Aberrant expression of Fas ligand on anti-DNA autoantibody secreting B lymphocytes in patients with systemic lupus erythematosus: “Immune privilege”-like state of the autoreactive B cells

H. Nagafuchi, S. Wakisaka, Y. Takeba, M. Takeno, T. Sakane, N. Suzuki

Departments of Immunology and Medicine, and Institute of Medical Science, St. Marianna University School of Medicine, Kanagawa, Japan

Abstract

Background

Fas/Fas ligand (FasL) system has been assigned a pivotal role in the development and maintenance of peripheral tolerance, and mice with defects in their Fas/FasL system develop lupus-like symptoms. In this study we examined FasL expression of peripheral blood lymphocytes in patients with systemic lupus erythematosus (SLE).

Methods

We assessed FasL mRNA and protein expression by reverse transcription (RT)-PCR and immunoblotting and immunocytochemical staining, respectively, in patients with SLE. Anti-DNA antibody secreting B cells were purified using biotin labeled DNA and streptavidin-bead.

Results

Expression of FasL protein was not or very weakly detected in freshly isolated PBMC in normal individuals. In contrast, freshly isolated SLE PBMC exhibited the enhanced expression of FasL protein without in vitro *stimulation. Not only purified T cells but also purified B cells expressed FasL on their cell surface spontaneously. In addition, freshly isolated anti-DNA autoantibody secreting B cells express FasL without in vitro stimulation.*

Conclusion

The results suggest that autoreactive B lymphocytes which aberrantly express FasL may kill Fas+ immunoregulatory T lymphocytes. Thus aberrantly expressed FasL may facilitate escape of the autoreactive B cells from the immune tolerance system, and may contribute to the sustained secretion of autoantibodies in patients with SLE.

Key words

Human, B lymphocytes, lupus, apoptosis.

Hiroko Nagafuchi, MD, PhD; Sueshige Wakisaka, MD, PhD; Yuko Takeba, DVM, PhD; Mitsuhiro Takeno, MD, PhD; Tsuyoshi Sakane, MD, PhD; and Noboru Suzuki, MD, PhD.

Please address correspondence and reprint requests to: Dr. Noboru Suzuki, Departments of Immunology and Medicine, St. Marianna University School of Medicine, 2-16-1, Sugao, Miyamae-ku, Kawasaki, Kanagawa 216-8511, Japan. E-mail: n3suzuki@marianna-u.ac.jp

Received on December 13, 2001; accepted in revised form on April 17, 2002.

Introduction

Systemic lupus erythematosus (SLE) is a prototype of the systemic autoimmune diseases; it depends on a genetic aspect, a sexual preference, and an unknown means of acquisition including impaired T cell regulation and B cell hyperactivity (1-7). The appearance of autoantibodies such as anti-DNA antibody in circulation is associated with the pathogenesis (4-6). Defects of central and peripheral tolerance play important roles in the development of such autoantibodies in this disease (3, 8).

Fas ligand (FasL) belongs to the TNF superfamily and is induced in activated lymphocytes and eliminates Fas-expressing lymphocytes, resulting in the down regulation of immune responses (9-11). It is suggested that Fas/FasL system play an important role in the clonal deletion of autoreactive T cells in the periphery and activation-induced cell death of T cells (9-11). The murine *lpr* and *gld* mutations are mutations of Fas and FasL genes and cause abnormal expression of Fas and dysfunction of FasL, respectively (12-14). The *lpr* and *gld* mice manifest SLE-like autoimmune diseases by producing autoantibodies including anti-DNA and rheumatoid factors (13, 14).

In addition, the induction of apoptosis by FasL in invading lymphocytes acts as a mechanism of immune privilege and is important in preventing graft rejection (15). Furthermore, FasL is expressed in certain malignancies and it has been implicated as a possible key mechanism in immune privilege of these tumors (16-18).

Defects of self tolerance have been assigned an important role in the development and maintenance of autoimmune responses in SLE (8). There have been several studies reporting Fas and FasL expression on lymphocytes in patients with SLE (19-24). In addition, soluble Fas was secreted to modulate Fas/FasL system in patients with SLE (25-27).

To determine which lymphocyte subpopulation(s) is responsible for FasL expression in patients with SLE, we carried out immunoblotting analysis, immunofluorescence analysis and RT-PCR analysis of peripheral blood lymphocytes. We found FasL expression of anti-DNA autoantibody secreting B lymphocytes in a majority of patients with SLE.

