Increased Circulating CD5$^+$ B Cells in Human SLE: No Correlation with the Level of Autoantibodies

전신성 홍반성 낭창 환자의 혈액내 CD5$^+$ B 세포의 증가: 자가항체 농도와의 비연관성

Jeong Soo Park*, Soo Youn Baek*, Gyu Tae Shin†, Dohun Kim†, Dong-Ho Nahm††, Kyung Ae Ma†, Jung Koo Youn** and Young-Ju Jang*

박정수* · 백수연* · 신규태† · 김도현† · 남동호†† · 마경애†
윤정구** · 장영주*

*Laboratory of Immunology, Institute for Medical Science, †Department of Nephrology, ††Department of Allergy and Clinical Immunology, and **Department of Microbiology, Ajou University School of Medicine, Suwon 442-749, Korea

*아주의대 의과학연구소 면역학연구실, †신장내과학교실, ††알레르기 및 임상면역학교실, **미생물학교실

There has been a controversy about the association of the levels of CD5$^+$ B cell subpopulation and autoantibodies in SLE. To date, there have been very few papers which compared the levels of the subpopulation with several kinds of autoantibodies. In the present work, we studied the correlations between the levels of circulating CD5$^+$ B cells and autoantibodies against dsDNA, ssDNA, RNA, thyroglobulin, and human IgG in 14 Korean patients with systemic lupus erythematosus (SLE). The levels of circulating CD5$^+$ B cells and total IgM in the sera were also compared in peripheral bloods of these patients and normal individuals. The patients had remarkably elevated levels of both circulating CD5$^+$ B cell subsets and titers of several autoantibodies as compared with normals. A close association of the levels of CD5$^+$ B cell subsets with the measures of any autoantibodies, however, was not observed. The levels of total IgM in the patients increased as compared with normal individuals. Our data indicate that the overally increased production of autoantibodies in these patients is due to a polyclonal B-cell activation, not to a specific stimulation of CD5$^+$ B cell subsets.

Korean J. Immunol. 22, 1: 23~30, 2000

Key Words: Autoantibodies, CD5, SLE, Polyclonal B cell activation

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the deposition of autoantibodies and immune complexes, leading to tissue damage. Autoantibodies reacting to self-components have been shown to play a pathogenic role in some autoimmune diseases. However, it is not clearly understood whether excess autoantibody productions reflect a specific stimulation of autoreactive clones[1,2] or a polyclonal activation of many different B cells[3,4]. The CD5 antigen which was first described as a molecule restricted to T lymphocyte is also expressed on some human B cell subset, called CD5$^+$ B cells[5]. Contradictory results have been obtained from different researchers about the roles of CD5$^+$ B cells in both human and murine SLE. Some reports emphasized the significance of the CD5$^+$ B cells in the pathogenesis of autoimmune diseases, but others observed they are not critical to the pathogenesis. To understand clearly the roles of CD5$^+$ B cells in health and disease states, a great deal of effort has been made.

Reprint request to: Young-Ju Jang, Laboratory of Immunology, Institute for Medical Science, Ajou University School of Medicine, Suwon 442-749, Korea. Tel: 0331) 219-4516, E-mail: jangyj@madang.ajou.ac.kr

One of the major abnormalities in NZB mice, the mouse model most commonly used as the investigations of human SLE[8], is the increased frequencies of Ly1$^+$ B cells (CD5$^+$ B cells in humans) in both spleens and peritoneal cavities[9]. These Ly1$^+$ B cells have been shown to be responsible for spontaneous secretion of IgM, which recognize autoantigens such as erythrocytes, thymocytes and ssDNA[6,7,10,11]. In MRL-lpr/lpr mice, an another mouse model of SLE, it has been reported that the autoantibody-producing cell precursors are bone marrow-derived Ly1$^+$ B cells[12]. We have also previously shown the increased levels of Ly1$^+$ B cells in spleens and peritoneal cavities of MRL-lpr/lpr mice in comparison with those of normal mice[13].

Human CD5$^+$ B cells are the predominant population of B cells in fetal lymphoid organs and neonatal cord bloods. The frequencies of CD5$^+$ B cells decreases during development, becoming a minor proportion (only 5 to 10 per cent) of B cells in the peripheral bloods of healthy adults[14~16]. It was reported that the B cell subsets spontaneously secrete autoantibodies in humans as in mice [17,18]. Because of the self-antigen binding activity of the antibodies they produce, it has been suggested that CD5$^+$ B cells may play an important role in autoimmune process in humans. It has been shown that circulating CD5$^+$ B cells were increased in numbers and they produced autoantibodies in Caucasian patients with rheumatoid arthritis (RA), Sjögren's syndrome (SS)[20,21].

