Fas Ligand Mutation in a Patient with Systemic Lupus Erythematosus and Lymphoproliferative Disease

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Abstract

The pathogenesis of systemic lupus erythematosus (SLE) is multifactorial and multigenetic. The apoptosis genes, *fas* and *fas* ligand (*fasL*), are candidate contributory genes in human SLE, as mutations of these genes result in autoimmunity in several murine models of this disease. In humans, *fas* mutations result in a familial autoimmune lymphoproliferative syndrome, but defects in FasL have not yet been identified. In this study, DNA from 75 patients with SLE was screened by single-stranded conformational polymorphism analysis for potential mutations of the extracellular domain of FasL. A heterozygous single-stranded conformational polymorphism for FasL was identified in one SLE patient, who exhibited lymphadenopathy. Molecular cloning and sequencing indicated that the genomic DNA of this patient contained an 84-bp deletion within exon 4 of the *fasL* gene, resulting in a predicted 28 amino acid in-frame deletion. Analysis of PBMC from this patient revealed decreased FasL activity, decreased activation-induced cell death, and increased T cell proliferation after activation. This is the first report of defective FasL-mediated apoptosis related to a mutation of the human FasL gene in a patient with SLE and suggests that *fasL* mutations are an uncommon cause of the disease. (*J. Clin. Invest.* 1996. 98:1107–1113.) Key words: Fas ligand • apoptosis • single-stranded conformational polymorphism • systemic lupus erythematosus • autoimmunity

Introduction

The genetic bases of autoimmune disease and lymphadenopathy in C3H/HeJ-*gld*/*gld* and MRL/*lpr*-*lpr* mice (1) have been identified as mutations of the *fas* ligand (*fasL*)[1] and *fas* apoptosis genes, respectively (2–4). Murine and human FasL are members of the TNF family, which exhibits highly conserved regions of sequence corresponding to antiparallel β strands in the extracellular carboxyl terminal domain (5, 6). Analysis of full-length cDNA clones from C3H-*gld*/*gld* mice revealed a single discordancy at base pair 889 (3, 4). This mutation, which is predicted to result in a phenylalanine to leucine substitution in the last β strand of FasL protein, greatly decreases the ability of FasL to induce Fas-mediated apoptosis.

Searches for mutations of *fas* or *fasL* in patients with autoimmune diseases and systemic lupus erythematosus (SLE) have revealed a mutation of the *fas* gene, which was associated with defective apoptosis, in autoimmune lymphoproliferative syndrome (ALPS) (7, 8), but no mutations in *fasL* have yet been reported. In patients with SLE, increased levels of activation-induced cell death (AICD), which can be mediated by interactions of Fas and FasL (9–14), have been reported (15, 16). In addition, increased levels of a soluble form of Fas, which is capable of inhibiting apoptosis, have been reported in patients with SLE (17–19), although some investigators report normal levels (20, 21). We report here that, of 75 patients with SLE screened, 1 patient carried a mutation of the *fasL* gene, which was expressed heterozygously, and had defective AICD and *fasL* apoptosis function.

Methods

Patient selection. DNA was obtained from freshly isolated PBMC from 75 patients who met the revised 1982 American College of Rheumatology (ACR, formerly, the American Rheumatism Association criteria for SLE; reference 22). One African-American patient was identified as having a unique *fas* polymorphism by single-stranded conformational polymorphism (SSCP).

Control patient population. DNA was obtained from freshly isolated PBMC from 52 sex- and race-matched normal control individuals.

Peripheral blood lymphocyte isolation. Peripheral blood (20 cc) was obtained in a preheparinized tube. PBMCs were isolated by Ficoll-Hypaque gradient density centrifugation and the high molecular weight DNA was extracted.

PCR–single-stranded conformational polymorphism analysis. SSCP analysis was carried out as described previously (23–25). Internal primers were designed for amplification of exon 4 of human FasL;

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1. *Abbreviations used in this paper:* 7-AAD, 7-amino actinomycin D; AICD, activation-induced cell death; ALPS, autoimmune lymphoproliferative syndrome; *FasL*, *fas* ligand; SLE, systemic lupus erythematosus; SSCP, single-stranded conformation polymorphism.

P1, 5′-CAGGCAAGTCCAACTCAAGG and P2, 5′-TGGAAAGAATCCCAAAGTGC. The entire exon 4 coding region was amplified with an annealing temperature of 60°C in the presence of α-^{32}P dCTP (Amersham Corp., Arlington Heights, IL). The PCR product was denatured in a buffer consisting of 95% formamide, 20 mM NaOH at 96°C for 3 min, quenched on ice, and then loaded onto a 10% glycerol–6% polyacrylamide nondenaturing gel. After electrophoresis at 20 W for 8 h at room temperature, the gels were transferred to 3M paper (Whatman Inc., Clifton, NJ), dried under vacuum, and autoradiographed.

