Toll-like Receptor Expression in Lupus Peripheral Blood Mononuclear Cells

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ABSTRACT. ***Objective.*** To investigate expression of members of the Toll-like receptor (TLR) family in peripheral blood mononuclear cells (PBMC) in patients with systemic lupus erythematosus (SLE).

Methods. We analyzed PBMC from 14 patients with SLE and 15 healthy subjects. The surface expressions of TLR2 and TLR4 and intracellular expression of TLR9 on PBMC were analyzed by flow cytometry.

Results. Although TLR4 expressions on CD14+ monocytes were not significantly different between healthy subjects and patients with SLE, TLR2 expressions on monocytes were reduced in patients with SLE compared to healthy subjects. Intracellular TLR9 expression levels of CD19+ B lymphocytes were significantly elevated in patients with SLE. However, the TLR9 expression levels of plasmacytoid dendritic cells were not significantly different between these patients and healthy subjects.

Conclusion. Our results show that human peripheral blood B cells express TLR9 and that its expression is increased in patients with SLE. This upregulated expression of TLR9 in B cells may be related to the abnormal B cell hyperactivity in patients with SLE. (First Release Jan 15 2007; J Rheumatol 2007;34:493–500)

Key Indexing Terms:
B LYMPHOCYTES
INTERFERON-α
SYSTEMIC LUPUS ERYTHEMATOSUS
TOLL-LIKE RECEPTOR

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the generation of autoantibodies against nuclear proteins and DNA[1]. These autoantibodies are thought to contribute to the pathogenesis of SLE, and the levels of anti-DNA antibodies correlate with the disease activity[2,3]. The deposition of antinuclear antibody and antigen immune complexes (IC) in tissue is thought to induce local activation of immune systems[4], which may perpetuate SLE. It has also been proposed that the type 1 interferon (IFN) system has a pivotal etiopathogenic role in SLE, since IFN-α correlates with disease activity of SLE[5,6]. Recent studies showed that the sera of patients with SLE selectively induce the production of IFN-α from the natural IFN-α-producing cells, which are identical to plasmacytoid dendritic cells (PDC)[7]. Further, DNA/anti-DNA antibody complexes in lupus sera can provide signals for IFN-α production through the Toll-like receptor-9 (TLR9), which responds to bacterial DNA sequences containing nonmethylated CpG motifs[8]. In humans, the TLR family consists of 10 members, each of which is involved in the recognition of pathogen-derived materials[9]. Recent data suggest that TLR also play an important role in autoimmunity[10]. It is important to determine whether circulatory mononuclear cells in patients with SLE express TLR that could be involved in PDC activation as well as IFN-α induction. We examined the expression of TLR in lupus circulating mononuclear cells using flow cytometry methods.

MATERIALS AND METHODS

Patients and controls. A total of 14 patients with SLE (12 women and 2 men, aged 36.1 ± 13.3 yrs) were enrolled in our study (Table 1), in addition to 15 healthy volunteers (12 women, 3 men, aged 37.7 ± 8.1 yrs) and 6 patients with rheumatoid arthritis (RA; 5 women, 1 man, aged 48.1 ± 11.4 yrs) as controls. Consecutive patients entering the rheumatology clinic who fulfilled the American College of Rheumatology 1982 revised classification criteria for SLE[11] were selected for this investigation. Among the 14 patients, 8 received prednisolone (PSL) as monotherapy (mean dosage 20.3 mg/day, range 5–60 mg/day). The remaining 5 patients were treated with both PSL (mean dosage 19.4 mg/day, range 7-50 mg/day) and cyclophosphamide (intermittent intravenous cyclophosphamide therapy), and one patient received PSL (5 mg/day) and azathioprine (50 mg/day). Disease activity was scored in all patients with SLE by SLE Disease Activity Index (SLEDAI)[12]. The study protocol was approved by the Ethics Committee of Nagasaki Medical Center.

Reagents for flow cytometry. Antibodies used for phenotype analysis were obtained from Beckman Coulter (Fullerton, CA, USA), and antibodies for TLR analysis were purchased from eBioscience (San Diego, CA, USA). Antibody for BDCA-2 was purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). Antibodies used in this study were as follows: FITC-labeled anti-human CD14, CD19, and BDCA-2; phycoerythrin (PE)-labeled anti-human TLR2, 4, and 9; and PC-5-labeled anti-human CD123.

Flow cytometry. Heparinized blood was collected and peripheral blood mononuclear cells (PBMC) were isolated by Ficoll gradient centrifugation. For staining for TLR9, PBMC were stained for cell surface antigen and fixed

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Table 1. Clinical data of patients with SLE.

Patient No.	Age, yrs	Sex	SLEDAI	Anti-dsDNA, IU/ml	CH50, IU/ml	PSL, mg/day	Cyclophosphamide	Azathioprine
1	49	F	6	141	30	25		
2	27	F	4	26	30	11		
3	29	F	9	17	12	14		
4	19	F	5	5	36	5		+
5	27	F	8	21	29	10		
6	24	F	14	30	15	30		
7	57	F	4	5	38	7	+	
8	45	F	12	33	25	12.5	+	
9	25	F	16	14	26	60		
10	33	F	12	19	23	50	+	
11	38	F	2	17	38	5		
12	55	M	10	32	41	15	+	
13	24	M	18	17	29	15	+	
14	54	F	10	4	37	7.5		

SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; anti-dsDNA: anti-double-stranded DNA antibody (normal 0.0–10.0 IU/ml); PSL: prednisolone.

and permeabilized using a commercial kit (eBioscience). Cells were then stained for intracellular TLR9 with a PE-labeled TLR9 monoclonal antibody (mAb; eBioscience). In brief, cells were stained for their surface markers using fluorescein isothiocyanate (FITC)-conjugated anti-human CD14, CD19, blood dendritic cell antigen (BDCA)-2, or PC-5-conjugated CD123 for 15 min. Cells were then washed with staining buffer, fixed, and permeabilized. After washing with staining buffer, cells were stained with PE-conjugated anti-human TLR9 (eBioscience). Fluorescence was measured on an Epics XL (Beckman-Coulter). The acquired data were analyzed with EXPO32 software (Beckman-Coulter).