However, several contradictory results have also been reported. Casali et al.[19] have showed that high affinity, monoreactive, IgG autoantibodies, which are the characteristics of autoantibodies found in SLE patients were produced by the conventional CD5$^-$ B cell subsets. It has been reported that CD5$^+$ B cells were not expanded in Caucasian patients with SLE[26]. Liu et al.[22] have shown that there was no correlations between the levels of CD5$^+$ B cells and the values of rheumatoid factor (RF) in Chinese patients with Rheumatoid Arthritis (RA). A study with Japanese RA patients has noted that 20~25% of them have circulating CD5$^+$ B cell levels higher than the Caucasian RA patients included in that study[23].

We investigated the levels of CD5$^+$ B cells in the peripheral bloods of Korean SLE patients by using two-color immunofluorescence analysis. We also addressed the question whether there were any correlations between the levels of circulating CD5$^+$ B cells and the serum titers of several autoantibodies in the patients. In addition, the role of polyclonal B cell activation was examined by measurment of the levels of total IgM and several autoantibodies in the sera by ELISA.

MATERIALS AND METHODS

Patients

Fourteen Korean patients with SLE registered at Ajou University Hospital were evaluated. Eleven patients were female and 3 were male. The mean age of these patients was 30 years, ranged from 14 to 42. All patients met the 'Revised classification criteria for SLE'[24], including autoantibody profile. A normal control group included 19 heathy volunteers. Fifteen volunteers were female and 4 were male. The mean age of these volunteers were 31 years, ranged from 23 to 41.

Direct-binding ELISA

To measure the titers of anti-dsDNA antibodies, anti-ssDNA antibodies, anti-RNA antibodies, anti-thyroglobulin antibodies, and anti-human IgG antibodies (RF) in patients' and normals' sera by direct ELISA, 96 well polystyrene microtiter plates (Nunc, Denmark) were coated with calf thymus dsDNA (Sigma chemical Co., MO, U.S.A.), calf thymus ssDNA (Sigma chemical Co.), thyroglobluin (Sigma chemical Co.), human IgG (Sigma chemical Co.) at each concentration of 5 μg/ml, or yeast RNA (USB, Germany) at 100 μg/ml overnight at 4℃. For blank wells, PBS were added instead of the coating antigens at the same condition. The plates were washed three times with PBS containing 0.05% Tween-20 (PBST) and blocked with 3% BSA-PBST for 2h at room temperature (RT). One hundred-fold dilutions of the sera in PBS were then added. After incubation for 1h at RT, the plates were washed three times with PBST, then incubated with a 1:30,000 dilution of alkaline phosphatase-conjugated goat anti-human IgM (Sigma chemical Co.) in 1% BSA-PBST for 2h at RT. After washing five times with PBST, a substrate solution of p-nitrophenyl phosphate (Sigma chemical CO.) was added. After 1 hr incubation at RT, absorbances were measured at 405 nm using ELISA reader (Bio-Rad, CA, U.S.A.). To measure total IgM in the sera, same procedures were performed as those for measuring the titers of autoantibodies, except 5 μg/ml of human IgM were used as coating antigen.

Preparation of peripheral blood mononuclear cells (PBMCs)

Mononuclear cells were isolated from peripheral bloods by centrifugation using Ficoll-Hypaque (Sigma chemical Co.). Cells were washed twice in serum-free DMEM medium. To remove red blood cells, the cell suspension was incubated with 0.17 M Tris-NH_4Cl for 2 mins at RT. Cells were then washed three times with 2% FBS/PBS and ad-

justed to 1×10^7/ml in 2% FBS/PBS. Cell viability was determined by Trypan blue exclusion. In all samples, more than 90% of cells were viable.

