g/dl)	1.67±0.59 (0.58-2.90, n=17)	1.76±0.48 (0.99-2.65, n=19)	1.23±0.39 (0.76-2.15, n=15)	1.56±0.51 (0.93-2.97, n=24)
IgG (mg/dl)	2,123±739 (678-3,770, n=17)	2,171±550 (1,250-3,060, n=19)	1,519±411 (962-2,530, n=15)	1,918±586 (1,170-3,690, n=24)
IgA (mg/dl)	401±211 (106-858, n=17)	407±227 (176-1,070, n=19)	319±142 (129-630, n=15)	433±188 (170-961, n=24)
IgM (mg/dl)	267±225 (84-1,100, n=17)	193±180 (6-847, n=19)	170±116 (53-458, n=15)	180±113 (58-605, n=24)
RF titer	101±102 (0-320, n=17)	100±203 (0-640, n=18)	8±25 (0-80, n=10)	125±284 (0-1,280, n=24)
anti-DNA titer	—	72±174 (0-640, n=16)	57±166 (0-640, n=15)	—
C3 (mg/dl)	64±17 (27-92, n=16)	56±21 (23-97, n=18)	50±15 (26-71, n=15)	—

Data are indicated as mean value $\pm$ one standard deviation. Range of the data and number of the patients are indicated in parentheses. Abbreviations are as follows: I⁺-SS=primary Sjögren's syndrome, II⁺-SS=secondary SS, SLE=systemic lupus erythematosus, RA=rheumatoid arthritis, ESR=erythrocyte sedimentation rates, γ -gl=gammaglobulins, and RF=rheumatoid factors.

Table 2 Total lymphocytes and their subpopulations

	lymphocyte counts(/ μ l)	total B-cells (CD 19 ⁺)(%)	total T-cells CD 19 ⁺ -CD 5 ⁺ (%)	CD 3 ⁺ (%)
control (n=22)	2,037±524	6.3±3.0	70.1±10.7	69.6±11.2
I ⁺ -SS (n=17)	1,673±547	8.9±7.1	65.4±14.6	63.8±15.9
II ⁺ -SS (n=20)	1,234±730 ^b	8.1±6.1	73.2±14.3	72.6±15.6
SLE (n=15)	1,383±886 ^a	11.7±8.6 ^a	65.7±15.3	63.8±17.3
RA (n=24)	1,601±811 ^a	9.2±6.5	59.0±15.5 ^a	57.3±18.1 ^a

The p values between control and patients are less than 0.05 (a) and 0.01 (b). Same abbreviations are employed in other Tables.

SLE patients, and were relatively higher in patients with I⁺-SS or II⁺-SS than those in controls.

4. Correlations of B-cells with serological parameters

The proportions of total B-cells positively correlated with IgM levels in I⁺-SS patients ($Y=46.9+24.8X$, $R=0.779$, $p<0.05$), and with ESR ($Y=21.0+2.4X$, $r=0.541$, $p<0.05$), total gammaglobulins ($Y=1.03+0.06X$, $r=0.728$, $p<0.05$) and IgG levels ($Y=1,337+64X$, $r=0.700$, $p<0.05$) in RA patients.

The percentages of CD5⁺ B-cells also positively correlated with total gammaglobulins ($Y=1.25+$

$0.17X$, $r=0.522$, $p<0.05$) and IgG ($Y=1,575+193X$, $r=0.506$, $p<0.05$) in RA patients.

The percentages of Tfr⁺ B-cells positively correlated with total gammaglobulins ($Y=1.16+1.31X$, $r=0.486$, $p<0.05$), IgM ($Y=87.5+306.5X$, $r=0.509$, $p<0.05$) and RF titers ($Y=-132+857X$, $r=0.569$, $p<0.05$) in RA patients, and with anti-DNA antibody titers ($Y=-46.3+105X$, $r=0.554$, $p<0.05$) in SLE patients.

5. Correlations of the dosage of PSL with B-cells

We further examined possible correlations of the dosage of PSL with B-cells. A significant positive

Table 3 Percentages of CD 5⁺ B-Cells

	CD 5 ⁺ CD 19 ⁺ cells (%)	(CD 5 ⁺ CD 19 ⁺ cells/CD 19 ⁺ cells) × 100 (%)
control (n=22)	1.6 ± 0.8	28.2 ± 11.3
I ⁺ -SS (n=17)	1.9 ± 1.3	23.5 ± 10.2
II ⁺ -SS (n=20)	1.5 ± 1.7	17.2 ± 10.1 ^b
SLE (n=15)	1.1 ± 0.8 ^a	10.1 ± 7.7 ^b
RA (n=24)	1.8 ± 1.5	19.3 ± 8.7 ^b

correlation was found between the dosage of PSL and percentages of Tfr⁺ B-cells in the whole patients ($Y = -0.08 + 0.06 X$, $r = 0.772$, $p < 0.05$, Figure 2), SLE ($Y = -0.28 + 0.08 X$, $r = 0.828$, $p < 0.05$) and RA patients ($Y = 0.11 + 0.03 X$, $r = 0.719$, $p < 0.05$), who were receiving PSL. A similar correlation was found in the II⁺-SS patients with PSL ($Y = 0.17 + 0.03 X$, $r = 0.507$, $p > 0.05$).