Cell culture. PBMC were isolated by Ficoll-Hypaque (Amersham Pharmacia, Tokyo, Japan) density gradient centrifugation. T cells were removed from PBMC by negative selection using anti-CD2 magnetic beads (Dynal, Oslo, Norway). T-cell-depleted mononuclear cells (MNC; 1×10^5 per 200 µl well) were cultured in RPMI-1640 (Gibco, NY, NY, USA) supplemented with 10% fetal bovine serum (Gibco BRL Invitrogen, Tokyo) in 96-well tissue culture plates. CpG oligodeoxynucleotide (ODN) 2006 (5'-TCG TCG TTT TGT CGT TTT GTC GTT-3') complete phosphorothioate (CpG ODN2006; InvivoGen, San Diego, CA, USA) was added at Day 0. T-cell-depleted MNC were also cultured with 96-well plates coated with 1 µg/ml purified goat anti-human IgG, IgA, and IgG (Southern Biotechnology, Birmingham, AL, USA) to trigger the B cell receptor (BCR) as controls. Cell cultures were maintained for 4 days and culture supernatants were collected and stored at –20°C. The amounts of IgG in culture supernatants were determined by antibody sandwich-type ELISA (Bethyl Laboratories, Montgomery, AL, USA).

Statistical analysis. Comparisons between groups were done using the nonparametric Mann-Whitney U-test. All statistics were performed with Stat View 7.0 (SAS Institute, Cary, NC, USA).

RESULTS

TLR2 and TLR4 expressions on CD14+ cells in patients with SLE. We compared the baseline expression of TLR2 and TLR4 on CD14+ monocytes in patients with SLE and healthy subjects. As shown in a representative histogram of monocytes, surface expressions of TLR2 were lower in patients with SLE than in healthy subjects (Figure 1A). The geometric mean fluorescence intensity of TLR2 was significantly lower in patients with SLE than in healthy subjects (Figure 1B). However, there was no statistical difference in TLR4 expression between patients with SLE and healthy subjects (Figures 2A, 2B). Although we also examined the baseline expression of TLR2 and TLR4 on B cells and T cells in patients with

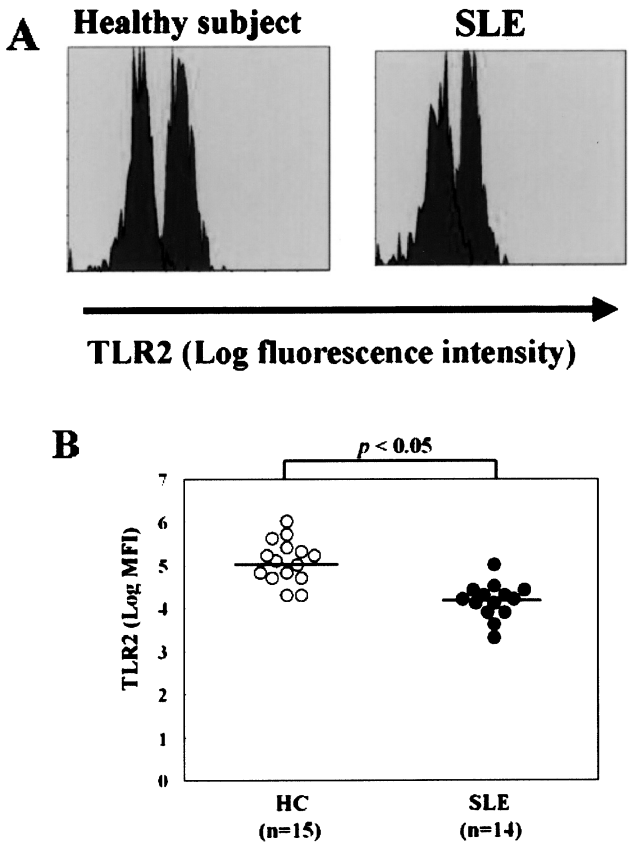


Figure 1. Diminished expression of TLR2 on CD14+ monocytes in patients with SLE. A. Histograms show baseline expression of surface TLR2 on monocytes in a representative patient with SLE and healthy subject. Darker areas indicate staining with isotype control mAb. B. Mean fluorescence intensity of surface TLR2 staining for CD14+ monocytes in patients with SLE (n = 14) and healthy subjects (HC; n = 15). Significance of differences between groups were analyzed by Mann-Whitney U-test.

SLE, we could not detect TLR2 and TLR4 expression on B or T cells in patients with SLE or in healthy subjects (data not shown).