cDNA and genomic cloning. The full-length 834-bp cDNA and 8.5-kb genomic DNA of human FasL were amplified using the PCR primers 5′-TGACTCACCAGCTGCCATGCAG (−16 to +6) and 5′-GGAAAGAATCCCAAAGTGCTTCTC (870 to 846). The PCR conditions for cDNA cloning were denaturation at 95°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s for a total of 35 cycles. Genomic cloning was carried out using the hot start method (United States Biochem. Corp., Cleveland, OH). The primers and dNTP solutions were incubated with AmpiWax beads at 80°C for 5 min followed by cooling to 4°C. After addition of the rTth DNA polymerase (United States Biochem. Corp.), PCR conditions were denaturation at 94°C for 1 min, and annealing and extension at 70°C for 10 min for a total of 35 cycles. Exon 4 of human FasL was amplified using P1 and P2 that include the entire coding region of exon 4 as described. The PCR products were cloned into the PCR II TA cloning vector (Invitrogen Corp., San Diego, CA).

Sequence analysis. cDNA clones were sequenced in both directions using overlapping primers and a Sequenase Kit (United States Biochem. Corp.) with M13 universal primers and human FasL internal primers; sp-1: 5′-AGAAGGCCTGGTCAAAGG-3′(112–129); sp-2: 5′-GTTCCCTCTCTTCTTCAG-3′(228–211); sp-3: 5′-TCTACCAGCCAGATGCAC-3′(349–366); sp-4: 5′-ACCTATTTGCTTCTCCAA-3′(396–379); sp-5: 5′-ACAGGCCACCCCAGTCCA-3′(394–411); sp-6: 5′-ACCTGTTAAATGGGCCAC-3′(453–436); sp-7; 5′-TCAGGCAAGTCCAACTCAAGG-3′(448–469); sp-8; 5′-TCCAAAGTATACTTCCGG-3′(576–593); sp-9; 5′-CATGTAGACCTTGTGGCTCAG-3′(636–619).

Activation-induced cell death. PBMCs were cultured in RPMI-1640 media supplemented with heat-inactivated 10% FCS, L-glutamine (2 mM), streptomycin (100 μg/ml), and penicillin (100 U/ml) at 37°C in an atmosphere of 95% air, 5% carbon dioxide at 90% relative humidity. For analysis of AICD, cells were cultured for 0, 24, or 48 h in round-bottom, 96-well plates at a density of 5×10^6/ml in the presence or absence of 50 μg/ml PHA (Sigma Chemical Co., St. Louis, MO) and 50 U/ml of IL-2 (Endogen, Inc., Cambridge, MA).

Cell death. Cells were collected by centrifugation at 1,000 rpm for 10 min at 4°C and incubated on ice for 30 min with 5 μg/ml of 7-amino actinomycin D (7-ADD) in FACS® buffer (Becton Dickinson & Co., San Jose, CA) (1% BSA, 0.1% NaAzide, PBS) (26). Stained cells were washed once with FACS® buffer, resuspended in 1% paraformaldehyde/PBS supplemented with 50 μg/ml of actinomycin D, and analyzed flow cytometry.

Cell proliferation. Cell proliferation was estimated in 96-well, flat-bottom plates (Costar Corp., Cambridge, MA) by addition of 0.5 μCi of [^{3}H]thymidine (Dupont-NEN, Boston, MA) 8 h before harvest, measurement of the uptake of [^{3}H]thymidine in a liquid scintillation counter, and calculation of the percentage increase in [^{3}H]thymidine uptake of treated compared to untreated cells. Each result represents the mean of triplicate samples.

Analysis of FasL activity by cytotoxic analysis. FasL activity was estimated by measuring the ability of the test cells to induce apoptosis of the Fas-positive human T lymphocyte cell line, CEM-6. The CEM-6 cells were labeled with 700 μCi of ^{51}Cr-sodium chromate (Amersham Corp.) for 1 h at 37°C. The cells were then washed three times with RPMI 1640 medium supplemented with dialyzed 10% FCS. The ^{51}Cr-labeled A20 cells were added to stimulated PBMC in a 96-well plate at different effector–target ratios. Supernatants were collected after 6 h and the amount of released ^{51}Cr measured using a gamma counter (Beckman Instruments, Inc., Fullerton, CA). Spontaneous release of ^{51}Cr was determined by incubating ^{51}Cr-labeled A20 cells with medium alone, and maximum release was determined by adding SDS to a final concentration of 0.05%. The percentage of specific ^{51}Cr release was calculated as follows: percent specific lysis = (experimental ^{51}Cr release − spontaneous ^{51}Cr release)/(maximum ^{51}Cr release − spontaneous ^{51}Cr release).