The percentages of CD5⁺ B-cells were negatively correlated with the dosage of PSL in the patients with II⁺-SS receiving the treatment ($Y = 1.15 - 0.04 X$, $r = 0.638$, $p < 0.05$, Figure 3). However, such correlations could not be identified in the whole patients and other patients' groups.

6. Correlations among B-cells

The percentages of total B-cells were significantly correlated with those of CD5⁺ B-cells in whole subjects including controls and patients ($Y = 0.47 + 0.13 X$, $r = 0.640$, $p < 0.05$), I⁺-SS patients ($Y = 0.58 + 0.15 X$, $r = 0.832$, $p < 0.05$), II⁺-SS patients ($Y = -0.37 + 0.23 X$, $r = 0.845$, $p < 0.05$) and RA patients ($Y = 0.13 + 0.18 X$, $r = 0.758$, $p < 0.05$), but not in SLE patients. However, the percentages of total B-cells were not correlated with those of CD5⁺ B-cells in total B-cells in any patients' groups.

The percentages of total B-cells positively correlated with those of Tfr⁺ B-cells in RA patients ($Y = 0.13 + 0.02 X$, $r = 0.631$, $p < 0.05$), but not in the other patients' groups. On the other hand, the percentages of total B-cells negatively correlated with those of Tfr⁺ B-cells in total B-cells in I⁺-SS

Table 4 Percentages of activated B-cells

	IL-2 R ⁺ CD 19 ⁺ cells (%)	Tfr ⁺ CD 19 ⁺ cells (%)	(Tfr ⁺ CD 19 ⁺ cells/CD 19 ⁺ cells) × 100 (%)
control (n=22)	0.05 ± 0.04	0.25 ± 0.19	5.2 ± 3.8
I ⁺ -SS (n=17)	0.04 ± 0.03	0.48 ± 0.28 ^a	8.9 ± 6.1
II ⁺ -SS (n=20)	0.07 ± 0.09	0.54 ± 0.44 ^b	14.0 ± 20.2
SLE (n=15)	0.04 ± 0.05	0.99 ± 0.88 ^b	12.3 ± 8.2 ^b
RA (n=24)	0.04 ± 0.05	0.30 ± 0.19	4.8 ± 2.5

Abbreviations are as follows: IL-2 R = interleukin-2 receptors and Tfr = transferrin receptors.

patients ($Y = 12.7 - 0.43 X$, $r = -0.502$, $p < 0.05$) and II⁺-SS patients ($Y = 26.4 - 1.48 X$, $r = -0.455$, $p < 0.05$).

The proportions of CD5⁺ B-cells also positively correlated with those of Tfr⁺ B-cells ($Y = 0.21 + 0.05 X$, $r = 0.420$, $p < 0.05$) in RA patients, but not in other patients' groups.

Since the distributions of ages and sexes were different between controls and patients' groups, we divided the 22 healthy controls into subgroups (13 adults younger than 37 years old and 9 adults older than 38 years old, and 11 females and 11 males) according to their mean age or sex. However, there was no significant difference in the lymphocyte counts, percentages of total B-cells, CD5⁺ B-cells, Tfr⁺ B-cells and CD19⁺ CD5⁺ or CD3⁺ T-cells, and percentages of CD5⁺ B-cells or Tfr⁺ B-cells in total B-cells between the subgroups: younger vs. older groups, or female vs. male groups.

IV. Discussion

Present studies demonstrated that the proportions of total B-cells in PB were significantly higher in SLE patients and relatively higher in patients with SS or RA than controls. The proportions of CD5⁺ B-cells were also higher in I⁺-SS or RA patients than controls. However, the proportions of CD5⁺ B-cells in total B-cells were significantly low in patients with II⁺-SS, SLE or RA. Our results, therefore, are different from the previous reports by

Figure 2 Correlation of transferrin receptor (TfR)⁺ B-cells with prednisolone treatment

The regression line in the figure was obtained from the whole patients including primary Sjögren's syndrome (○), secondary Sjögren's syndrome (●), systemic lupus erythematosus (SLE; ■) and rheumatoid arthritis (RA; ×), who were receiving prednisolone. Similar regression lines were observed in the patients with SLE or RA when each patients' group was analyzed.

others who demonstrated a significant or relative increase of CD5⁺ B-cells in the PB of patients with I⁻-SS, RA or SLE²⁻³). In addition, the CD5⁺ B-cells were significantly decreased in the PB of our SLE patients, although they were receiving PSL treatment, except for one patient.