Staining of PBMCs

One hundred μl of the PBMC suspension (total 1×10^6 cells) and 5 μl of monoclonal anti-human CD5-Fluorescein (FITC) (Sigma chemical Co.) were added to a Falcon 2058 tube and were mixed gently. Unstained cells for the control were prepared by mixing 5 μl of PBS instead of the fluorochrome to the cells. The mixture was incubated for 30 mins at RT in the dark place, and 2 ml of cold 2% FBS/PBS (staining solution) was added. The sample was centrifugated at 400 g for 5 mins. After the supernatant was aspirated, cell pellet was resuspended in 100 μl of staining solution and 5 μl of monoclonal anti-human CD19 R-Phycoerythrin (PE) (Sigma chemical Co.), and the same procedures were performed as those for the anti-human CD5-FITC staining. The cell pellet was resuspended in 1% paraformaldehyde in PBS for fixation until FACS analysis.

Flow cytometric analysis

Analyses were performed with Coherent INNOVA 304-laser FACSVantage Flow Cytometer (Beckton Dickinson, CA, U.S.A.) equipped with logarithmic amplifiers to measure light scatter and the amount of pairs of fluorochrome-labeled monoclonal reagents bound to individual cells. During the analysis, a Macintosh Quadra 650 (Apple Computer, CA, U.S.A.) and CellQuest program were used to collect and store individual measurements on 10,000 cells for later analysis. Two-color staining data were presented as "dot plots" in which each dot represents one or more events (cells or particles). To generate statistics for a dot plot, quadrants were defined to divide dot plots into four sections. Division of quadrant was determined with controls including unstained and single stained PBMC with either FITC-antibody or PE-antibody. The statistics of each quadrants were calculated as percentage of the total events in a quadrant compared to the whole plot.

Statistical analyses

The statistical significance of differences between the levels of $CD5^+$ B cells in patients and those in normals, and the levels of total IgM in two groups were assessed by the Student's t-test. The statistical significance of correlations of the levels of $CD5^+$ B cells and the titers of the autoantibodies were analyzed by Spearman's rank analysis.

RESULTS

Levels of $CD19^+$ B cells expressing CD5 antigen (CD 5^+ B cells; $CD19^+$ $CD5^+$ cells) in SLE patients were markedly elevated as compared with normal individuals; mean percentages of $CD5^+$ B cells in total B cells were

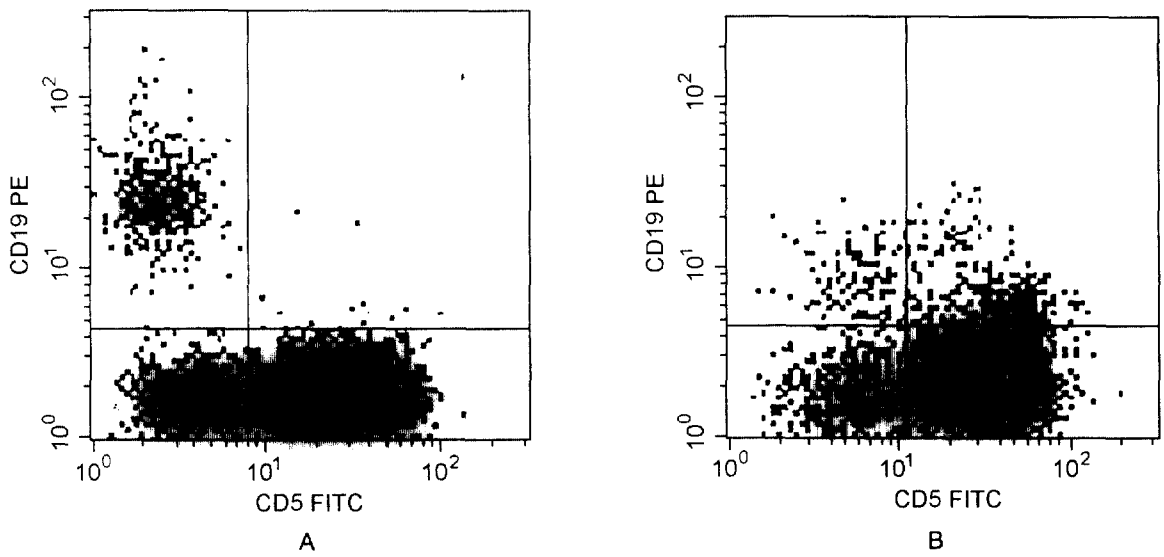

Figure 1. Representative two-color immunofluorescent stain profile of circulating CD5+ B cells in normal individuals (A) and patients with SLE (B). Fluorescence signal of CD5-FITC is displayed on X axis, CD19-PE on Y axis (both in log scale). Based on control sample (unstained cells), dot plots were divided into four quadrants: unstained cells (lower left); cells with both fluorochrome (upper right) or stained with either FITC-CD5 (lower right) or PE-CD19 (upper left). Numbers of upper right and upper left quadrants were added to calculate the number of total B cells. Numbers of upper right divided by those of total B cells are the percentage of $CD5^+$ B cells.