Expression of Fas ligand of normal and mutant allele. The full-length human cDNA encoding both the normal and mutated Fas ligand allele from the SLE patient were cloned into the pcDNA1 expression vector (Invitrogen Corp.). After cloning, the sequence was confirmed to match the original sequence for the normal and mutated Fas ligand from the patient. Transient transfections were performed using the PerFect™ transfection kit (Invitrogen Corp.) according to the manufacturer's protocol. Plasmid DNA was prepared for transfection by adjusting the concentration to 1 mg/ml in sterile water. The lipid was prepared by mixing 24 μl of the Optimum™ lipid solution (2 mg/ml) with 2 ml lipid buffer solution in a siliconized 10-ml polystyrene tube (2059; Fisher Scientific Co., Fairlawn, NJ). 8 μl of the plasmid DNA was mixed with 2 ml of lipid buffer in a fresh polystyrene tube, resulting in a final concentration of 4 μg/ml and a 6:1 (wt/wt) ratio of lipid to plasmid. For cotransfection experiments, the DNA was mixed before combining with the lipid solution. The cells were prepared for transfection as recommended. Briefly, 10^5 Cos-7 cells were added to each well in a six-well microtiter plate. This resulted in a 50% confluent cell layer after culture for 24 h. Cells were washed in serum-free medium twice and once with 2 ml PBS to remove serum that may interfere with transfection. 2 ml of transfection solution was added per well. The cells were then incubated for 4 h and then the transfection solution was removed and replaced with DMEM + 10% FCS. The cells were incubated for an additional 72 h. The supernatant was collected and stored at −20°C until assayed.

Transfection efficiency. Transfection efficiency was determined using a β-galactosidase reporter gene cotransfected with the Fas ligand expression vectors using the PerFect™ transient transfection technique. The pcDNA3.1/His/lacZ (Invitrogen Corp.) β-galactosidase reporter gene activity was assayed after a 24-h culture in separate experiments using cells transfected as described above. Cells were fixed in 2% formaldehyde/0.2% glutaraldehyde in PBS, washed, and then stained by incubation with X-Gal for 12 h. The transfection efficiency was determined by counting the percentage of stained cells in at least five random fields of view. The transfection efficiency was equivalent (10–12%) in all cotransfection experiments.

Results

Clinical evaluation. The patient exhibiting an SSCP for FasL was a 64-yr-old, African-American male with SLE manifested by malar rash, arthritis, serositis, renal disease, leukopenia, positive LE cell preparation and anti-DNA antibodies, and a positive antinuclear antibody.

The patient first presented at 52 yr with a temperature of 102.4°F, weight loss, polyarthralgias, fatigue, and pleuritic chest pain. A physical examination revealed multiple anterior cervical nodes, a 1 × 1 cm left supraclavicular node, bilateral 2 × 3 cm firm axillary nodes, and bilateral inguinal lymphadenopathy. The spleen was not enlarged by physical exam or by liver–spleen scan. At the initial presentation, laboratory evaluation revealed H/H = 10.6/30.5, WBC count of 3,100, 1^+ proteinuria, positive antinuclear antibody with a titer of 1:300 in a homogeneous pattern and a Complement hemolytic 50% (CH50) = 130 (150–250 nl). A bone-marrow aspiration revealed LE cells, and chest x-ray revealed bilateral pleural effusion. A biopsy of the right anterior cervical lymph node revealed atypical lymphoid hyperplasia with reactive germinal centers and atypical large mononuclear cells in cortical areas.

An ophthalmologic examination revealed cotton-wool spots near the optic disc, which were considered consistent with lupus retinopathy.

The patient initially responded well to treatment to prednisone, which resulted in resolution of weight loss, fever, arthralgia, fatigue, and pleuritis. Due to persistent proteinuria for 4-yr refractory to prednisone therapy, the patient was started on 2 mg/d chlorambucil for 3 yr. At this point, proteinuria resolved and the creatinine clearance was stable at 64 ml/min and the patient was maintained on 5 mg/d prednisone.

Lymphoproliferative disease. 20 of the 75 SLE patients were followed prospectively for development of lymphadenopathy. 12 patients were noted to exhibit lymphadenopathy at some time during the course of SLE and 8 exhibited no detectable lymphadenopathy during a disease time course, ranging from 10 to 22 yr. Lymphadenopathy was reported once in 7 patients, twice in 1 patient, 3 times in 1 patient, and with active disease in 3 patients. The size and extent of lymphadenopathy in SLE patients with lymphadenopathy was always less compared with the patient with the Fas ligand mutation.

Identification of a FasL polymorphism by SSCP analysis. DNA from 75 patients with SLE (42 Caucasian and 33 African American) and 52 sex- and race-matched normal controls were analyzed for polymorphisms and potential mutations of the fourth exon of the FasL by SSCP analysis using a labeled FasL fourth-exon probe. All patients and normal controls exhibited an SSCP migration pattern consisting of two upper bands (Fig. 1). In addition, one African-American SLE patient exhibited two unique lower bands indicating the potential presence of a heterozygous mutation of the FasL. None of the other 75 SLE patients exhibited an SSCP polymorphism for the fourth exon of FasL.