The reasons for the discrepancies in the results between ours and others might be explained by the differences in either the subjects including patients and controls examined, antibodies or methods employed: the first group determined the CD5⁺ B-cells in the B-cell-enriched fraction separated by sheep erythrocyte-rosetting technique from the PB of RA patients²). They employed a double immunofluorescent technique using CD5 antibody and anti-human immunoglobulins antibodies, and enumerated the cells microscopically. Their mean per-

centage of CD5⁺ B-cells in total B-cells in RA patients was equivalent to our results, whereas the value in controls was markedly lower than those in the present study or later studies by others. The second group examined the CD5⁺ B-cells in the PBMC from patients with RA or other connective tissue diseases by two-color flowcytometry using CD5 antibody and anti- μ chain MoAb or CD19 antibody³). Their mean percentage of CD5⁺ B-cells in total B-cells in RA patients was similar to our results, but that in controls was lower than ours. The third group also determined the CD5⁺ B-cells in the PBMC from patients with I⁻-SS, RA or SLE by two-color flowcytometry using CD5 and CD19 MoAb⁴), which was considered to be almost identical to our method. Their mean proportion of CD5⁺ B-cells in total B-cells in patients with I⁻-SS or

Figure 3 Correlation of CD5⁺ B-cells with prednisolone treatment in patients with secondary Sjögren's syndrome

The regression line in the figure was obtained from the patients with secondary Sjögren's syndrome being treated with prednisolone. They consisted of eleven patients associated with systemic lupus erythematosus (■) and three patients with the other systemic diseases (●).

RA was higher than that in the present study, although the value in controls was similar to our results. Furthermore, the fourth group also employed the 2-color flowcytometry using CD5 and CD19 MoAb⁹. Their mean proportions of CD5⁺ B-cells in total B-cells were remarkably lower in the PBMC from healthy adults, SLE or RF-positive RA as compared with the results by ours and others.

The proportions of total B-cells in PB are quite lower than those of T-cells, and CD5⁺ B-cells are a minor subset of B-cells. Therefore, B-cell-enriched fraction used in the first report might lose a certain subset such as CD5⁺ B-cells. The microscopic method may have some difficulties to identify the CD5⁺ B-cells which are considered as CD5^{dim} cells. The low expression of CD5 antigen on B-cells probably explains the differences among the results obtained by using a flowcytometer. However, the reactivity of CD5 MoAb employed in the present study would not be the cause, since its reactivity to

T-cells were considered to be adequate as compared with the values obtained by using CD3 MoAb. CD19 MoAb will be more appropriate for the determination of total B-cells than anti-human immunoglobulins antibodies or anti- μ chain MoAb, since the MoAb can recognize a wide range of B-cells. Treatments and severity of the diseases at the time of study might be responsible for the differences, but they seemed to be similar among the studies including ours.

B-cells expressing activation antigens were simultaneously determined in the present study. The percentages of IL-2 R⁺ B-cells were within negligible range in the PB of controls and patients. However, we could demonstrate that the proportions of Tfr⁺ B-cells were significantly high in the PB of patients with I⁺-SS, II⁺-SS or SLE. The present results in I⁺-SS confirmed those reported by others¹¹, who determined IL-2 R and Tfr on the B-cells in I⁺-SS. However, some of the results reported by other groups who examined activated B-cells in the PB of patients with SS, SLE or RA by other methods⁸⁻¹⁰ are different from those of the present study: one group examined B-cells which spontaneously produce immunoglobulins in the PB of SS or SLE patients⁹. They found that the cells were increased in the PB of SLE patients, but not in the PB of SS patients. In the other study, activated B-cells determined by B 532 antibody were not increased in the PB of SS patients, although the cells were increased in the lymphocytes separated from salivary glands of the patients⁹. Another study determined surface IgM and IgD on lymphocytes as well as rosette-forming cells with murine erythrocytes in the PB suggested an increase of activated B-cells in active RA patients¹⁰. The discrepancies could be explained by the differences of activation stages of B-cells determined by each method.

We further analyzed possible correlations of the B-cells with serological parameters or with PSL treatment. The proportions of total B-cells or of CD5⁺ B-cells positively correlated with ESR, total gammaglobulin, IgG or IgM levels in patients with RA or I⁺-SS. In the patients with II⁺-SS, PSL

seemed to decrease the CD5⁺ B-cells, and the cells were also decreased in SLE patients receiving PSL treatment. The proportions of Tfr⁺ B-cells also positively correlated with total gammaglobulin and IgM levels or RF titers in RA. More interestingly, the cells were considered to reflect the activity of diseases, since Tfr⁺ B-cells positively correlated with the dosage of PSL in each patients' group.

In addition, we examined possible correlations among the B-cells determined. The percentages of CD5⁺ B-cells correlated with total B-cells in patients with I⁻-SS, II⁻-SS or RA. However, CD5⁺ B-cells correlated with Tfr⁺ B-cells in only RA patients. Therefore, the results may suggest that CD5 antigen might not be an activation antigen of B-cells or stimulations to B-cells are different among the patients' groups : CD5⁺ B-cells which produce

low-affinity and polyreactive antibodies¹⁾ are being activated in RA, while CD5⁻ B-cells producing high-affinity and monoreactive antibodies are being activated in SLE or SS patients.

Finally, our present results did not support the previous evidences reported by others¹⁹⁾ who demonstrated deficient CD5 antigen-expression on T-cells of the PB from patients with I⁻-SS, since no dissociation was observed in the expression of CD5 and CD3 antigens on T-cells in our patients.

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