Increased expression of TLR9 on B cells in patients with SLE. We also examined whether lupus B cells express TLR9 in patients with SLE. We identified B cells as CD19+ populations (Figures 3A, 3B). As shown in Figure 3C, CD19+ B cells express TLR9 constitutively. In a representative histogram of B cells, intracellular expression of TLR9 was higher in patients with SLE than in healthy subjects. The geometric mean fluorescence intensity of TLR9 in B cells is shown in Figure 3D. TLR9 expression levels in B cells were significantly increased in patients with SLE compared to healthy subjects. In contrast, these increased expressions of TLR9 in B cells were not observed in the patients with RA, another autoimmune disease (Figures 4A, 4B). We evaluated the relationship between TLR9 expression on B cells and the treatment regimens in patients with SLE. However, there was no significant difference of TLR9 expression in the presence or absence of moderate doses of corticosteroids (PSL > 20 mg/day) or immunosuppressants (Figure 5). Similarly, there was no significant difference of TLR2 expression on CD14+ monocytes in the presence or absence of moderate doses of corticosteroids or immunosuppressants (Figure 6). We also evaluated the correlations of the TLR9 expression levels in B cells with clinical measures such as the SLEDAI, hemolytic complement (CH50), and titers of anti-dsDNA antibodies. However, there was no significant correlation between TLR9 expression levels and these clinical measures.

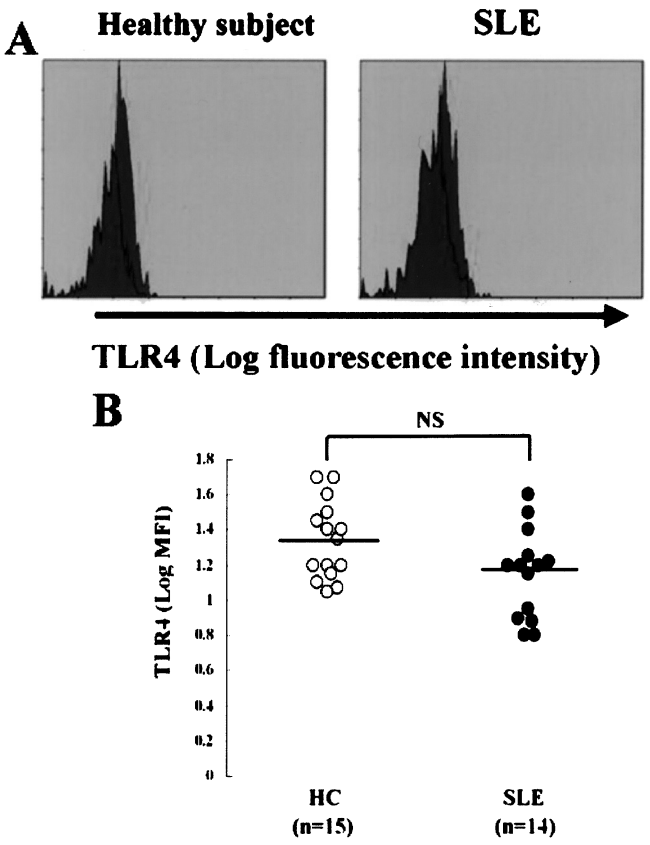


Figure 2. Expression of TLR4 on CD14+ monocytes in patients with SLE. A. Histograms show baseline expression of surface TLR4 on monocytes in a representative patient with SLE and healthy subject. Darker areas indicate staining with isotype control mAb. B. Mean fluorescence intensity of surface TLR2 staining for CD14+ monocytes in patients with SLE (n = 14) and healthy subjects (HC: n = 15). Significance of differences between groups were analyzed by Mann-Whitney U-test.

TLR9 expression in lupus PDC. It was demonstrated that TLR9 mediates the activation of PDC by SLE-IC. To investigate TLR9 expression on PDC, we performed double-staining of PBMC, with a mixture of mAb against CD123 and BDCA-2. PDC, identified as CD123/BDCA-2 double-positive populations[13], were gated (Figure 7) and analyzed for TLR9 expression. In order to make low proportions of peripheral blood PDC visible especially in patients with SLE, at least 100,000 cells were acquired. Figure 6A shows a representative result for the TLR9 expression of gated BDCA-2+, CD123+ cells from healthy subjects and patients with SLE. No significant difference between healthy subjects and patients with SLE was observed with regard to the TLR9 expression on PDC (Figure 8).

IgG production following CpG stimulation in B cells. To determine the ability of CpG ODN to stimulate immunoglobulin production by lupus B cells, T-cell-depleted MNC were cultured in the presence of CpG ODN. Cultured supernatants were harvested on Day 5 of culture and analyzed for the presence of IgG by ELISA. CpG ODN stimulated the production of IgG from control and lupus B cells. The increase in IgG production was 1.6-fold by control B cells, and an equivalent 1.9-fold increase in lupus B cells. This difference was not statistically significant. Under stimulation through BCR, the increase in IgG production was 5.6-fold in controls and 6.4-fold in SLE, and no significant difference was observed. These findings indicate that lupus B cells respond to CpG ODN stimulation; however, the ability to respond to CpG ODN in lupus B cells was not different from that in control B cells (Table 2, Figure 9).

DISCUSSION

Type 1 IFN has been proposed to have a pivotal etiopathogenic role in SLE, since serum levels of IFN-α correlate with the disease activity of SLE[5,6]. IFN-α may contribute to lupus autoimmune processes by differentiating B lymphocytes and following autoantibody production[14]. TLR are a family of pattern-recognition receptors that evolved to detect microbial infection[9]. These receptors recognize conserved molecular products derived from different classes of microorganisms[15]. Although TLR detect molecular patterns of microbial origin, some TLR and their ligands have emerged as important regulators of immunity relevant to effector responses to autoimmunity[16].