DNA and cDNA characterization of the FasL mutation. Full-length FasL genomic and cDNA clones were obtained from the patient exhibiting the unique SSCP pattern and sequenced in both directions. There was an 84-bp deletion corresponding to bp 472–555 of the cDNA clone and an identical deletion in exon 4 of the genomic clones (Fig. 2, *A–C*). There were no other mutations in either the cDNA clone or the genomic clones of the SLE patient compared to the published human FasL sequence (27). The mutation occurred heterozygously since PCR amplification of genomic DNA from the patient yielded both a 384-bp, normal-size exon 4 FasL product as well as the product with the 84-bp deletion (Fig. 2 *B*). Identical results were obtained using these primers for PCR amplification of cDNA from patient and controls indicating that transcription of both the normal and mutant FasL genes occurred. The 84-bp deletion was predicted to result in an in-frame deletion of 28 amino acids and a truncated extracellular domain of FasL (Fig. 2 *C*).

Abnormal proliferation and activation-induced apoptosis. To determine if the heterozygous expression of the mutated FasL resulted in abnormal apoptosis, PBMCs from the SLE patient and normal controls were stimulated with PHA (50 μg/ml). PBMCs obtained from the SLE patient with the FasL mutation underwent a higher PHA-stimulated response than PBMCs obtained from controls (Fig. 3). At day 0, freshly isolated PBMCs from both normal controls and the SLE patient with the FasL mutation exhibited low apoptosis as indicated by flow-cytometric analysis of 7-AAD (Fig. 4). After stimulation for 2 d or 4 d, the level of apoptosis in both the stimulated and unstimulated cells obtained from the patient with SLE with the Fas mutation, and the unstimulated control cell, remained low, whereas the level of apoptosis in stimulated control cells was greatly increased.

Functional analysis of FasL. To determine if the FasL produced by PBMCs from the SLE patient exhibited a functional defect, freshly isolated PBMCs were stimulated with PHA and IL-2, and then analyzed for their ability to kill ^{51}Cr-labeled, Fas-positive CEM-6 target cells. Stimulated PBMCs obtained

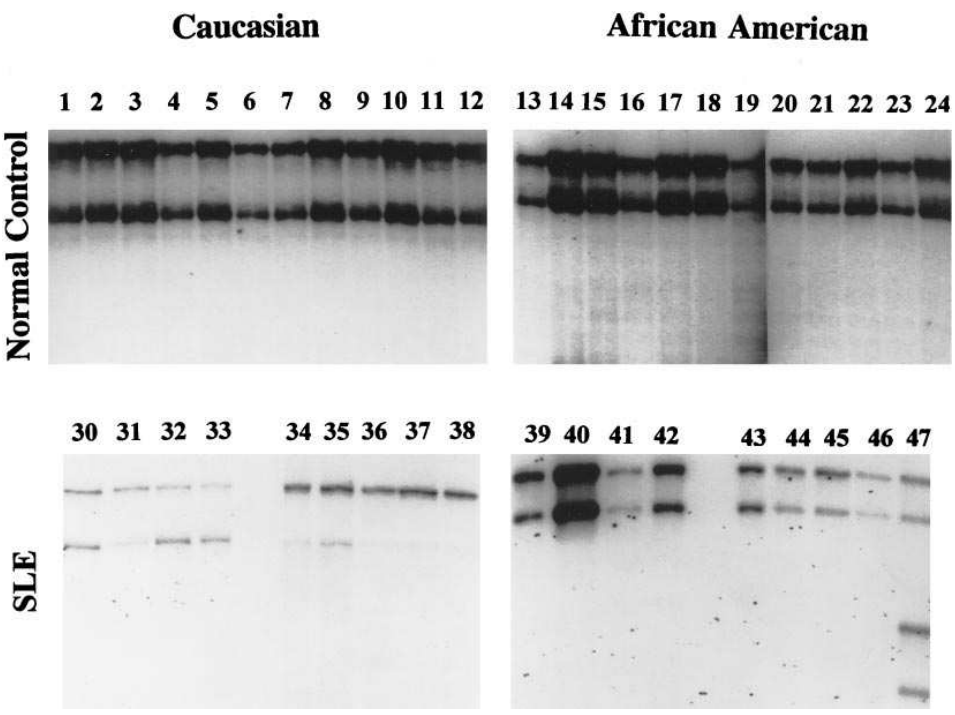

Figure 1. PCR-SSCP analysis of SLE patients and normal controls. 75 patients with SLE and 55 normal controls were screened by SSCP analysis of the extracellular domain of FasL using a pair of FasL primers that include most of the coding region found in the fourth exon of FasL. A typical SSCP analysis for 12 Caucasian and 12 African American normal controls and 9 Caucasian and 9 African American SLE patients are shown. One patient (*lane 47*) out of 75 was identified as having an abnormal SSCP pattern indicating the possible presence of a heterozygous mutation in the FasL gene.