Patients and methods

Patients

We studied 20 patients, all women, who fulfilled the 1982 revised criteria for the classification of SLE by American Rheumatism Association (28). The mean age (+ SD) of these patients was 35.1 + 9.2 yr. (Range 14-55). Human studies committee approval and individual informed consent from each patient were obtained before we conducted the present study. The 20 patients have been treated with low doses of corticosteroid therapy (less than 10 mg Predonison/day). None has been treated with high doses of corticosteroid therapy nor immunosuppressive therapy.

Nineteen healthy female volunteer blood donors served as control subjects. Their mean age (+ SD) was 36.2 + 6.3 yr. (Range 25-52).

Cell separation and cell cultures

PBMC were obtained by Ficoll-Hypaque centrifugation of heparinized blood from patients with SLE and normal healthy adults. Freshly isolated PBMC were suspended in RPMI1640 medium containing 10% FCS, Penicillin and Streptomycin (Life Science, Tokyo, Japan). T cells and B cells were purified by means of a SRBC rosette technique, followed by depletion of monocytes by a Petri dish adherence procedure. B cells were further purified by panning with anti-CD3 (OKT3; Ortho Diagnostic Systems, Raritan, NJ), OKM1(Ortho Diagnostic Systems) and anti-CD56 mAbs (Leu19; Becton Dickinson Monoclonal Center, Mountain View, CA) (29). T cells were treated to deplete NK cells by panning with anti-CD56 mAb. Cells firmly adhering to the petri dishes were recovered by vigorous pipetting and were used as monocytes. The T cells, B cells, and monocytes are more than 95%, 93%, and 90% positive for the relevant cell surface antigen, respectively.

Separation of anti-DNA antibody secreting B cells

Purified B cells from patients with SLE were incubated with biotin-labeled DNA, followed by streptavidin-beads. DNA binding B cells were separated by MACS apparatus as previously described (29, 30). All purification procedures were carried out using Ca^{++} Mg^{++} free PBS with 0.01% sodium azide at 4°C in order not to cause unwanted lymphocyte activation during the cell purification procedure. The resulting DNA-binding fraction and non DNA-binding fraction were more than 99% positive and less than 1% positive for the DNA-binding B cells, respectively, confirmed by DNA-biotin/streptavidin-peroxidase reaction of the cytospin preparations. With this method, we recovered the DNA-binding B cells from patients with SLE who have high titer of circulating anti-DNA antibody, but never obtained the DNA-binding B cells from normal individuals.

RNA extraction and reverse transcription (RT)-PCR

FasL cDNA was amplified by a PCR based technique (31). In brief, total RNA of PBL and purified lymphocyte subpopulations was reverse transcribed. Full length FasL cDNA was amplified by PCR using a sense primer gacgcatgcgaattcatgcagcgcccttcaattaccca and an antisense primer gtggtcgacttagagcttatataagccgaaaaagc. For amplification of the extracellular region, a sense primer ggcgcatgcgaattccagctcttccacctacagaaggag was used. β-actin primers (a sense primer gtggggcgccccaggcac, an antisense primer ctcctaatgtcacgcacgat) were used to compare and monitor efficient cDNA synthesis between different samples. PCR amplification of the cDNA was performed using a 35-cycle program consisting of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. The DNA fragments were separated on 1.5% agarose gels and visualized by staining with ethidium bromide (31).

Preparation of rabbit affinity-purified anti-human FasL antibody

PCR product of the extracellular region was digested with Sph I and Sal I and ligated into pQE30 (QIAGEN, Hilden, Germany) for expression in bacterial cells. We performed DNA sequencing of the plasmid to confirm fidelity of FasL cDNA (30). A bacterial strain, M15 [pREP4], was transformed with pQE30 carrying the extracellular domain of FasL cDNA (pQE30-FasL EX1, amino acids 103-281) by electroporation. After stimulation with 2 mM isopropylthio-galactoside for 3 h, bacterial cells were lysed in lysis buffer. Thereafter, extracellular domain of FasL protein tagged with 6 x histidine at its N-terminus (termed FasL EX1) was affinity-purified with Ni-NTA resin. Recombinant FasL EX1 eluted from the resin was further purified for immunization by preparative SDS-PAGE. Then the SDS-PAGE gel purified FasL EX1 was used as an antigen for immunizing rabbits (New Zealand White rabbit) according to the standard protocol. Anti-FasL antibody was affinity-purified with immobilized FasL EX1 as described (31). We confirmed the specificity of the resultant antibody by neutralizing the antibody with soluble FasL, but not irrelevant soluble antigen in the immunoblotting analysis.