Recent data indicate that immune complexes containing

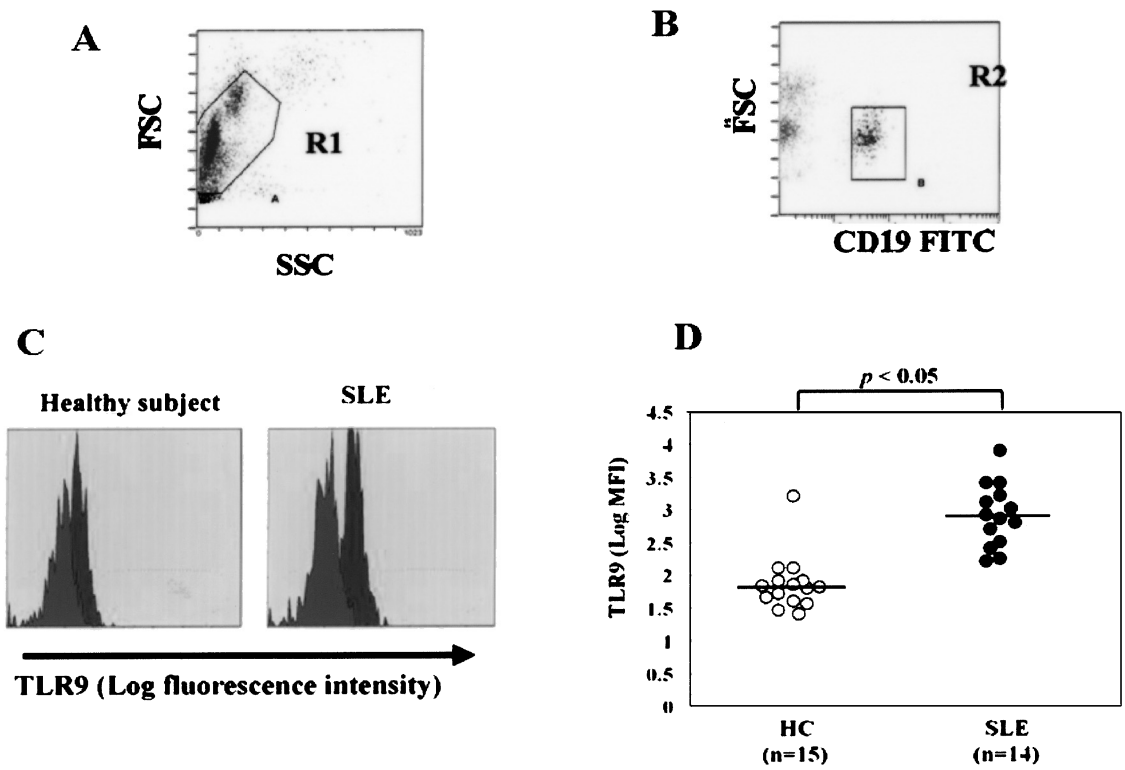
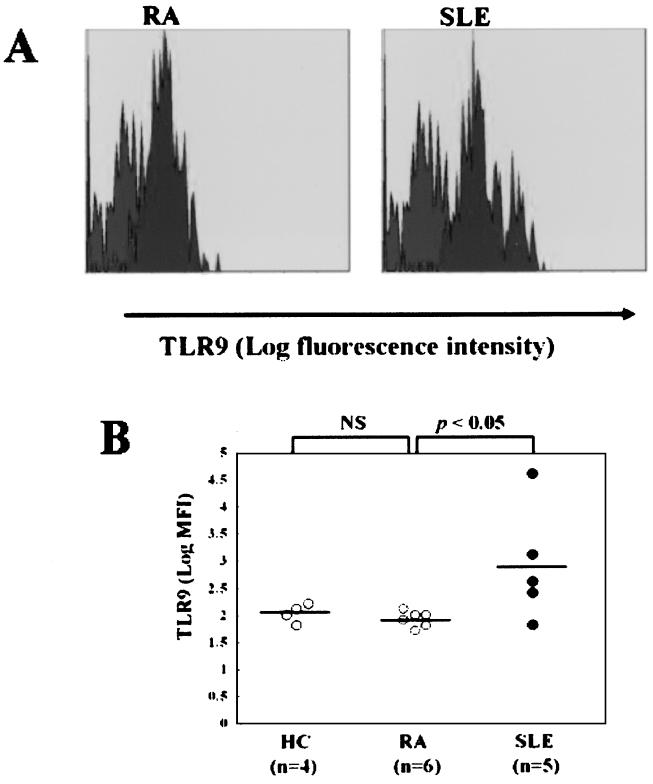


Figure 3. Upregulated expression of TLR9 on CD19+ B cells in patients with SLE. A, B. Isolation of CD19+ B cells by flow cytometry. PBMC were assessed by forward scatter (FS) and side scatter (SS) measures. Cells gated in R1 (A) were further analyzed for anti-CD19 FITC-labeled mAb. CD19+ cells were gated (R2) and defined as B cells (panel B). C. Histograms depicting baseline expression of intracellular TLR9 on B cells in a representative patient with SLE and healthy subject. Darker areas indicate staining with isotype control mAb. D. Mean fluorescence intensity of intracellular TLR9 staining for CD19+ B cells in patients with SLE (n = 14) and healthy subjects (HC; n = 15). Significance of differences between groups were analyzed by Mann-Whitney U-test.



nucleic acid and lupus IgG can induce IFN-α secretion from PDC[8]. Further, Means, *et al* showed that SLE-IC stimulates PDC to produce IFN-α in a TLR9-dependent manner[17]. The ability of PDC to secrete IFN-α depends on cellular sensors that detect the presence of bacterial DNA or IC[18], and TLR9 expression may account for the IFN-α production from PDC. We investigated the expression of TLR9 on circulating PDC in patients with SLE. Our data show that the expression of TLR9 in PDC was not different between patients with SLE and healthy subjects. Previous investigations showed that the numbers of circulating PDC are decreased in patients with SLE[19], but large numbers of activated PDC infiltrate skin lesions and actively produce IFN-α[20]. These findings suggest that PDC seem to be activated by IC in the pathogenic lesions and circulating PDC may not reflect these immune conditions. Further investigations concerning the TLR9 expression in tissue-infiltrating PDC are needed in patients with SLE.