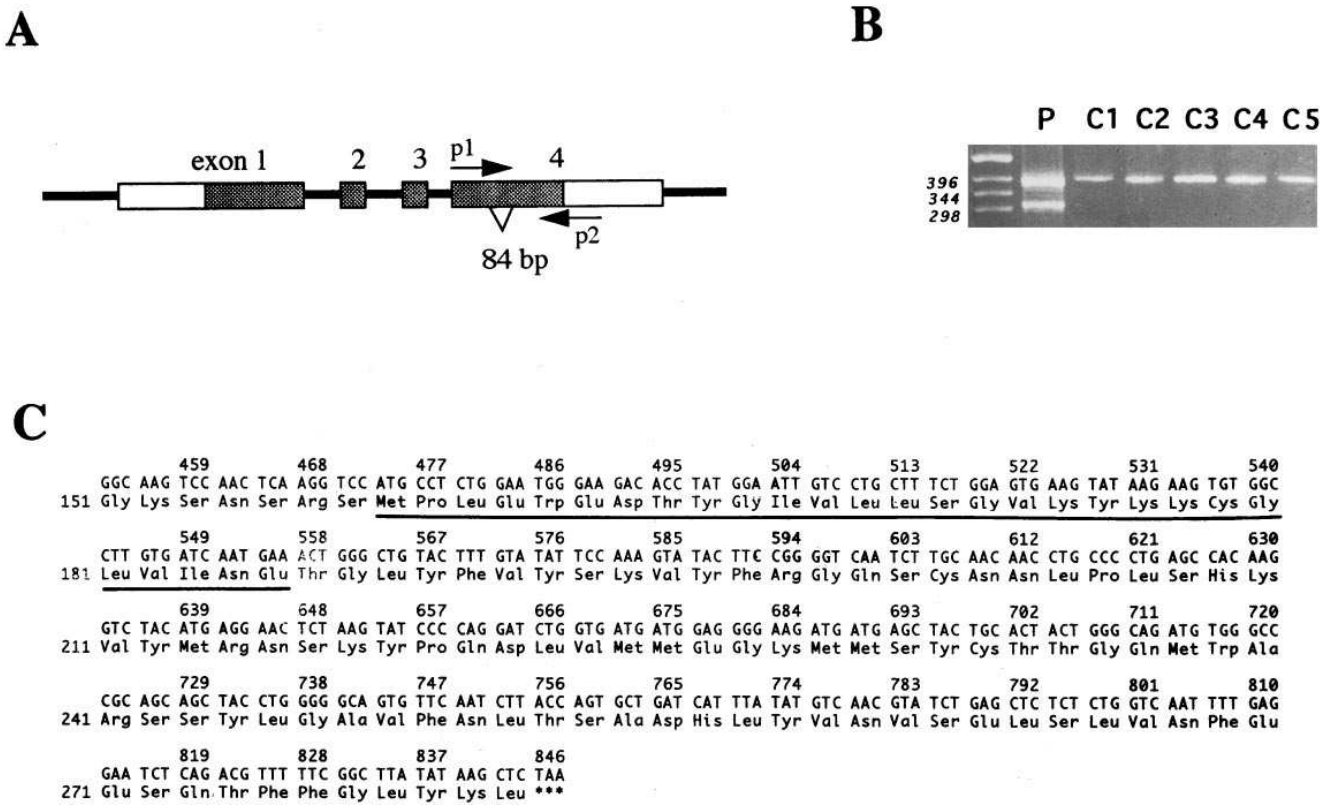

Figure 2. Structure of human FasL genomic DNA and sequence analysis. (*A*) Structure of the FasL genomic DNA, location of primers used for PCR amplification, and the location of the deletion. (*B*) Amplification of genomic DNA from the patient (*P*) with the unique SSCP analyses and five other SLE patients (*C1–C5*) using primers p1 and p2. (*C*) The 84-bp deletion (*underlined*) leads to a 28 aa in-frame truncation of human FasL exon 4.

from the patient with SLE with the FasL mutation exhibited lower levels of FasL activity in this assay than stimulated PBMCs from either eight other SLE patients (Fig. 5 *A*) or from six normal control individuals (Fig. 5 *B*).

Functional analysis of cloned normal and mutant FasL alleles. To determine if the normal and mutant FasL alleles from the SLE patient exhibited different Fas apoptosis function, the full-length cDNA alleles were cloned into the pcDNA1 expression vector. The clones were cotranfected with the β-galactosidase reporter gene into Cos-7 cells, and supernates were collected 72 h later. The FasL apoptosis function of the supernatant was determined by incubation with ^{51}Cr-labeled Fas^{+} CEM-6 target cells. Supernatant from the Cos-7 cells that were transiently transfected with the normal allele exhibited high specific cytotoxic activity when incubated with the CEM-6 target cells (Fig. 6). In contrast, supernate collected from the Cos-7 cells after transient transfection with the mutant allele exhibited very low activity in killing the ^{51}Cr-labeled CEM-6 target cells. Supernatant was also collected from Cos cells that were cotransfected with an expression vector containing the normal allele and the expression vector containing the mutant allele. The low specific cytotoxicity of the normal allele was suppressed by the presence of the mutant allele, suggesting that the mutant allele can function as a dominant negative. Transfection efficiency was ~10–12% for all transfection experiments as indicated by cotransfection with a β-galactosidase reporter construct with the expression vector for the normal allele, mutant allele, or both normal and mutant alleles (Fig. 6 *B*).

