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ORIGINAL ARTICLE

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CD19/22 balance relates to improvement of disease activity in systemic lupus erythematosus

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Abstract B cells in patients with systemic lupus erythematosus (SLE) are hyperactivated and B-cell receptor signal transduction may be affected by various response regulators. CD19 and CD22 play a major role as regulators of B-cell response. Therefore, we examined CD19 and CD22 expressions on B cells of patients with SLE, and how they were related to disease activity. Thirty-one patients with active SLE were selected and enrolled in this study. Evaluation of CD19 and CD22 expressions on B cells was performed prior to and after treatments with flow cytometry analysis. Disease activity was determined according to the SLE disease activity index score. CD19 and CD22 expressions on B cells in SLE patients revealed no significant differences when compared with the controls. However, improvement of SLE was recognized among patients with an increased ratio of CD22-positive cells. Our results suggest that this balance is a useful marker for determining improvement of SLE disease activity, although the CD19/22 balance does not contribute to the pathogenesis of SLE.

Key words B cell · CD19 · CD22 · Systemic lupus erythematosus (SLE)

Introduction

B cells in patients with systemic lupus erythematosus (SLE) are hyperactivated, and induce various autoantibodies. The causation of B-cell activation is mainly due to T-cell–B-cell interaction, accompanied with B-cell antigen receptor (BCR) ligation or costimulation, i.e., the interaction between CD154 and CD40,[1] CD28 and CD80/86,[2] or other molecules such as CD27/70 and CD134/CD134 ligand.[3]

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B-cell antigen receptor signal transduction is involved in the activation of B cells. CD19 and CD22 are cell-surface glycoproteins of the Ig superfamily. They are expressed on B cells and function as response regulators.[4,5] CD19 amplifies the BCR signal transduction through the interaction with Src-family protein tyrosine kinase (PTK), and downstream molecules.[6–9] In contrast to CD19, CD22 is involved in a negative regulatory effect on BCR signal transduction through SHP-1 recruitment.[5,10]

Previous studies have suggested the relationship between CD19/22 and autoimmune diseases such as SLE.[11–13] Therefore, we hypothesized that the imbalance of CD19/22 expression on B cells contributes to the pathogenesis of SLE. In this study, we determined and evaluated the ratio of CD19/22 expressions on B cells in SLE patients.

Patients and methods

We examined 31 patients with SLE who met the ACR criteria. The subjects consisted of 30 female patients and 1 male patient, aged 15 to 68 years with a mean age of 35 years, and duration of disease ranging from less than 1 year to 22 years with a mean duration of 5.9 years. Their clinical features are summarized in Table 1. Blood samples were drawn during both the active and improved states of the disease. Their disease activities were determined according to the SLE disease activity index (SLEDAI) score.

Peripheral blood mononucleocytes (PBMCs) were prepared from the collected blood samples, and stained by fluorescein isothiocyanate-labeled anti-CD19 or anti-CD22 monoclonal antibody, and PE-labeled anti-CD20 monoclonal antibody (Becton Dickinson, Franklin Lakes, NJ, USA) as the mature B-cell marker. Then flow cytometry analysis was performed with a Vantage FACS (fluorescence-activated cell sorting) station (Becton Dickinson).

The expression of CD19 and CD22 is indicated by the ratio of CD19 to CD20, CD22 to CD20, and CD19 to CD22. The changes in the CD19/CD22 ratio were then calculated prior to and after treatment and the patients were divided

Table 1. Patients' profiles

		Main clinical feature		Complication	
SLE	$n = 31$	Nephritis	19	Sjs	4
		CNS lupus	3	APS	3
		Serositis	3	IP	2
		Arthritis	3	HPS	1
		Thrombocytopenia	2	ITP	1
		Pancytopenia	1	PRCA	1
				Other	1
		Total	31	Total	13
NC	$n = 10$				

Thirty-one patients with SLE were examined, 13 of whom also had various complications
SLE, systemic lupus erythematosus; NC, normal controls; CNS, central nervous system; Sjs, Sjögren syndrome; APS, anti-phospholipid syndrome; IP, interstitial pneumonitis; HPS, hemophagocytic syndrome; ITP, idiopathic thrombocytic purpura; PRCA, pure red cell anemia