Figure 4. Expression of TLR9 on CD19+ B cells in patients with RA and SLE. A. Histograms show baseline expression of intracellular TLR9 on B cells in representative patients. Darker areas indicate staining with isotype control mAb. B. Mean fluorescence intensity of intracellular TLR9 staining for CD19+ B cells in healthy subjects (HC, n = 4), patients with RA (n = 6), and with SLE (n = 5). Significance of differences between groups were analyzed by Mann-Whitney U-test.

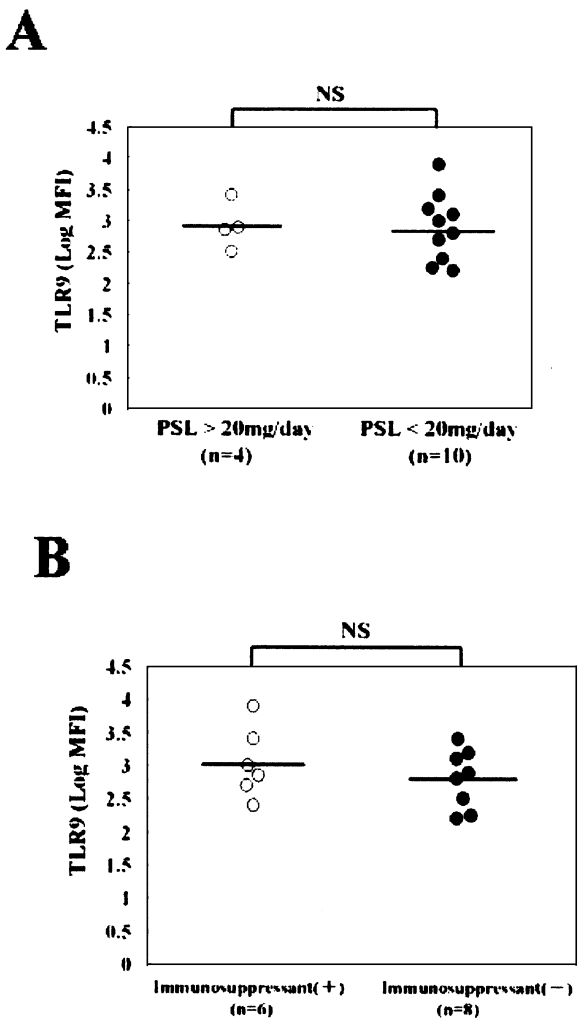


Figure 5. Expression of TLR9 on CD19+ B cells in patients with SLE. A. Mean fluorescence intensity (MFI) of intracellular TLR9 staining for CD19+ B cells in patients with SLE treated with > 20 mg/day prednisolone (PSL; n = 4) or < 20 mg/day PSL (n = 10). Significance of differences between groups were analyzed by Mann-Whitney U-test. B. MFI of intracellular TLR9 staining for CD19+ B cells in patients with SLE treated with (n = 6) or without immunosuppressants (n = 8). Significance of differences between groups were analyzed by Mann-Whitney U-test.

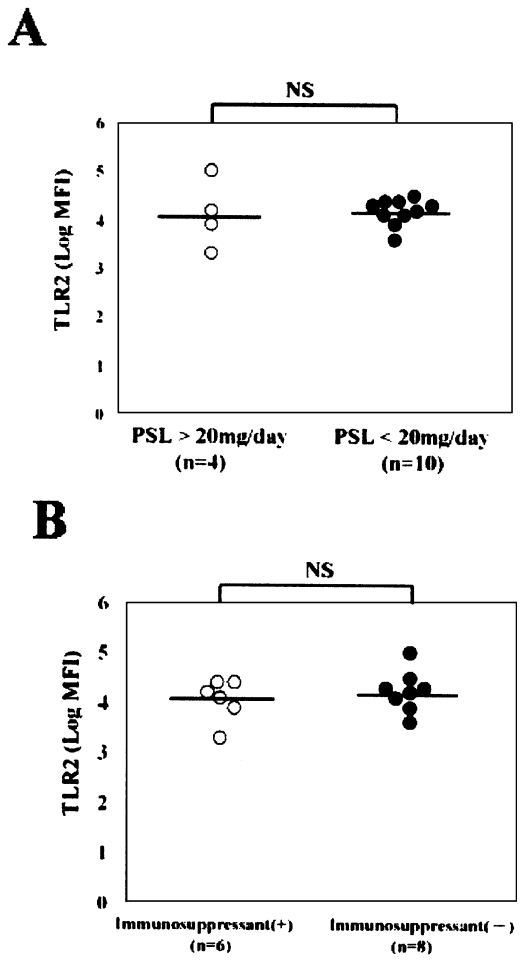


Figure 6. Expression of TLR2 on CD14+ monocytes in patients with SLE. A. Mean fluorescence intensity (MFI) of intracellular TLR2 staining for CD14+ B monocytes in patients with SLE treated with > 20 mg/day prednisolone (PSL; n = 4) or < 20 mg/day PSL (n = 10). Significance levels for differences between groups were analyzed by Mann-Whitney U-test. B. MFI of intracellular TLR2 staining for CD14+ monocytes in patients with SLE treated with (n = 6) or without immunosuppressants (n = 8). Significance of differences between groups were analyzed by Mann-Whitney U-test.