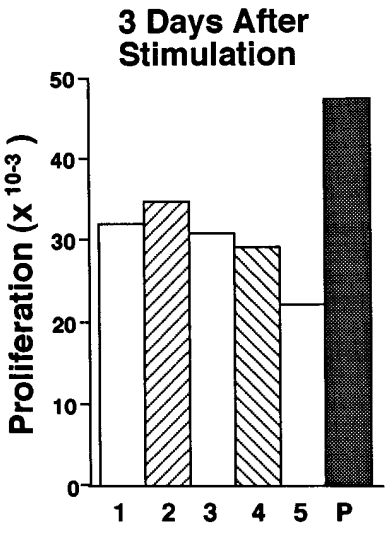

Figure 3. Mitogen-induced proliferation of PBMCs. Freshly isolated PBMC from normal controls (*1–5*) and the SLE patient (*P*) were cultured with PHA for 3 d. Proliferation was determined by incorporation of tritiated thymidine added for the final 8 h of culture. There was an increase in the uptake of tritiated thymidine by the cells of the SLE patient (*P*) compared with those of five normal controls ($P <$ 0.01). Each result represents the mean of two separate experiments performed in triplicate.

Discussion

SSCP has been used to identify point mutations in a number of genes (23–25) and PCR-SSCP recently has been used to iden-

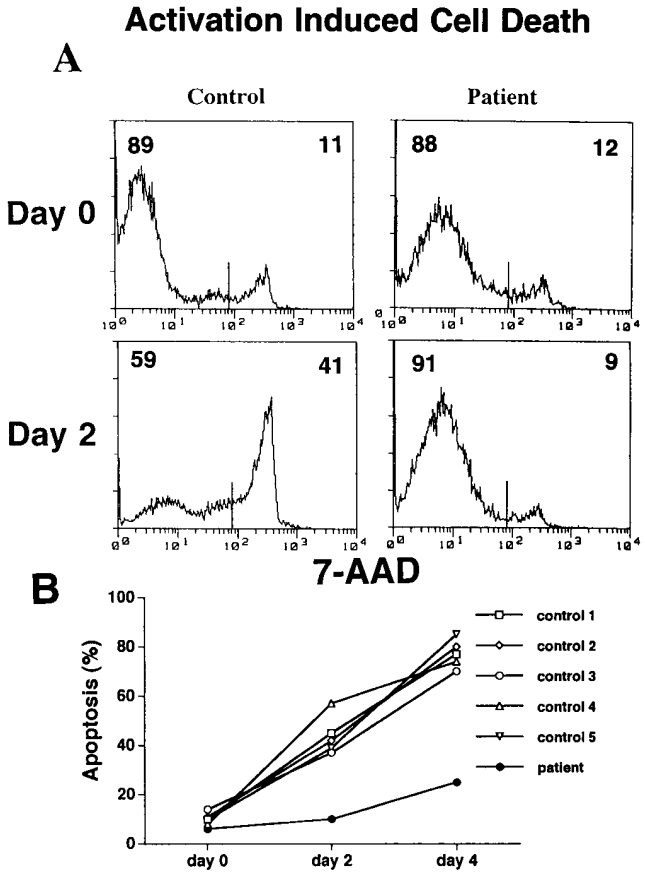

Figure 4. Defective activation-induced apoptosis. Measurement of cell death using 7-AAD-staining. (*A*) Apoptosis analysis of unstimulated cells and cells stimulated with PHA/IL-2 for 2 d from one control and the SLE patient with the FasL mutation. (*B*) Percent apoptosis, determined by 7-AAD staining at 0, 2, and 4 d after stimulation with PHA/IL-2 of PBMC obtained from five normal controls and the SLE patient. Analysis was carried out on two different samples from the SLE patient with similar results.