SDS-PAGE and immunoblotting analysis

$1x10^7$ PBMC or purified lymphocytes were lysed in 100 μl Nonidet P40 lysis buffer containing protease inhibitors (1 mg/ml PMSF, 5 mM EDTA, 2 mg/ml aprotinin, and 2 mg/ml leupeptin). Cell lysates were electrophoresed on 4-20% SDS-PAGE gels (31). Proteins were electro-transferred to polyvinylidene difluoride membranes (Millipore, Beetford, MA). The membrane was probed with rabbit affinity-purified anti-human FasL antibody, followed by incubation with biotin-labeled anti-rabbit IgG antibody and streptavidin-peroxidase (31). Visualization was carried out by chemiluminescence (Amersham, Tokyo, Japan).

Immunofluorescence staining

Cell staining with mAbs and data analysis were performed as previously described with minor modification (32). Briefly, purified lymphocytes were sequentially incubated with hamster anti-human FasL mAb (Medical & Biological Laboratories, Nagoya, Japan), biotin-conjugated rabbit anti-hamster IgG and streptavidin-phycoerythrin for 30 min at 4°C. After washing, stained cells were analyzed by a flow cytometer (FACScan analyzer; Becton-Dickinson FACS System, San Jose, CA).

Immunocytochemical staining

The purified B cell subpopulations were span to adhere onto the slides. The samples were fixed with cold acetone for 15 min and were blocked with 2% skim milk for 30 min. The samples were incubated with the first antibody, mouse anti-human FasL mAb (BD Transduction, Lexington, KY) for overnight at 4°C. All subsequent procedures were performed using NBA kit (Non-biotin amplification system; Zymed Laboratories, South San Francisco, CA) as suggested by the manufacturer.

Results

Expression of FasL protein on PBMC of SLE patients

We first carried out immunoblotting analysis to detect FasL protein of PBMC by using rabbit affinity-purified anti-human FasL antibody we have developed (31). In normal individuals, unstimulated PBMC do not or very weakly express FasL protein (Fig. 1). Of note is the fact that in vast majority (18/20) of SLE patients, freshly isolated PBMC express FasL protein spontaneously (Fig. 1).

Lymphocyte subpopulations responsible for aberrant FasL expression in patients with SLE

To determine which lymphocyte subpopulation accounts for the increase in FasL expression in patients with SLE, PBMC were separated into T cells and B cells. We found that not only T cells but also B cells express excessive FasL protein, when compared with normal counterparts (Fig. 2). Monocytes from normal individuals and SLE patients expressed similar amounts of FasL spontaneously (Fig. 2) (33).

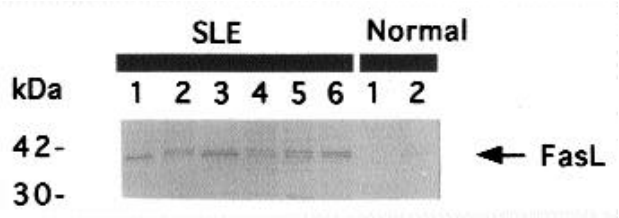

Fig. 1. Aberrant FasL protein expression on peripheral blood lymphocytes in patients with SLE.
Freshly isolated PBMC from 6 patients with SLE were immediately lysed. As controls, normal PBMC from 2 donors were included. The cell lysates were analyzed by immunoblotting using affinity-purified anti-human FasL antibody. Results shown are representative of five independent experiments with similar results. We found that 18 patients showed enhanced FasL expression out of 20 patients studied when compared with normal PBMC.

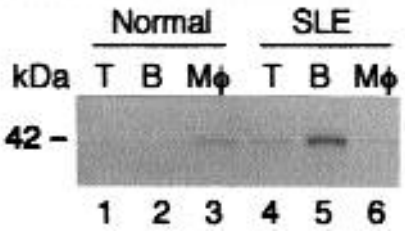

Fig. 2. Detection of FasL protein in SLE T cells, B cells and monocytes.
Purified T cells,B cells and monocytes were immediately lysed and analyzed by immunoblotting. Normal T cells and B cells did not express FasL spontaneously. SLE B cells and T cells showed enhanced FasL expression. Normal monocytes and SLE monocytes expressed comparable amounts of FasL. Results shown are representative of four independent experiments employing six SLE patients with similar results.

We have further confirmed FasL mRNA expression of both SLE T and B cells by the RT-PCR technique (Fig. 3).

FACS analysis of FasL expression in patients with SLE

We next focused our attention on cell surface expression of FasL on T and B lymphocytes of SLE patients. We performed flow cytometric analysis of FasL expression by using human FasL specific monoclonal antibody (Fig. 4). We found that both T cells and B cells express cell surface FasL in patients with SLE. In contrast, normal T and B cells did not express FasL on the cell surface spontaneously (data not shown).