The high serum levels of IFN-α in patients with SLE were found to promote the differentiation of B cells as well as to activate PDC[21]. Similar to PDC, B cells express a limited set of TLR, including TLR7 and TLR9[22]. Leadbetter, *et al* showed that autoreactive B cells are activated by IgG-chromatin IC and require the synergistic engagement of B cell receptor and TLR9[23]. It is postulated that engagement of the BCR by an autoantibody-antoantigen IC triggers the endocytosis of IC that then results in the efficient delivery of chromatin fragments to endosome-associated TLR9[23]. We consistently observed the expression of TLR9 in freshly isolated human peripheral blood B cells, and our first major observation was the upregulated expression of TLR9 in peripheral blood B cells from patients with SLE. More recently, it was postulated that the engagement of TLR receptors, particularly TLR9, on B cells seems to play an important role in B cell activation and autoantibody production[24]. The importance of the TLR9-dependent pathways will depend on the levels of TLR9 expression. The differential expression of TLR9 may correlate with the responsiveness to CpG DNA, and the altered TLR9 expression could potentially affect the B cell immune response to chromatic or IC in patients with SLE.

CpG ODN have been shown to activate B cells via the family of TLR9[25]. Our data indicated that both control and lupus B cells responded to CpG DNA stimulation. The ability of lupus B cells to produce IgG was higher compared to control B cells; however, the difference was not significant. Since our B cell preparations contain mononuclear cells (T-cell-depleted mononuclear cells), the abnormal response of non-B cell pop-

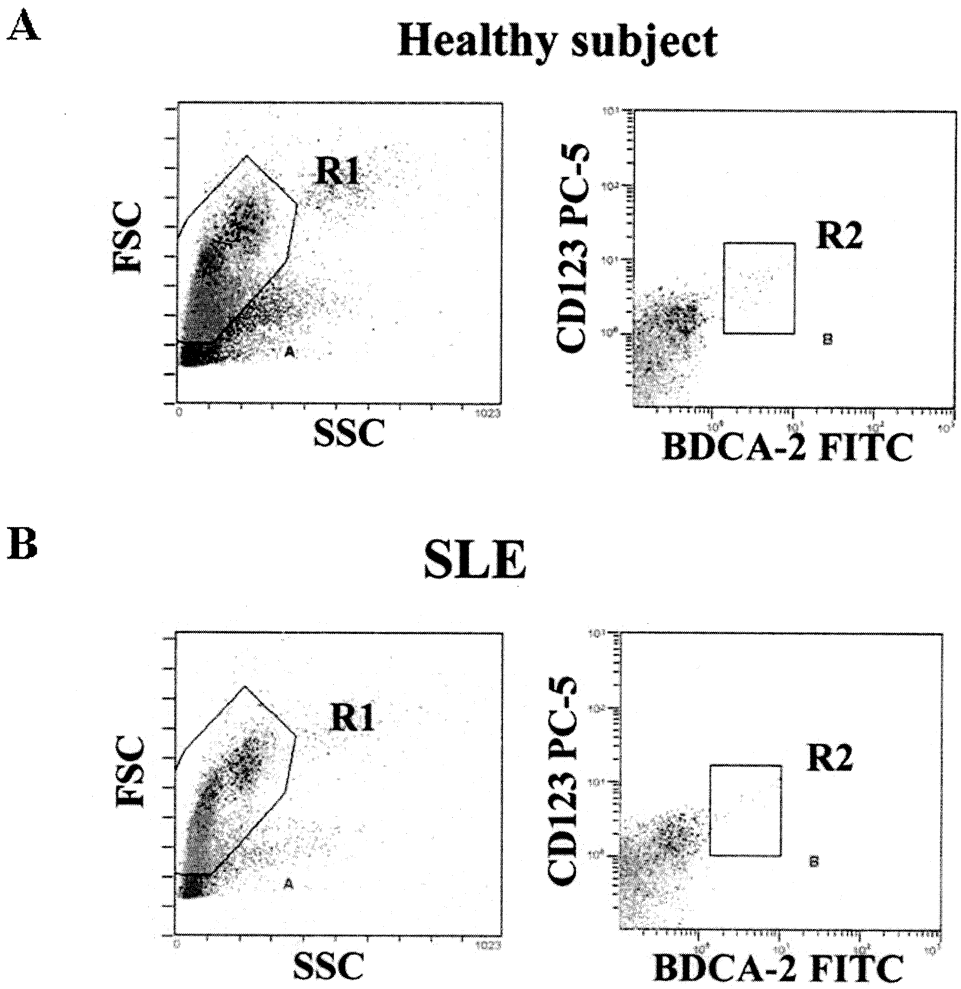


Figure 7. Identification of circulating plasmacytoid dendritic cells (PDC) by flow cytometry in peripheral blood from a representative patient with SLE and healthy subject. Mononuclear cells isolated from a healthy subject (A) and patient with SLE (B) were assessed by forward scatter (FS) and side scatter (SS). The R1-gated events were then analyzed for BDCA-2 and CD123, and BDCA-2+, CD-123+ double-positive cells (R2) were defined as PDC.

ulations to CpG DNA contributed to this phenomenon as described previously[26].