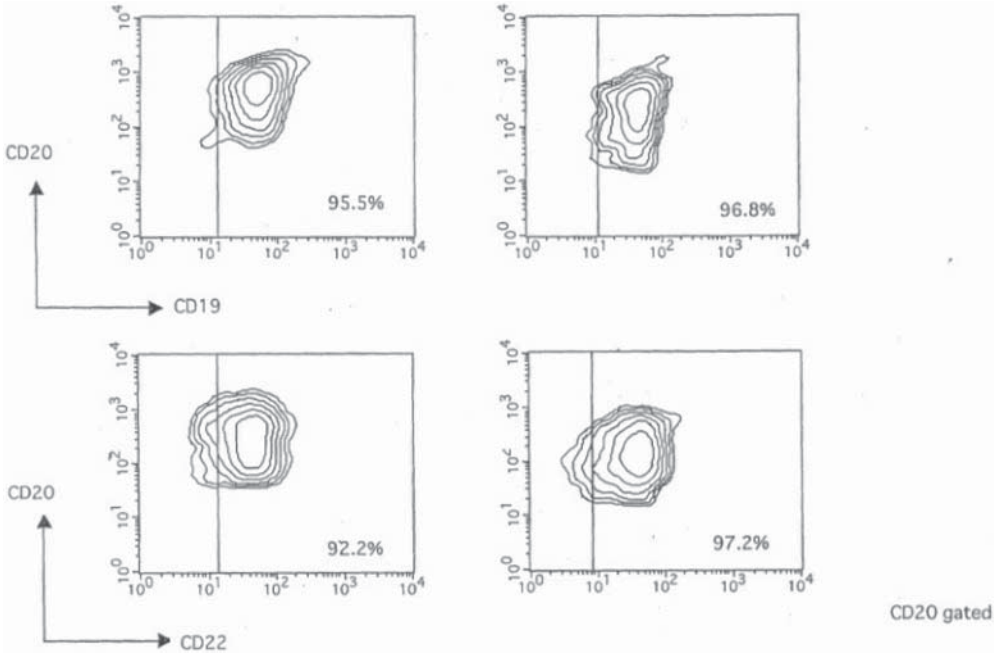


Fig. 1. Representative fluorescence-activated cell sorting pattern of CD22 and CD19 expression on mature B cells in peripheral blood of a systemic lupus erythematosus (SLE) patient

into three groups, A, B, and C, according to the change in the CD19/22 ratio. The three groups were categorized according to standard error, which was revealed to be 3.2%. To further ensure accurate measurements, the standard error was doubled. In brief, group A consisted of patients with greater than 6.4% increase in the CD19/22 ratio (CD19 relatively increased), group C of patients with more than 6.4% decrease in CD19/22 ratio (CD22 relatively increased), and group B of those who did not belong to groups A or C.

Results

First, we examined the expression of CD19 and CD22 on CD20-positive mature B cells. The representative FACS pattern is shown in Fig. 1. As for the results, neither CD19 nor CD22 expressions on mature B cells reveal any significant differences between SLE patients and control subjects. Thus, the findings suggest there were no essential abnormalities in CD19 and CD22 expression on B cells in SLE patients, as shown in Figs. 2 and 3.

Next, we evaluated the ratio of CD19 and CD22 expression on mature B cells. No clinical feature was correlating with the CD19/CD22 ratio; autoantibody production was also not correlating (data not shown). Finally, we evaluated the change of this ratio according to treatments. The patients were divided into three groups according to the change in variation as described above. Each case is shown in Fig. 4. Then improvement in disease activity was evaluated for each group. When the statistical significance in SLEDAI score decrease was evaluated for each group, patients in group C revealed a significant decrease in SLEDAI

score compared to patients in group A ($P = 0.017$), as shown in Fig. 5. Thus, patients with a decreased CD19/22 ratio exhibited decreases in disease activity, and good response to treatment. We also evaluated the difference in the change of the CD19/22 ratio in relation to treatment methods, but there was no significant difference demonstrated (data not shown).