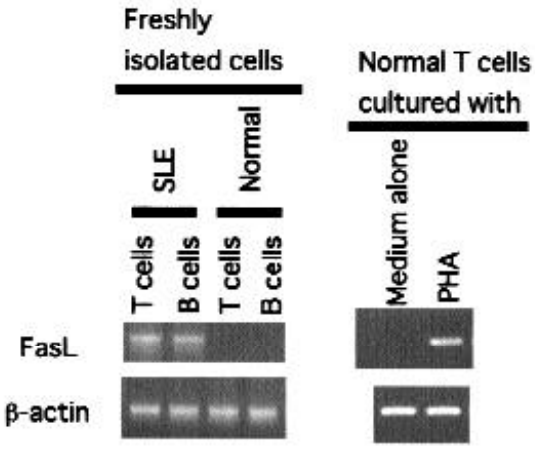

Fig. 3. RT-PCR analysis of FasL mRNA expression of SLE T and B cells.
Purified T cells and B cells were immediately lysed for total RNA extraction. Some of the T cells were stimulated with PHA (1 μg/ml) for 8 hours. After cDNA synthesis,PCR was performed. We found that both freshly recovered T cells and B cells express FasL mRNA in patients with SLE. Results shown are representative of five independent experiments employing 12 SLE patients with similar results.

Anti-DNA autoantibody secreting B cells express FasL in patients with SLE

It should be clarified whether aberrant FasL expression is somehow associated with the development of autoimmune responses in patients with SLE. To this end, we took advantage of our previous finding that polyclonal anti-DNA antibody secreting B cells can be purified by using biotin-labeled DNA and streptavidin-beads (29, 30). Thus, we purified DNA binding B cells and non-DNA binding B cells from SLE B cells. We confirmed that the DNA binding B cells, but not non-DNA binding B cells, expressed A30-Jκ2 mRNA which encodes the cationic anti-DNA antibody light chain (data not shown) and secretes anti-DNA antibody (DNA binding B cells, anti-DNA antibody secretion assayed by ELISA, 4.2 u/ml; non-DNA binding B cells, anti-DNA antibody secretion 0 u/ml) (29). We found that FasL is spontaneously expressed in a small number of non-DNA binding B cells (Fig. 5 and Table I). In four cases out of four cases studied, freshly isolated anti-DNA secreting B cells expressed FasL without any stimulation (Fig. 5). Thus, it is evident that anti-DNA secreting B cells preferentially express FasL in patients with SLE.

Discussion

SLE is a disease characterized by the production of autoantibodies directed against various self components. The profile of autoantibodies in SLE suggests that tolerance to multiple unrelated antigens may be lost (19). To determine whether SLE has a defect in Fas/FasL system which maintains peripheral tolerance, we have evaluated FasL expression of lymphocytes in circulation in patients with SLE.

We found that SLE whole B cells spontaneously express cell surface FasL. In our preliminary experiments, we found that soluble FasL spontaneously secreted by SLE whole B cells induced apoptotic cell death of Fas expressing Jurkat cells (data not shown). At pre-

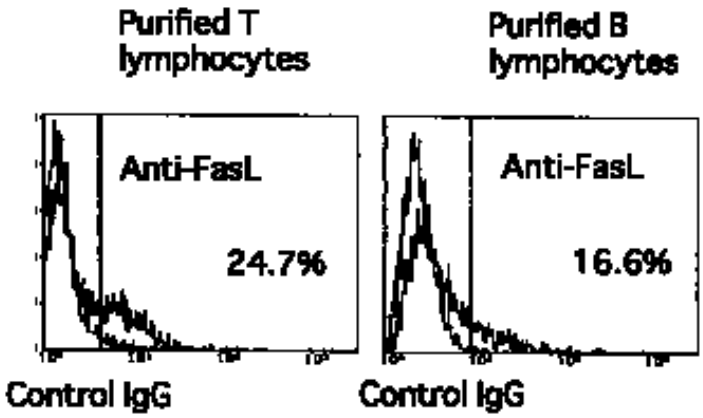

Fig. 4. Flow cytometric analysis of cell surface FasL expression of SLE T cells and B cells.
Purified T cells and B cells were stained with anti-FasL mAb. Irrelevant hamster IgG was used to define control staining. Both T cells and B cells express FasL in patients with SLE.% positive cells were indicated in the figure. Results shown are representative of four independent experiments employing four SLE patients with similar results.