The exact immune mechanisms underlying the upregulation of TLR9 on lupus B cells remain to be elucidated in our study. Bernasconi, *et al* indicated that in human naive B cells, TLR9 is expressed at low to undetectable levels, but its expression is rapidly upregulated by BCR triggering[27]. In contrast, memory B cells expressed TLR9 at constrictively high levels[28]. SLE is characterized by polyclonal B cell activation[29], and these alternations of B cell activation or differentiation status may account for the upregulation of TLR9 expression. We could not show the interaction between increased TLR9 expression on B cells and lupus disease activity. Our study included 14 patients with SLE with low to moderate disease activity; therefore, further large-scale investigations of patients with SLE are needed to elucidate the relationship between TLR9 expression on B cells and lupus disease activity.

In summary, we showed that TLR9 expression in B lymphocytes was increased in patients with SLE. This upregulated TLR9 expression may activate B lymphocytes through the interaction between TLR9 and its ligands and may be related to the pathogenesis of lupus.

REFERENCES

1. Ruiz-Irastorza G, Khamashta MA, Castellino G, Hughes GR. Systemic lupus erythematosus. Lancet 2001;357:1027-32.
2. Hahn BH. Antibodies to DNA. N Engl J Med 1998;338:1359-68.
3. ter Borg EJ, Horst G, Hummel EJ, Limburg PC, Kallenberg CG. Measurement of increases in anti-double-stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus erythematosus. A long-term, prospective study. Arthritis Rheum 1990;33:634-43.
4. Ebling FM, Hahn BH. Pathogenic subsets of antibodies to DNA. Int Rev Immunol 1989;5:79-95.
5. Ronnblom L, Alm GV. An etiopathogenic role for the type I IFN system in SLE. Trends Immunol 2001;22:427-31.

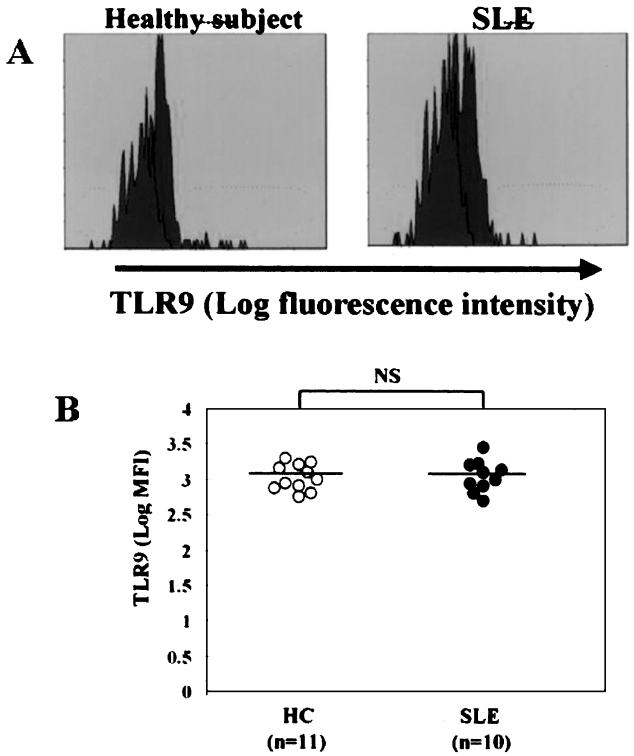


Figure 8. Expression of TLR9 on plasmacytoid dendritic cells (PDC) in patients with SLE. A. Histograms show baseline expression of intracellular TLR9 on PDC in a representative patient with SLE and healthy subject. Darker areas indicate staining with isotype control mAb. B. MFI of surface TLR9 staining for PDC in patients with SLE (n = 14) and healthy subjects (HC; n = 15). Significance of differences between groups were analyzed by Mann-Whitney U-test.

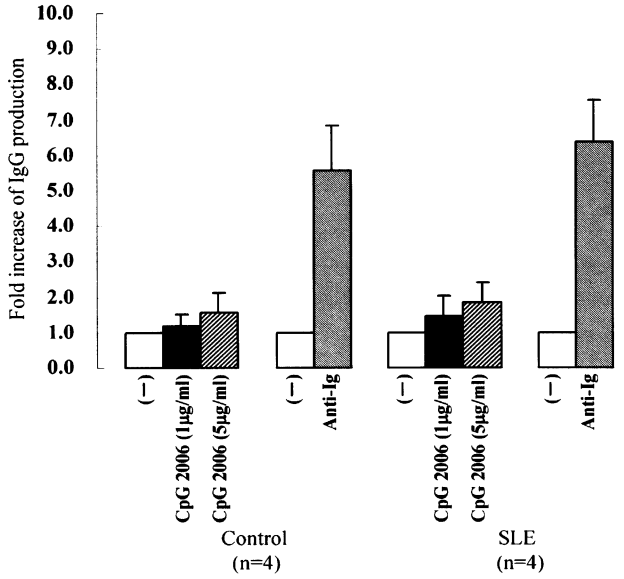


Figure 9. Secretion of IgG by PBMC from patients with SLE (n = 4) and healthy subjects (control; n = 4) after stimulation with CpG ODN. Freshly isolated T cell-depleted MNC were cultured with CpG ODN and anti-IgG. The amounts of IgG in culture supernatants were determined by ELISA. The IgG secreted from untreated MNC was assigned the value of 1.0 and data were expressed as fold induction. These data represent mean ± SD of 4 independent samples.