16.9% in patients and 7.8% in normals (Figure 1A and B). Difference between the two groups showed statistical significance ($p<0.05$) in student's t-test. Only 3 SLE patients represented less than 10% values. There were no significant differences in numbers of total PBMC between the patients and normals. Numbers of total PBMC were $5.5\times10^5\pm1.0$ cells/ml of bloods in patients, ranged from 1.8×10^5 to 8.3×10^5. The numbers in normals were $5.3\times10^5\pm2.8$ cells/ml of bloods, ranged from 2.1×10^5 to 6.8×10^5. Percentages of $CD19^+$ B cells in PBMC also were not much different between two groups; in patients, they were $8.1\pm0.1\%$ of lymphocytes, ranged from 5.3 to 11.4%; in normals, they were $8.9\pm2.5\%$ of lymphocytes, ranged from 3.8 to 13.8%. In normals, the average levels of the $CD5^+$ B cells were between 5 and 10% in total B cells (Figure 1B) which distribution was similar to previously reported data[14,15]. In SLE patients, the levels of the $CD5^+$ B cell showed a wide range of distribution, which is 5.8 to 41.8%.

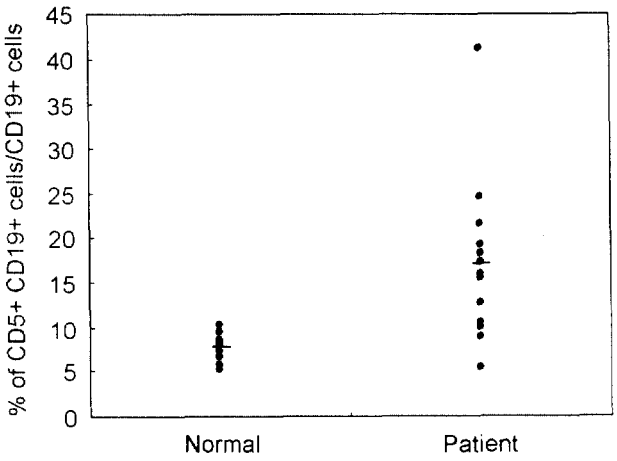

Figure 2. Circulating B cells in the peripheral bloods of normal individuals and patients with SLE. PBMCs were separated and stained with anti-human CD19-PE and anti-human CD5-FITC, and double stained PBMCs were analyzed with flow cytometry. Results are given as percentages of $CD5^+$ B cell subsets in total B cells. Bars indicate the mean values.

Titers of the autoantibodies against several autoantigens in the sera of patients were higher than those of normal individuals (Table 1 and Figure 2). In each case of autoantibodies, there were statistically significant differences between two groups ($p<0.05$). In the blank controls, the O.D. values were less than 0.1. Titers of anti-human IgG (RF) among other autoantibodies tended to be increased most in these patients. The overall increment of the several autoantibodies suggest a possibility of polyclonal B-cell activation in these patients.

Correlations of the levels of circulating $CD5^+$ B cells

Table 1. The profiles of patients with SLE

No.	Sex	Age	Disease duration	Drug dose (mg/day)	The titer of autoantibodies to[a]					% of $CD5^+$ $CD19^+$ cells/total $CD19^+$ cells[d]
					dsDNA	ssDNA	RNA	Thyroglobulin	Human IgG	
1	F	32	13 yr	PSN[b] (7.5)	±	±	±	±		9.66
2	F	37	2 yr	PSN (5)	+	+	+	±	+++	18.68
3	F	39	2 yr	None	++	++	+	++	+++	5.84
4	F	34	4 yr	PSN (5)	+	+	+	+	+	24.96
5	M	32	3 yr	PSN (12.5)	±	+	±	±	±	22.02
6	M	40	1 yr	PSN (5)	±	±	±	±	+	15.93
7	F	14	1 wk	PSN (40)	+	+	±	+	+++	10.88
8	F	30	6 mon	PSN (10)	+	+	±	±	+	10.53
9	F	31	6 yr	PSN (12.5)	++	++	+	+	++	18.45
10	F	21	1 yr	PSN (5)	+++	+++	+	+++	+++	19.56
11	F	42	4 yr	PSN (5)	±	±	±	±	±	17.61
12	F	34	1 yr	DFC[c] (12)	++	++	+	±	+++	16.20
13	F	19	1 wk	None	+	+	±	++	+++	13.09
14	M	19	3 yr	DFC (24)	+	±	±	±	+++	41.76