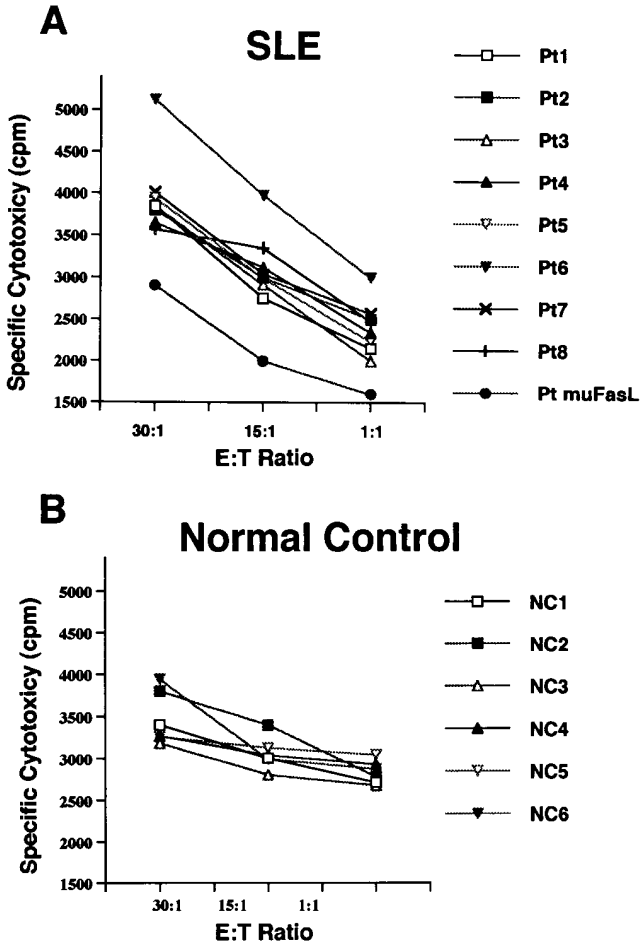

Figure 5. FasL function. FasL activity as determined by the specific lysis of different effector cell:target cell ratios (*E:T*) of PHA-stimulated PBMCs to ^{51}Cr-labeled FasL–sensitive CEM-6 cells. PBMCs from the SLE patients and normal controls were stimulated with PHA for 48 h. (*A*) Specific cytotoxicity of the SLE patient with the FasL mutation compared with eight other SLE patients. (*B*) Specific cytotoxicity of six normal control individuals.

tify mutations in CD40 ligand (28). The present experiments use SSCP to screen patients with SLE for a defect in the fourth exon of FasL and a heterozygous mutation was found in 1 of 75 patients, but in none of the 52 normal control patients. This does not exclude the possibility that a FasL mutation might be observed with a low frequency of $<$ 1–2% of normal controls, and a larger patient population would need to be analyzed to determine if mutations of FasL were more frequent in SLE patients compared with control patients. Thus, SSCP is a simple procedure capable of detecting heterozygous mutations of FasL.

Signaling through Fas requires the expression of FasL as a trimer that crosslinks three Fas molecules (29). The formation of a Fas trimer causes conformational changes in the intramembrane and intracellular regions of Fas and binding of signal transduction proteins including FADD and RIP (30–32). It has been proposed that the defective apoptosis associated with heterozygous mutations in individuals with ALPS is due to defective trimerization of Fas due to a dominant negative effect of the mutant allele (7, 8). The present experiments have analyzed FasL function in a patient with SLE containing a heterozygous mutation of FasL. There was high apoptosis function in Cos-7 cell supernates expressing the normal allele and low FasL apoptosis function in Cos-7 cell supernates expressing the mutant allele. The mutant allele appeared to act as a dominant negative since there was low FasL function of Cos-7 cells expressing both the normal allele and the mutant allele. We therefore propose that the heterozygous mutation of FasL in the patient with SLE inhibits production of the functional FasL trimer that is essential for apoptosis signaling through Fas (3, 4).

The extent of lymphadenopathy was increased in the patient with SLE with the mutant FasL allele compared to other SLE patients. 20 of the 75 SLE patients in this study were followed prospectively for development of lymphadenopathy for 10–22 yr. Although lymphadenopathy was a common feature of SLE and 12 patients exhibited lymphadenopathy at some time during the course of SLE, the size and extent of lymphadenopathy in SLE patients with lymphadenopathy was always smaller compared with the patient with the FasL mutation (P.H. Schur, unpublished observations). However, the lymph-