Table 2. Immunoglobulin production after 4 days of culture with CpG oligodeoxynucleotide. Results are mean (SD) of duplicate samples.

	IgG, ng/ml			
	None	CpG 2006 (1 μg/ml)	CpG 2006 (5 μg/ml)	Anti-Ig
Control				
1	125.2 (21.7)	135.1 (17.4)	212.6 (17.9)*	417.2 (44.7)*
2	106.2 (9.5)	155.5 (25.1)*	195.4 (32.1)*	517.2 (69.1)*
3	97.6 (11.6)	105.6 (9.9)	121.5 (17.8)*	617.5 (55.2)*
4	79.1 (14.8)	85.5 (13.2)	96.5 (9.5)	715.2 (69.9)*
SLE				
1	105.4 (7.2)	252.4 (19.2)*	289.2 (17.7)*	781.2 (91.1)*
2	132.4 (16.3)	167.5 (12.1)	245.1 (21.9)*	824.6 (102.9)*
3	108.8 (8.9)	118.2 (9.4)	162.5 (12.7)*	654.9 (78.1)*
4	152.7 (11.1)	191.9 (17.1)*	233.5 (29.1)*	932.7 (115.4)*

* $p < 0.05$ vs No stimulation.

6. Ronnblom L, Alm GV. Systemic lupus erythematosus and the type I interferon system. Arthritis Res Ther 2003;5:68-75.
7. Ronnblom L, Alm GV. A pivotal role for the natural interferon alpha-producing cells (plasmacytoid dendritic cells) in the pathogenesis of lupus. J Exp Med 2001;194:59-63.
8. Lovgren T, Eloranta ML, Bave U, Alm GV, Ronnblom L. Induction of interferon-alpha production in plasmacytoid dendritic cells by immune complexes containing nucleic acid released by necrotic or late apoptotic cells and lupus IgG. Arthritis Rheum 2004;50:1861-72.
9. Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol 2003;21:335-76.
10. Pasare C, Medzhitov R. Toll-like receptors: linking innate and adaptive immunity. Adv Exp Med Biol 2005;560:11-8.
11. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
12. Urowitz MB, Gladman DD. Measures of disease activity and

damage in SLE. Baillieres Clin Rheumatol 1998;12:405-13.
13. Dzionek A, Fuchs A, Schmidt P, et al. BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. J Immunol 2000;165:6037-46.
14. Le Bon A, Schiavoni G, D'Agostino G, Gresser I, Belardelli F, Tough DF. Type 1 interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. Immunity 2001;14:461-70.
15. Kopp E, Medzhitov R. Recognition of microbial infection by Toll-like receptors. Curr Opin Immunol 2003;15:396-401.
16. Rifkin IR, Leadbetter EA, Busconi L, Viglianti G, Marshak-Rothstein A. Toll-like receptors, endogenous ligands, and systemic autoimmune disease. Immunol Rev 2005;204:27-42.
17. Means TK, Latz E, Hayashi F, Murali MR, Golenbock DT, Luster AD. Human lupus autoantibody-DNA complexes activate DCs through cooperation of CD32 and TLR9. J Clin Invest 2005;115:407-17.
18. Bauer S, Wagner H. Bacterial CpG-DNA licenses TLR9. Curr Top Microbiol Immunol 2002;270:145-54.
19. Scheinecker C, Zwolfer B, Koller M, Manner G, Smolen JS. Alterations of dendritic cells in systemic lupus erythematosus: phenotypic and functional deficiencies. Arthritis Rheum 2001;44:856-65.
20. Farkas L, Beiske K, Lund-Johansen F, Brandtzaeg P, Jahnsen FL. Plasmacytoid dendritic cells (natural interferon-alpha/beta-producing cells) accumulate in cutaneous lupus erythematosus lesions. Am J Pathol 2001;159:237-43.
21. Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Immunol 2005;23:275-306.
22. Hornung V, Rothenfusser S, Britsch S, et al. Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J Immunol 2002;168:4531-7.
23. Leadbetter EA, Rifkin IR, Hohlbaum AM, Beaudette BC, Shlomchik MJ, Marshak-Rothstein A. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. Nature 2002;416:603-7.
24. Viglianti GA, Lau CM, Hanley TM, Miko BA, Shlomchik MJ, Marshak-Rothstein A. Activation of autoreactive B cells by CpG dsDNA. Immunity 2003;19:837-47.
25. Bauer S, Kirschning CJ, Hacker H, et al. Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. Proc Natl Acad Sci USA 2001;98:9237-42.
26. Zeuner RA, Klinman DM, Illei G, et al. Response of peripheral blood mononuclear cells from lupus patients to stimulation by CpG oligodeoxynucleotides. Rheumatology Oxford 2003;42:563-9.
27. Bernasconi NL, Onai N, Lanzavecchia A. A role for Toll-like receptors in acquired immunity: up-regulation of TLR9 by BCR triggering in naive B cells and constitutive expression in memory B cells. Blood 2003;101:4500-4.
28. Kikuchi K, Lian ZX, Yang GX, et al. Bacterial CpG induces hyper-IgM production in CD27(+) memory B cells in primary biliary cirrhosis. Gastroenterology 2005;128:304-12.
29. Renaudineau Y, Pers JO, Bendaoud B, Jamin C, Youinou P. Dysfunctional B cells in systemic lupus erythematosus. Autoimmun Rev 2004;3:516-23.