[a] The titers of autoantibodies were measured by direct binding ELISA. The titers of autoantibodies are expressed on the basis of the difference in O.D. values in comparison with the negative control. When the O.D. value was less than 2.0 times to that of blank, it was marked with ±, 2.1 to 4 times with +, 4.1 to 6 times with ++, and more than 6.1 times with +++. [b] PSN; prednisolone, [c] DFC; deflzacort, n.k.; not known.

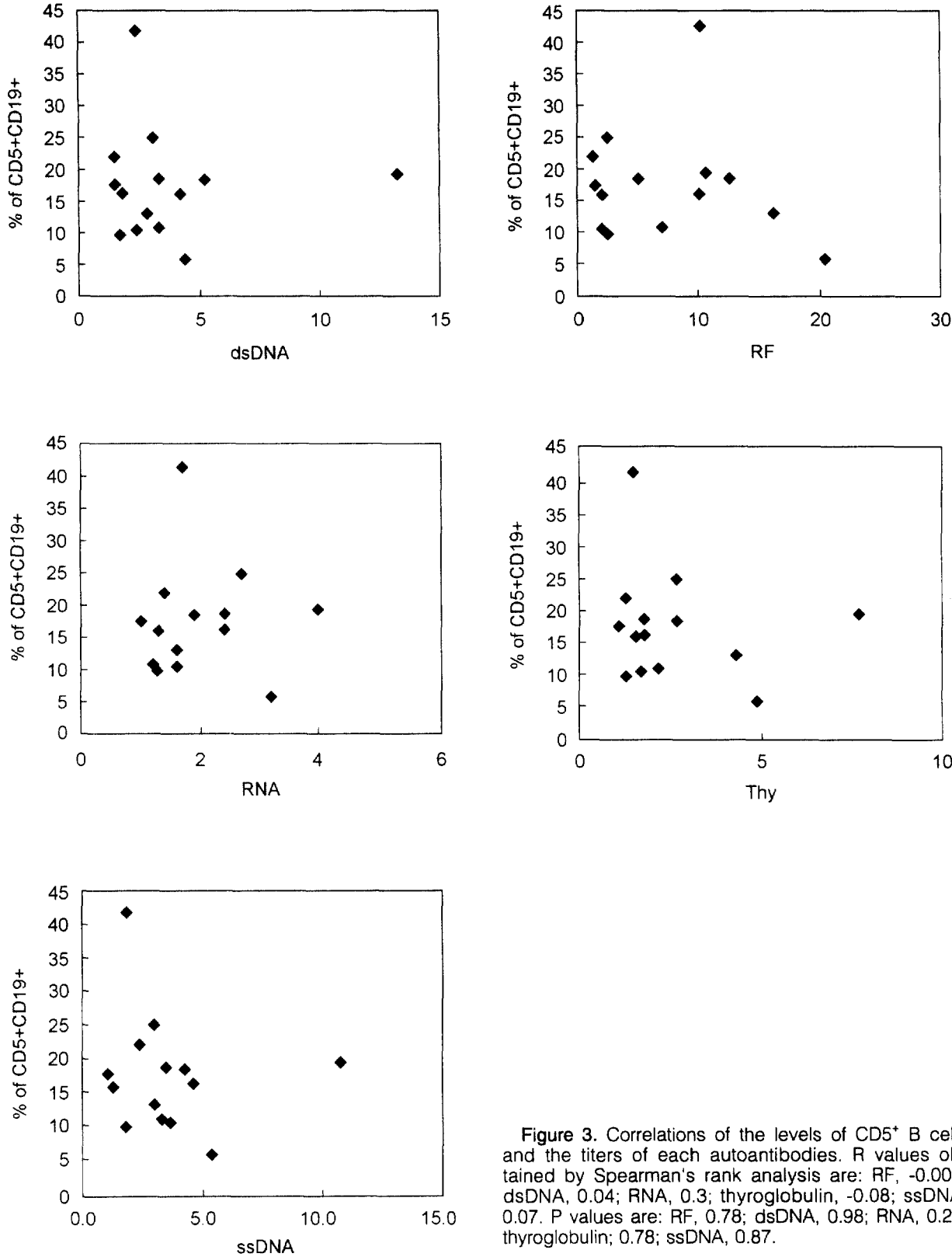

Figure 3. Correlations of the levels of CD5+ B cells and the titers of each autoantibodies. R values obtained by Spearman's rank analysis are: RF, -0.004; dsDNA, 0.04; RNA, 0.3; thyroglobulin, -0.08; ssDNA, 0.07. P values are: RF, 0.78; dsDNA, 0.98; RNA, 0.25; thyroglobulin; 0.78; ssDNA, 0.87.

and the titers of the autoantibodies were analyzed by Spearman's rank analysis (Figure 3). A close association of them with the measures of any autoantibody was not observed, although a relatively better correlation was shown with the levels of anti-RNA autoantibody than with others.

The levels of total IgM in the sera of the patients and normal individuals were compared by ELISA (Figure 4).

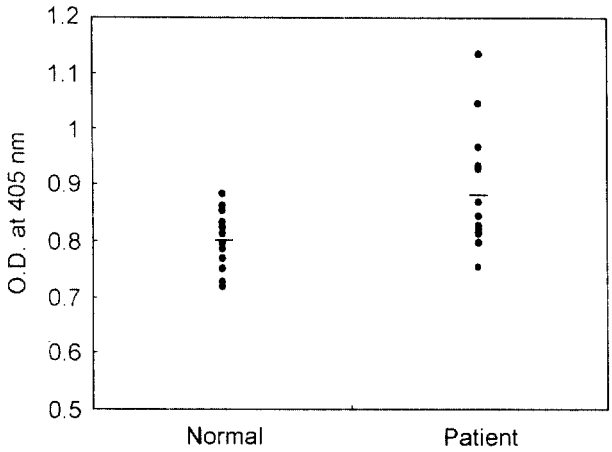

Figure 4. Levels of total IgM in the sera of normal individuals and patients. Results from ELISA are given as O.D. values at 405 nm. Each points in the graphs are averages from four repeat wells. Data are representative of three experiments. Bars indicate the mean values.