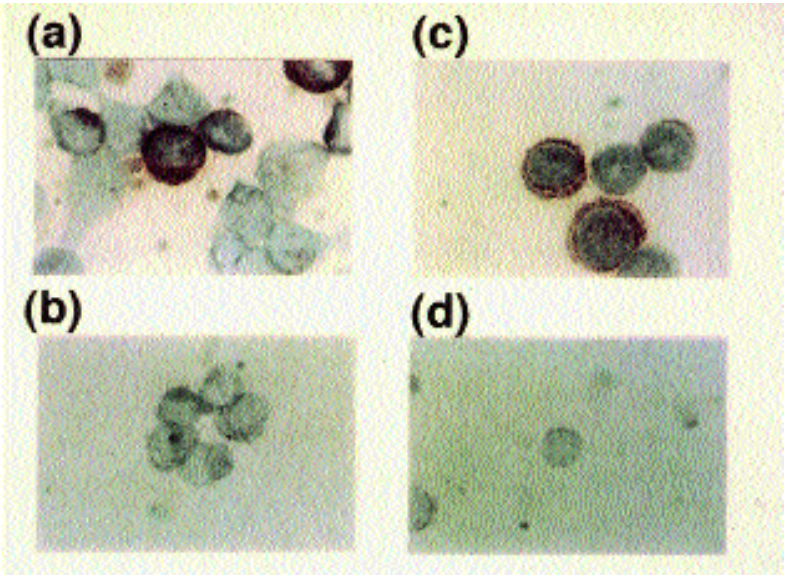

Fig. 5. Immunocytochemical staining of human FasL protein in DNA binding B cells and non-DNA binding B cells in patients with SLE.
DNA-binding B cells and non-DNA binding B cells were recovered as previously described (25). All purification and staining procedures were carried out immediately at 4°C to avoid unwanted activation of the cells. We confirmed that DNA binding, but not non-DNA binding, B cells expressed anti-DNA associated Ig Vκ RNA and secrete anti-DNA antibody. Color development was carried out by using non-biotin amplification system. (**a**) Non-DNA binding B cells, anti-FasL antibody; (**b**) Non-DNA binding B cells, control IgG; (**c**) DNA binding B cells, anti-FasL antibody; and (**d**) DNA binding B cells,control IgG. Results shown are representative of four independent experiments employing 4 SLE patients with similar results.

sent, it is difficult to obtain sufficient numbers of anti-DNA antibody secreting B cells of SLE patients to test apoptosis inducing activity on Fas expressing T cells of their culture supernatants.

Expression of FasL in B cells and malignant B cell lymphoma/leukemia has already been reported (34-36). In addition, virus infection induces FasL expression of B lymphocytes, leading to apoptosis induction (37, 38).

Even though anti-DNA autoantibody secreting B cells predominantly expressed FasL, B cells secreting antibodies with other specificity also expressed FasL (Table I). Thus, the fact that anti-DNA secreting B cells express FasL may reflect the preferential activation of autoreactive B cells in patients with SLE. We carefully compared the FasL expression and the disease duration of each patient. We did not find any clear-cut association between them.

FasL is expressed preferentially, but not exclusively on autoantibody secreting B cells in patients with SLE, and thus the B cells may have been protected from immunoregulatory Fas positive T lymphocytes. The situation of the B cells may be similar with "immune privilege". Indeed, FasL inhibits T cell function in immune-privileged organs such as the eye and testis (15, 16). FasL expression is also associated with increased apoptosis of tumor-infiltrating lymphocytes *in vivo*, thereby also contributing to the immune privilege of the tumor (17,18). There is a line of evidence that showed FasL expressing B cells down-regulate T cell immunity (38-41). Therefore, we speculate that the abundant presence of FasL in the autoantibody secreting B lymphocytes of SLE patients may contribute to immune privilege, perhaps by fostering the apoptosis of activated Fas-expressing immunoregulatory T lymphocytes. Thus, excessive FasL expression of autoreactive B cells may facilitate the "escape" of autoreactive B lymphocytes from the peripheral tolerance mechanism by immunoregulatory T lymphocytes.

FasL-defective mice develop an anti-DNA antibody response and disease manifestations (12-14). FasL may contribute to the development of autoantibody responses by two distinct pathways; deficient FasL expression of immunoregulatory T cells facilitates the escape of autoreactive lymphocytes from the immune surveillance system by failure of killing the autoreactive lymphocytes; excessive expression of FasL on autoreactive B lymphocytes also facilitates escape from the immune surveillance system by protecting themselves from immunoregulatory T cells ("immune privilege"). These results suggest that it is important to maintain appropriate FasL expression on lymphocytes to prevent autoimmunity.