Discussion

The expression of CD19 and CD22 on the B-cell surface plays major roles as regulatory molecules in their activation. B cells in CD19-deficient mice are hyporesponsive to most transmembrane signals and have been shown to be significantly deficient in proliferative activity, clonal expansion, and differentiation, while B cells in CD19 transgenic mice are hypersensitive to transmembrane signals and revealed increased proliferative activity or Ig production.[12,14,15] Previous studies have shown that mice with altered expression of these molecules induce autoantibody production and develop autoimmunity[15,16] On the other hand, B cells in CD22-deficient mice revealed reduction in number, shorter lifespan with enhanced apoptosis, and decreased surface IgM expression with increased major histocompatibility complex class II antigen expression, which resembles those of B cells overexpressing CD19.[17] Thus, the balance of CD19 and CD22 is important in the regulation of B-cell activation.[18,19]

In SLE with abnormal B-cell activation, it is postulated that the ratio of CD19/22 is increased. However, in this study there was no significant difference between patients and the controls. Therefore, we postulate that the imbalance in the CD19/22 ratio is not the direct cause of SLE. The balance in the ratio of CD19/22 is rather related to the

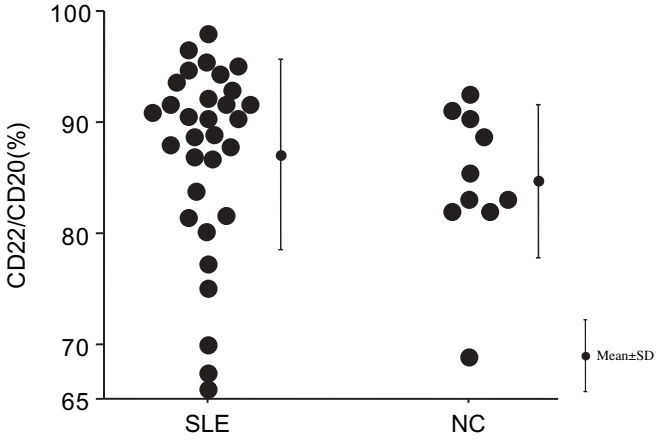


Fig. 2. CD22 expression on CD20-positive cells. The expression of CD22 on the surface of CD20-positive cells showed no significant difference between SLE patients and healthy donors

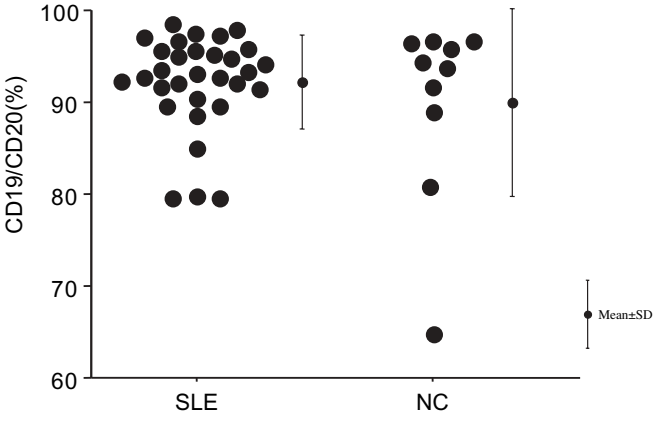
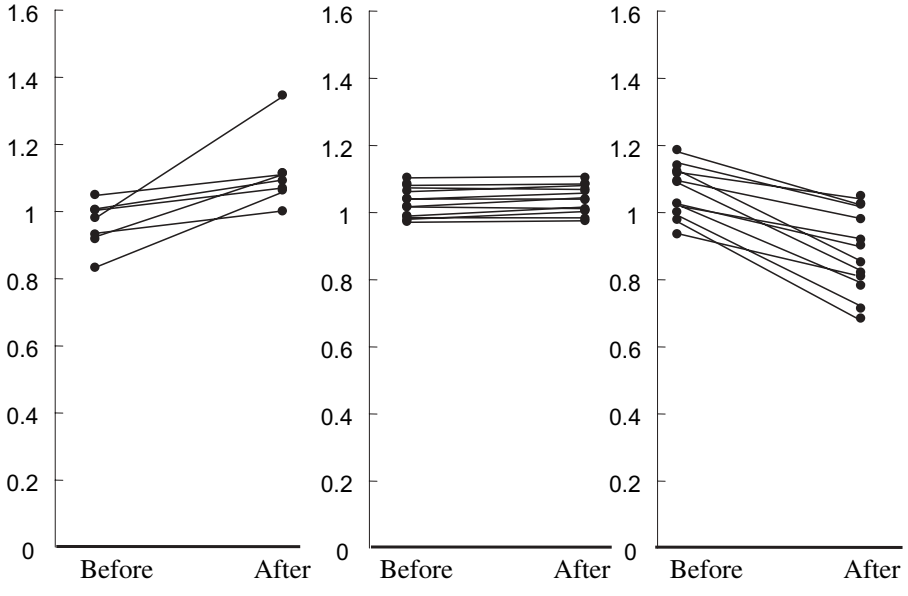