Although difference was not remarkable, the IgM levels of the patients tended to be slightly higher than those of the normals. IgM levels which was marked as O.D. at 405 nm was 0.9 ± 0.10 in patients and they were 0.8 ± 0.05 in normals. Difference between the two groups were in a range of significance ($p=0.045$) in student's *t-test*. In addition to the results of the increased titers of autoantibodies in the patients' sera, these results also suggest a role of polyclonal B-cell activation in the pathogenesis of SLE.

DISCUSSION

We show in this paper that both $CD5^+$ B cells and autoantibodies are present in higher levels in the peripheral bloods of the Korean SLE patients than in those of normal individuals although levels of circulating $CD5^+$ B cells are not correlated with titers of autoantibodies. There have been several other reports on elevated $CD5^+$ B cell levels in patients with SLE[27~30], although a few studies with Caucasian patients with SLE showed contradictory results, including the data reporting that $CD5^+$ B cells in patients with SLE were not expanded[19,26]. We suggest that an association of the high levels of $CD5^+$ B cells and autoantibodies with SLE disease might be related to underlying autoimmune process or an indicator of activation of immune system.

The levels of circulating $CD5^+$ B cells were not correlated with titers of any autoantibodies against dsDNA, ssDNA, RNA, thyroglobulin or IgG. Our data argue against several previous reports, which suggested $CD5^+$ B cells might be responsible for producing autoantibodies in humans[17,19] and mouse models[6,10,11]. Our results emphasize the significance of conventional $CD5^-$ B cells in the production of autoantibodies which were suggested by other groups[12,19,25].