It has been reported that Fas expression on lymphocytes obtained from patients

Table I. Preferential expression of FasL on anti-DNA autoantibody secreting B cells.

	FasL + cells	FasL- cells	% positive cells
Case 1			
DNA binding B cells	100	100	50%
Non-DNA binding B cells	43	157	21.5%
Case 2			
DNA binding B cells	116	84	58%
Non-DNA binding B cells	53	147	26.5%

DNA binding and non-DNA binding B cells were purified as described and the spontaneous expression of FasL was assessed by immunocytochemical staining. More than 200 cells were counted to calculate the percentage of FasL positive cells. DNA binding B cells secreted anti-DNA antibodies, whereas non-DNA binding B cells did not (see text). The results of 2 representative experiments out of 4 are shown.

with SLE is increased (19). Following activation of SLE T cells *in vitro*, no defects in up-regulation of the Fas and in the ability of the activated cells to undergo apoptosis following ligation of the Fas receptor with the monoclonal anti-Fas antibody were detected (19). Thus, immunoregulatory SLE T lymphocytes may be induced to undergo apoptosis after cellular interaction with FasL expressing autoreactive B cells. Even though we could not test directly whether anti-DNA antibody secreting B cells aberrantly kill Fas expressing autologous T cells, because of the low cell yield of the B cells.

In agreement with our finding that SLE lymphocytes express FasL, other investigators have reported that elevated serum levels of FasL in patients with SLE (42).

We could not find any clear relationship between FasL expression and disease activity, treatment, duration of the disease and serological parameters. It has been reported that corticosteroids reduce FasL expression on lymphocytes. We found that a vast majority of our patients who had been taking low doses of Predonison (less than 10 mg/day) expressed FasL spontaneously, suggesting that the low doses of corticosteroids did not affect FasL expression of lymphocytes. We have studied some patients with high corticosteroid therapy. However, their circulating lymphocytes were dramatically reduced. Thus, we were unable to recover sufficient lymphocytes for the analysis. In turn, we have conducted *in vitro* experiments where the effects of dexamethason on FasL expression were studied and confirmed the reduced FasL expression by corticosteroids.

TABLE 1 ?

Abnormal FasL expression was evident in both active SLE patients and inactive SLE patients. However, we and others found that normal resting B cells never expressed detectable levels of FasL (31,34). We think that FasL expressing B cells might be activated B cells in vivo, and the majority of FasL expressing B cells might be spontaneously activated B cells. Nonetheless, clear-cut correlation between disease activity and the FasL expression was not observed in patients with SLE. We have previously reported that disease activity did not correlate with the extent of spontaneous B cell activation in patients with SLE (43,44). The spontaneous B cell activation is one of the driving forces to the development of active disease. However, other factors such as T cell immune responses, genetic predisposition, sex hormones, and environmental factors including ultraviolet may contribute to exacerbation of the disease. In addition, therapeutic intervention made straight forward interpretation of the results difficult in patients with SLE. In any events, we found that some patients with inactive disease expressed FasL predominantly. Similarly, it has been reported that the lack of significant correlation of Fas expression with the composite index of disease activity, the SLEDAI, or with other serological parameters of activity (19).

In summary, our present study suggest that aberrantly expressed FasL may contribute to the "immune privilege" like state of autoreactive B cells, and may help the persistent survival of autoreactive B cells in patients with SLE.