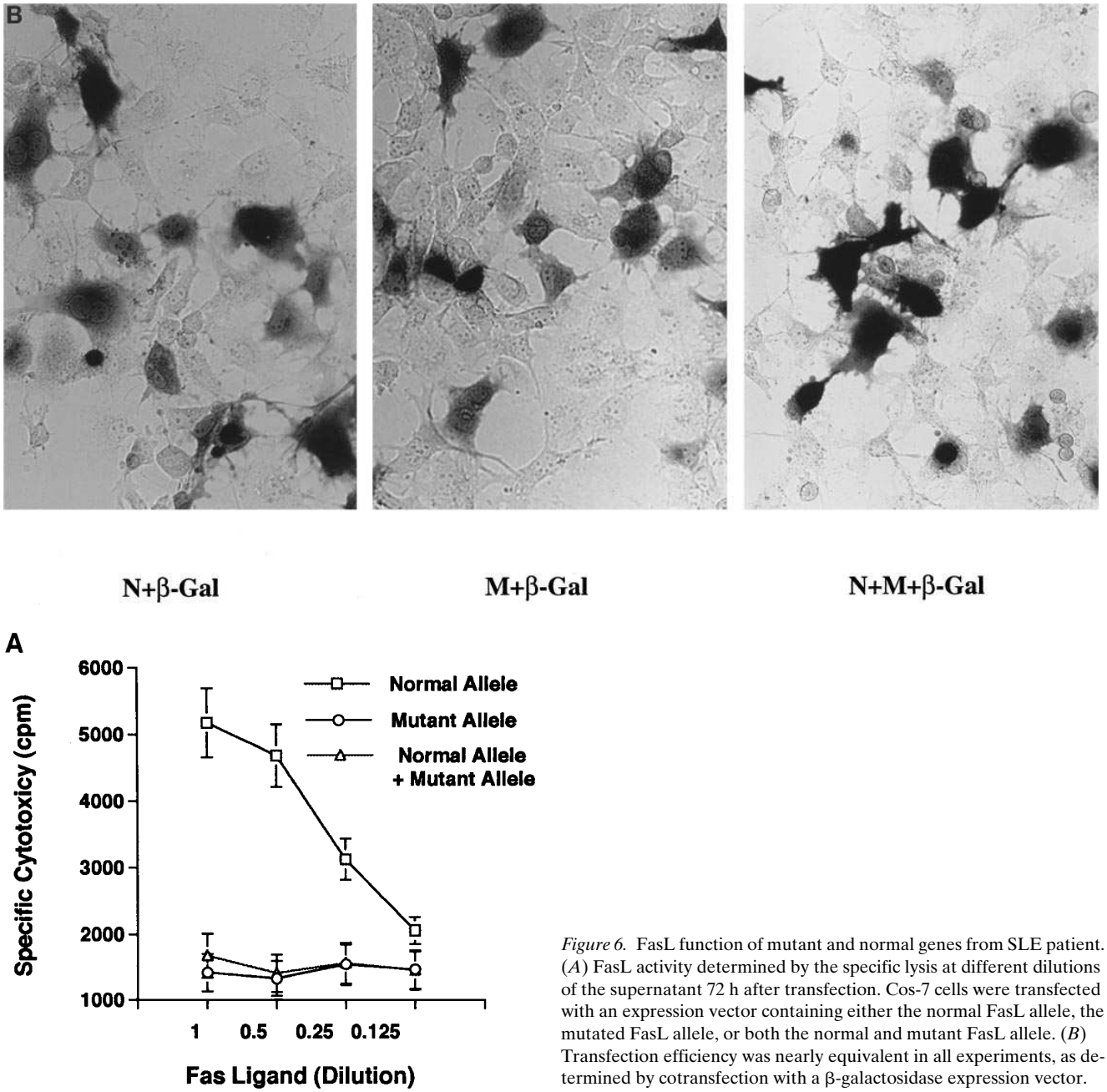

Figure 6. FasL function of mutant and normal genes from SLE patient. (*A*) FasL activity determined by the specific lysis at different dilutions of the supernatant 72 h after transfection. Cos-7 cells were transfected with an expression vector containing either the normal FasL allele, the mutated FasL allele, or both the normal and mutant FasL allele. (*B*) Transfection efficiency was nearly equivalent in all experiments, as determined by cotransfection with a β-galactosidase expression vector.

adenopathy of the patient with the FasL mutation appears to be less than that observed in the patient with ALPS (7, 8). Also, the patient with SLE and the FasL mutation did not exhibit an increase in $CD4^-CD8^-CD3^+$ T cells compared with other SLE patients and normal controls, whereas the ALPS patients do exhibit an increased percentage of $CD4^-CD8^-CD3^+$ T cells (data not shown). Despite these differences between the SLE patient with the heterozygous FasL mutation and the ALPS patients with the heterozygous Fas mutation, the patient reported here may more appropriately be considered an ALPS patient since the lymphadenopathy in adults with autoimmune disease may be less developed than observed in children. Therefore, we conclude that this elderly male patient with lymphadenopathy and autoimmunity represents an adult type of ALPS, and such patients may exhibit heterozygous mutations in Fas or FasL. A larger number of elderly male or female patients with lymphadenopathy and autoimmunity need to be analyzed to determine if such a subpopulation of patients exist.

SLE is a multifactorial disease that is thought to have a multigene basis together with a gene dosage effect (33). The findings of mutated *fas* and *fasL* genes indicated that defective apoptosis after activation may be one of the factors that predispose to development of SLE. The role of defective apoptosis in human SLE has been controversial, however, since statistically significant AICD apoptosis defects or statistically significant alterations in Fas expression in peripheral blood in patients with SLE have not been detected (34, 35). It is possible that defective Fas or FasL production may only occur in a small number of patients or in patients with a certain subtype

of disease and that a statistically significant difference would not be noticed in studies of small numbers of SLE patients exhibiting heterogeneous disease. Increased expression of the apoptosis-inhibiting gene, *Bcl-2*, has been reported in PBMC from patients with SLE (36, 37). A cell line established from a patient with SLE has been found to exhibit decreased AICD and to produce high levels of a soluble form of Fas, which lacks the transmembrane exon (38). Thus, defective apoptosis resulting from any one of several mechanisms could lead to autoimmune disease. We found a mutation of FasL in 1 of 75 patients with SLE, which indicates that this is an infrequent cause of SLE, but other mutations of FasL may exist in certain patients but not be detected by SSCP analysis.

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