$CD5^+$ B cells have been found in high numbers in fetal spleens, patients of chronic lymphocytic leukemia, recipients of bone marrow allografts, and patients with autoimmune diseases, such as RA or SS[14,20,21]. Although $CD5^+$ B cells have been implicated in autoimmunity in several studies in mice and humans, the relationship between the $CD5^+$ B cells and autoantibody production and the involvement of the B cell subsets in the pathogenesis of SLE have not been defined clearly yet. Previous observations that high affinity, monoreactive, IgG autoantibodies are produced by the conventional $CD5^-$ B cell subset[12,19,25] are well supported by our results that levels of circulating $CD5^+$ B cells are not correlated with levels of any autoantibodies in Korean patients with SLE. Our results indicate autoantibody production is not critically dependent on the expansion of the $CD5^+$ B cell subset as suggested previously[33]. We suggest that polyclonal B cell activation might be responsible for the increased production of autoantibodies and abnormal expansion of $CD5^+$ B cells observed in our study. A generalized polyclonal B cell activation of many different B cells, instead of expanding a specific B cell clones, might contribute to the pathogenesis of human SLE.

Various aspects about the levels of $CD5^+$ B cells in autoimmune diseases have been reported in different ethnic groups with RA and SLE. From the family and twin studies it has been shown that the levels of circulating $CD5^+$ B cells appeared to be under a genetic influence [31,32]. Japanese patients with RA have showed higher frequencies of circulating $CD5^+$ B cells than Caucasian RA patients[23]. Furthermore, Liu et al.[22] showed that there was no correlation between levels of $CD5^+$ B cells and values of RF in Chinese patients with RA. In Caucasian SLE patients, the levels of circulating $CD5^+$ B cells were not higher than those of normal individuals[19]. Our results obtained from Korean patients with SLE, however, show the higher levels of the circulating $CD5^+$ B cells in comparison to normals. The higher levels of circulating $CD5^+$ B cells in addition to those of autoantibodies may predispose to an increased risk of developing SLE in Koreans. The results might suggest the possibility of the influence of genetic background on the tendency of increased $CD5^+$ B cells in peripheral bloods in SLE patients. Whether such a different trend could also be identified among other ethnic backgrounds awaits a further investigation.

Acknowledgements

This work was supported by a grant GR96-225 from the Ministry of Education, Republic of Korea