References

1. DEAN GS, TYRRELL-PRICE J, CRAWLEY E, ISENBERG DA: Cytokines and systemic lupus erythematosus. *Ann Rheum Dis* 2000; 59: 243-51.
2. LIOSSIS SN, TSOKOSGC: Molecular aspects in the pathogenesis of human systemic lupus erythematosus. *Arch Immunol Ther Exp* 2000; 48: 11-19.
3. SHOENFELD Y, ALARCON-SEGOVIA D, BUSKILA D *et al.*: Frontiers of SLE: Review of the 5th International Congress of Systemic Lupus Erythematosus, Cancun, Mexico, April 20-25, 1998. *Semin Arthritis Rheum* 1999; 29: 112-30.
4. HAHN BH: Antibodies to DNA. *N Engl J Med* 1998; 338: 1359-68.
5. CONRAD K, TAN EM, HUMBEL RL, SHOENFELD Y: Autoantibodies – diagnostic, pathogenic and prognostic relevance. *Clin Exp Rheumatol* 1997; 15: 457-65.
6. RADIC MZ, COCCA BA, SEAL SN: Initiation of systemic autoimmunity and sequence specific anti-DNA autoantibodies. *Crit Rev Immunol* 1999; 19: 117-26.
7. WU CY, CHIOU YH, CHIU PC, HSIEH KS: Severe pulmonary hemorrhage as the initial manifestation in systemic lupus erythematosus with active nephritis. *Lupus* 2001; 10: 879-82.
8. MASON LJ, ISENBERG DA: Immunopathogenesis of SLE. *Baillieres Clin Rheumatol* 1998; 12: 385-403.
9. JU ST, PANKA DJ, CUI H *et al.*: Fas(CD95)/FasL interactions required for programmed cell death after T-cell activation. *Nature* 1995; 373: 444-8.
10. VAN PARIJS L, PEREZ VL, ABBAS AK: Mechanisms of peripheral T cell tolerance. *Novartis Found Symp* 1998; 215: 5-40.
11. ALDERSON MR, TOUGH TW, DAVIS-SMITH T *et al.*: Fas ligand mediates activation-induced cell death in human T lymphocytes. *J Exp Med* 1995; 181: 71-7.
12. RUSSELL JH, RUSH B, WEAVER C, WANG R: Mature T cells of autoimmune lpr/lpr mice have a defect in antigen-stimulated suicide. *Proc Natl Acad Sci USA* 1993;90:4409-13.
13. WEINTRAUB JP, GODFREY V, WOLTHUSEN PA, CHEEK RL, EISENBERG RA, COHEN PL: Immunological and pathological consequences of mutations in both Fas and Fas ligand. *Cell Immunol* 1998;186: 8-17.
14. TAKAHASHI T, TANAKA M, BRANNAN CI *et al.*: Generalized lymphoproliferative disease in mice, caused by a point mutation in the Fas ligand. *Cell* 1994; 76: 969-76.
15. GRIFFITH TS, BRUNNER T, FLETCHER SM, GREEN DR, FERGUSON TA: Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science* 1995; 270:1189-92.
16. STUART PM, GRIFFITH TS, USUI N, PEPOSE J, YU X, FERGUSON TA: CD95 ligand (FasL)-induced apoptosis is necessary for

corneal allograft survival. *J Clin Invest* 1997; 99: 396-402.