Fig. 3. CD19 expression on CD20-positive cells. The expression of CD19 on the surface of CD20-positive cells showed no significant difference between SLE patients and healthy donors

Fig. 4. Categorization of patients. Patients were categorized according to the change in ratio of CD19/CD22. Group A consisted of patients with greater than 6.4% for increased CD19/22 (i.e., CD19 relatively increased), group C of those with more than 6.4% of decreased CD19/22 ratio (CD22 relatively increased), while group B consisted of patients who did not belong to groups A or C



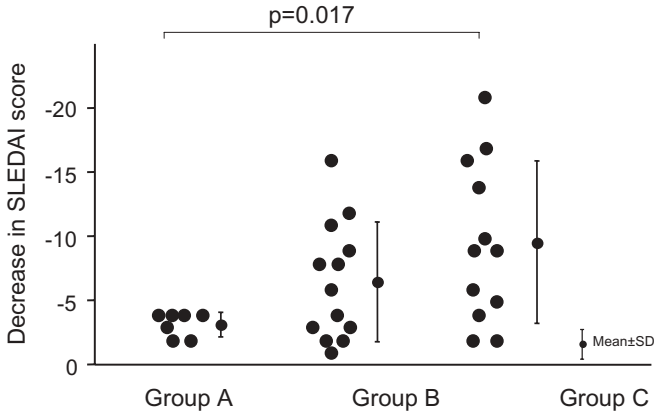


Fig. 5. Decrease in SLE disease activity index (*SLEDAI*) score. Improvement of disease activity was measured by the decrease of SLEDAI score. Patients belonging to group C revealed significant decrease of SLEDAI score compared with patients belonging to group A. Thus, patients with a relative increase of CD22 expression on B cells revealed good response to treatment

disease activity of SLE. In brief, patients with decreased CD19/22 ratio exhibited an improvement of disease activity. This result suggests that a relative increase of CD22 expression is important for the improvement of disease activity, regardless of the type of therapy administered. We had also postulated that intravenous cyclophosphamide pulse therapy would affect the CD19/22 ratio more than steroids; it was surprising that no difference was shown. But more surprisingly, the CD19/22 decrease still correlates with a lowering of disease activity. Therefore, the CD19/22 balance may be an important marker for determining overall improvement in SLE.

Recently, CD20 depletion therapy has been reported to be effective for SLE that is resistant to conventional treatments. A previous study shows that through B-cell depletion therapy, significant B-cell depletion in peripheral blood was observed, which correlated with clinical improvement, but no changes in anti-dsDNA/C3 were claimed.[20] This is supportive evidence demonstrating that lowering B-cell activity is important but does not always correlate with clinical features, autoantibody production, or other data. In fact, CD20 depletion therapy is also effective for autoantibody titer-independent symptoms like central nervous system lupus.

Previous studies have stressed that molecules are involved in the activation of B cells, for example CD80 and CD86.[2] Their presence or absence in the abnormality of B-cell regulation in SLE patients is also important. Our previous study revealed that interleukin-16, as a regulatory cytokine, increased during the active phase of SLE.[21] The findings of these studies, including the present one, suggest that these regulatory mechanisms are involved in the improvement of disease activity, and may give rise to answers to questions about the relationship between regulatory mechanisms and diseases.

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