REFERENCES

1) Hardin JA. The lupus autoantigens and the pathogenesis of systemic lupus erythematosus. Arthritis. Rheum. 1986: 24: 457-460
2) Santulli-Marottos S, Retter MW, Gee R, Mamula MI, and Clarke SH. Autoreactive B cell regulation: Peripheral induction of developmental arrest by lupus-associated autoantigens. Immunity. 1998: 8: 209-219
3) Klinman DM, and Steingerg AD. Systemic autoimmune disease arises from polyclonal B cell activation. J. Exp. Med. 1987: 165: 1755-1760
4) Mason LJ, and Isenberg DA. Immunopathogenesis of SLE. Bailliseres. Clin. Rheumatol. 1998: 12(3): 385-403
5) Caligaris-Cappio F, Gobbi M, Bofill M, and Janossy G. Infrequent normal B lymphocytes express features of B-chronic lymphocytic leukemia. J. Exp. Med. 1982: 155: 623-628
6) Hayakawa K, Hardy RR, Honda M, Steinberg AS, and Herzenberg LA. Ly1 B cells; functionally distinct lymphocytes that secrete IgM antibodies. Proc. Natl. Acad. Sci. USA. 1984: 81: 2492-2498
7) Kantor AB. The development and repertoire of B-1 cells (CD5 B cells). Immunol. Today. 1991: 12(11): 389-391
8) Theofilopoulos AN, Kofler R, Singer PA, and Dixon FJ. Molecular genetics of murine lupus models. Adv. Immunol. 1989: 46: 61-109
9) Herzenberg LA, Stall AM, Sidman C, Moore WA, Parks DR, and Herzenberg LA. The Ly-1 B cell lineage. Immunol. Rev. 1986: 93: 81-102
10) Ye YL, Chuang YH, and Chiang BL. In Vitro and in vivo functional analysis of CD5+ and CD5- B cells of autoimmune NZB X NZW F1 mice. Clin. Exp. Immunol. 1996: 106: 253-258
11) Ye YL, Suen JL, Chen YY, and Chiang BL. Phenotypic and functional analysis of activated B cells of autoimmune NZBX NZW F1 mice. Scand. J. Immunol. 1998: 47: 122-126
12) Reap EA, Sobel ES, Jennette JC, Cohen PL, and Eisenberg RA. Conventional B cells, not B1 cells, are the source of autoantibodies in chronic graft-versus-host disease. J. Immunol. 1993: 151: 7316-7323
13) Jang YJ, Youn JK, Park JS, and Kim YT. Studies on Ly-1+ B cells in autoimmune-prone strain of MRL/lpr mice. Ajou. Med. J. 1997: 2: 40-45
14) Youinou P, Mackenzie L, Jouquan J, Le Goff P, and Lydyard PM. CD5 positive B cells in patients with rheumatoid arthritis: phorbol ester mediated enhancement of detection. Ann. Rheum. Dis. 1987: 46: 17-22
15) Kipps TJ. The CD5 B cell. Adv. Immunol. 1989: 47: 117-185
16) Lydyard PM, Lamour A, MacKenzie LE, Jamin C, Mageed RA, Youinou P. CD5+ B cells and the immune system. Immunol. Lett. 1993: 38: 159-166
17) Hardy RR, Hayakawa K, Shimizu M, Yamasaki K, and Kishimoto T. Rheumatoid factor secretion from human Leu-1+ B cells. Science. 1987: 236(4797): 81-83
18) Casali P, Burastero SE, Nakamura M, Inghirami G, and Notkins AL. Human lymphocytes making rheumatoid factor and antibody to ssDNA belong to Leu-1+ B-cell subset. Science. 1987: 236: 77-81
19) Casali P, Burastero SE, Balow JE, and Notkins AL. High affinity antibodies to ssDNA are produced by CD5- B cells in systemic lupus erythematosus patients. J. Immunol. 1989: 143: 3476-3483
20) Maini RN, and Zyberk CP. The significance of CD5+ B cells in rheumatic diseases. Scand. J. Rheumatol. Suppl. 1988: 76: 237-242
21) Pers J, Jamin C, Predine-Hug F, Lydyard P, and Youinou P. The role of CD5-expressing B cells in health and disease. Int. J. Mol. Med. 1999: 3: 239-245
22) Liu ST, Wang CR, Liu MF, Li JS, Lei HY, and Chuang CY. The study of circulating CD5 positive B lymphocytes in Chinese patients with rheumatoid arthritis. Clin. rheumatol. 1996: 15: 250-253
23) Hardy RR, and Hayakawa K. Development and physiology of Ly-1 B and its human homolog, Leu-1B. Immunol. Rev. 1986: 93: 53
24) Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, and Winchester RJ. The 1982 received criteria for the classification of systemic lupus erythematosus. Arthritis. Rheum. 1982: 25: 1271-1277
25) Casali P. Polyclonal B cell activation and antigen-driven antibody response as mechanisms of autoantibody production in SLE. Autoimmunity. 1990: 5: 147-150
26) Plater-Zyberk C, Maini RN, Lam K, Kennedy TD, Janossy G. A rheumatoid arthritis B cell subset expresses a phenotype similar to that in chronic leukemia. Arth. Rheu. 1985: 28: 971
27) Becker H, Weber C, Storch S, and Federin K. Relationship between CD5+ B lymphocytes and the activity of systemic autoimmunity. Clin. Immunol. Immu-

nopathol. 1990: 56: 219-225
28) Kazbay K, and Osterland C. The frequency of Leu-1+ B cells in autoantibody positive and negative autoimmune diseases and in neonatal cord blood. Clin. Exp. Rheumatol. 1990: 8: 231-235
29) Smith HR, and Olson RR. CD5+ B lymphocytes in systemic lupus erythematosus and rheumatoid arthritis. J. Rheumatol. 1990: 17: 833-835
30) Markeljevic J, Batinic D, Uzarevic B, Bosikov J, Cikes N, Babic-naglic D, Horvat Z, and Marusic M. Peripheral blood CD5+ B cell subset in the remission phase of systemic connective tissue disease. J. Rheumatol. 1994: 21: 2225-2230
31) Kipps TJ, and Vaughan JH. Genetic influence on the levels of circulating CD5 B lymphocytes. J. Immunol. 1987: 149: 1060-1064
32) Youinou P, Mackenzie L, Katsikis P, Merdrignac G, Isenberg DA, Tuaillon N, Lamour A, Le Goff P, Jouquan J, and Drogou A. The relationship between CD5-expressing B lymphocytes and serologic abnormalities in rheumatiod arthritis patients and their relatives. Arthritis. Rheum. 1990: 33: 339-348
33) Via CS, and Hand werger BS. B-cell and T-cell function in systemic lupus erythematosus. Curr. Opin. Rheumatol. 1993: 5: 570-574