17. O'CONNELL J, BENNETT MW, O'SULLIVAN GC *et al.*: Fas ligand expression in primary colon adenocarcinomas: evidence that the Fas counter attack is a prevalent mechanism of immune evasion in human colon cancer. *J Pathol* 1998; 186: 240-6.
18. GASTMAN BR, ATARSHI Y, REICHERT TE *et al.*: Fas ligand is expressed on human squamous cell carcinomas of the head and neck, and it promotes apoptosis of T lymphocytes. *Cancer Res* 1999; 59: 5356-64.
19. MYSLER E, BINI P, DRAPPA J *et al.*: The apoptosis-1/Fas protein in human systemic lupus erythematosus. *J Clin Invest* 1994; 93: 1029-34.
20. SAKATA K,SAKATA A,VELA-ROCH N *et al.*: Fas (CD95)-transduced signal preferentially stimulates lupus peripheral T lymphocytes. Eur *J Immunol* 1998; 28: 2648-60.
21. GEORGESCU L, VAKKALANKA RK, ELKON KB, CROW MK: Interleukin-10 promotes activation-induced cell death of SLE lymphocytes mediated by Fas ligand. *J Clin Invest* 1997; 100: 2622-33.
22. MCNALLY J, YOO DH, DRAPPA J *et al.*: Fas ligand expression and function in systemic lupus erythematosus. *J Immunol* 1997; 159: 4628-36.
23. KOVACS B, LIOSSIS SN, DENNIS GJ, TSOKOS GC: Increased expression of functional Fas-ligand in activated T cells from patients with systemic lupus erythematosus. *Autoimmunity* 1997; 25: 213-21.
24. LORENZ HM, GRUNKE M, HIERONYMUS T *et al.*: *In vitro* apoptosis and expression of apoptosis-related molecules in lymphocytes from patients with systemic lupus erythematosus and other autoimmune diseases. *Arthritis Rheum* 1997; 40: 306-17.
25. CHENG J, ZHOU T, LIU C *et al.*: Protection from Fas-mediated apoptosis by a soluble form of the Fas molecule. *Science* 1994; 263: 1759-62.
26. KNIPPING E, KRAMMER PH, ONEL KB, LEHMAN TJ, MYSLER E, ELKON KB: Levels of soluble Fas/APO-1/ CD95 in systemic lupus erythematosus and juvenile rheumatoid arthritis. *Arthritis Rheum* 1995; 38: 1735-7.
27. GOEL N, ULRICH DT, ST CLAIR EW, FLEMING JA, LYNCH DH, SELDIN MF: Lack of correlation between serum soluble Fas/APO-1 levels and autoimmune disease. *Arthritis Rheum* 1995; 38: 1738-43.
28. 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.
29. HARADA T, SUZUKI N, MIZUSHIMA Y, SAKANE T: Usage of a novel class of germline Ig variable region gene for cationic anti-DNA autoantibodies in human lupus nephritis and its role for the development of the disease. *J Immunol* 1994; 153: 4806-15.
30. BLAISON G, KUNTZ JL, PASQUALIJL: Molecular analysis of V kappa III variable regions of polyclonal rheumatoid factors during rheumatoid arthritis. *Eur J Immunol* 1991; 21: 1221-7.
31. SUZUKI N, ICHINO M, MIHARA S, KANEKO S, SAKANE T: Inhibition of Fas/Fas ligand-mediated apoptotic cell death of lymphocytes *in vitro* by circulating anti-Fas ligand autoantibodies in patients with systemic lupus erythematosus. *Arthritis Rheum* 1998; 41: 344-53.
32. KASHIWAKURA J, SUZUKI N, NAGAFUCHI H *et al.*: Txk, a nonreceptor tyrosine kinase of the Tec family, is expressed in T helper type 1 cells and regulates interferon gamma production in human T lymphocytes. *J Exp Med* 1999; 190: 1147-54.
33. KIENER PA, DAVIS PM, RANKIN BM *et al.*: Human monocytic cells contain high levels of intracellular Fas ligand: rapid release following cellular activation. *J Immunol* 1997; 159: 1594-8.
34. HAHNE M, RENNO T, SCHROETER M *et al.*: Activated B cells express functional Fas ligand. *Eur J Immunol* 1996; 26: 721-4.
35. MULLAUER L,MOSBERGER I,CHOTT A: Fas ligand expression in nodal non-Hodgkin's lymphoma. *Mod Pathol* 1998; 11: 369-75.
36. SCHATTNER EJ, ELKON KB, YOO DH *et al.*: CD40 ligation induces Apo-1/Fas expression on human B lymphocytes and facilitates apoptosis through the Apo-1/Fas pathway. *J Exp Med* 1995; 182: 1557-65.
37. SAMUELSSON A, SONNERBORG A, HEUTS N, COSTER J, CHIODI F: Progressive B cell apoptosis and expression of Fas ligand during human immunodeficiency virus type 1 infection. *AIDS Res Hum Retroviruses* 1997; 13: 1031-8.
38. TANNER JE, ALFIERI C: Epstein-Barr virus induces Fas (CD95) in T cells and Fas ligand in B cells leading to T-cell apoptosis. *Blood* 1999; 94: 3439-47.
39. TIAN J, ZEKZER D, HANSSEN L, LU Y, OLCOTT A, KAUFMAN DL: Lipopolysaccharide-activated B cells down-regulate Th1 immunity and prevent autoimmune diabetes in nonobese diabetic mice. *J Immunol* 2001; 167: 1081-9.
40. TINHOFER I, MARSCHITZ I, KOS M *et al.*: Differential sensitivity of CD4+ and CD8+ T lymphocytes to the killing efficacy of Fas (Apo-1/CD95) ligand+ tumor cells in B chronic lymphocytic leukemia. *Blood* 1998; 91: 4273-81.
41. LUNDY SK, BOROS DL: Fas ligand-expressing B-1a lymphocytes mediate CD4(+)-T-cell apoptosis during schistosomal infection: induction by interleukin 4 (IL-4) and IL-10. *Infect Immun* 2002; 70: 812-9.
42. NOZAWA K,KAYAGAKI N, TOKANO Y, YAGITA H, OKUMURA K, HASIMOTO H: Soluble Fas (APO-1, CD95) and soluble Fas ligand in rheumatic diseases. *Arthritis Rheum* 1997; 40: 1126-9.
43. SAKANE T, MURAKAWA Y, SUZUKI N *et al.*: Familial occurrence of impaired interleukin-2 activity and increased peripheral blood B cells actively secreting immunoglobulins in systemic lupus erythematosus. *Am J Med* 1989; 86: 385-90.
44. SAKANE T, SUZUKI N, TAKADA S *et al.*: B cell hyperactivity and its relation to distinct clinical features and the degree of disease activity in patients with systemic lupus erythematosus. *Arthritis Rheum* 1988; 31: 80-87.