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Previous reports that diabetogenic lymphocytes did not induce diabetes in nonobese diabetic (NOD)-lpr mice suggested the critical role of Fas-Fas ligand (FasL) interaction in pancreatic β cell apoptosis. However, recent works demonstrated that FasL is not an effector molecule in islet cell death. We addressed why diabetes cannot be transferred to NOD-lpr mice despite the nonessential role of Fas in β cell apoptosis. Lymphocytes from NOD-lpr mice were constitutively expressing FasL. A decrease in the number of FasL+ lymphocytes by neonatal thymectomy facilitated the development of insulitis. Cotransfer of FasL+ lymphocytes from NOD-lpr mice completely abrogated diabetes after adoptive transfer of lymphocytes from diabetic NOD mice. The inhibition of diabetes by cotransferred lymphocytes was reversed by anti-FasL Ab, indicating that FasL on abnormal lymphocytes from NOD-lpr mice was responsible for the inhibition of diabetes transfer. Pretreatment of lymphocytes with soluble FasL (sFasL) also inhibited diabetes transfer. sFasL treatment decreased the number of CD4+CD45RB<sup>low</sup> cells and increased the number of propidium iodide-stained cells among CD4+CD45RB<sup>low</sup> cells, suggesting that sFasL induces apoptosis on CD4+CD45RB<sup>low</sup> “memory” cells. These results resolve the paradox between previous findings and suggest a new role for FasL in the treatment of autoimmune disorders. Our data also suggest that sFasL is involved in the deletion of potentially hazardous peripheral “memory” cells, contrary to previous reports that Fas on unmanipulated peripheral lymphocytes is nonfunctional.

Materials and Methods

Fas-deficient NOD-lpr mice

NOD mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and were maintained in a specific pathogen-free environment in the vivarium of Samsung Medical Center. The incidence of diabetes in female and male NOD mice was about 70% and 30%, respectively, at 24 wk of age. NOD mice were bred with MRL-lpr/lpr mice and F<sub>1</sub> mice were backcrossed to NOD mice. N2 (backcross 1, BC1) mice heterozygous for Fas with an early transposable element (ETn) were selected by Southern blot or PCR typing of tail DNA and then backcrossed repeatedly to NOD mice to derive NOD-lpr/wild (lpr/w) BC8 mice. They were intercrossed to derive NOD-lpr/w as an experimental group and NOD-lpr/w mice as a control group. Heterozygous and homozygous mice were typed again by using Southern blot analysis or PCR amplification of tail DNA. For Southern blot analysis, 10 µg of tail DNA was digested with EcoRI and run on 0.6% agarose gel. After capillary transfer for 18 h, the membrane was denatured with 0.4 N NaOH followed by neutralization in 0.2 M Tris (pH 7.5), 2× SSC, 0.1% SDS. After UV cross-linking and prehybridization at 65°C, the membranes were hybridized for 18 h with a EcoRI-HindIII fragment of murine Fas cDNA labeled with [32P]dCTP (Amersham, Buckinghamshire, U.K.). After washing with 2× SSC, 2× SSC, 0.1% SDS and then with 0.1× SSC, 0.1% SDS, the membranes were exposed to x-ray films. A 13-kb band indicated the presence of the normal Fas allele, and a 11-kb band indicated that of a mutated Fas allele because of a new EcoRI restriction site in the inserted ETn sequence (9) (data not shown). PCR was conducted using a primer set to select heterozygotes for the mutated Fas allele (forward, CAGCAGGAAATCTTATGAGCT; reverse, CTGG CAAACGCAACGGTTCG). Another primer set was used to distinguish heterozygotes and homozygotes for the mutated Fas allele (forward, CAGCAGGAAATCTTATGAGCT; reverse, CGAGAGATGCTAAGCAGCAG) (2). MHC haplotype of the BC mice was determined by PCR amplification of tail DNA using a primer set specific for I-E<sub>α</sub> (forward, ATGACGCTCCTGATGG; reverse, GGAGAGACAGCAGCCTCAGC) (10) to confirm homozygosity for H-2<sup>β2</sup>. A one-way MLC was employed to verify genetic homogeneity between NOD mice and NOD-lpr/lpr (-lpr/w) mice.
All animal studies were approved by the Institutional Review Board of Samsung Medical Center.

Adoptive transfer

Adoptive transfer of diabetes was conducted according to a previous report (11). In brief, 2 × 10⁷ splenocytes from diabetic female or male NOD mice were infused into the tail vein of each 8- to 10-wk-old NOD or NOD-lpr/lpr (−/−) mouse of the same sex. Recipient mice were sublethally irradiated (750 rad) using a Cesium irradiator (IBL 437, CIS Biointernational, Gif-sur-Yvette, France) 16 h before the transfer of splenocytes. The incidence of diabetes was above 90% at 4 wk after adoptive transfer to NOD mice in our previous experiments. For cotransfer experiments, 2 × 10⁷ splenocytes from 4- to 5-mo-old NOD-lpr/lpr mice and the same number of splenocytes from diabetic NOD mice of the same sex were transferred together to the irradiated recipient NOD mice of the same sex. Splenocytes from 4- to 5-mo-old NOD-lpr/w littermate mice were cotransferred in control experiments.

RNase protection assay

RNA from lymphocytes was hybridized with a 32P-labeled riboprobe prepared by in vitro transcription of a linearized expression vector harboring FasL cDNA. The intensity of the protected band after hybridization was standardized against that of the mouse β-actin band.

Neonatal thymectomy of NOD-lpr/lpr mice

Neonatal thymectomy of NOD-lpr/lpr mice was performed as described previously (12). In brief, the thymus was removed by a wire loop through a small longitudinal incision over the sternum before 1.5 days after birth. The thymus was removed by a wire loop through and also by inspecting the thymus at the time of sacrifice. The mice with the frequency of CD3⁺ T cells in heparinized peripheral blood at 7–8 wk of age and also by inspecting the thymus at the time of sacrifice. The mice with CD3⁺ T cell percentage above twice that of nude mice were excluded from the study. Donor lymphocytes were transferred to 8- to 10-wk-old neonatal thymectomized NOD-lpr/lpr of the same sex after sublethal irradiation.

Insulitis scoring

To determine the severity of insulitis, >30 pancreatic islets from three or more parallel sections of different cut levels were analyzed per mouse. The degree of insulitis was classified into four categories: 0, no insulitis; 1, periinsulitis with or without minimal lymphocytic infiltration in islets; 2, invasive insulitis with islet destruction (>50%); 3, islet destruction (>50%).

Anti-FasL Ab treatment

A hybridoma producing K10 anti-FasL Ab (13) was injected i.p. into nude mice pretreated with Pritane (Sigma, St. Louis, MO). Ab was purified using a protein A-Sepharose (Pharmacia, Uppsala, Sweden) column. Bound IgG was eluted with 50 mM glycine-HCl, pH 2.5. The collected fraction was dialyzed against PBS and then filter-sterilized. Ab (1 mg) was injected i.p. three times a week into NOD mice before and after cotransfer. Control NOD mice were treated with the same dose of mouse IgG (Sigma) during the cotransfer experiments. Validity of the anti-FasL Ab preparation was confirmed by an almost complete abrogation of hepatitis after injection of 15 mg/kg Con A (Sigma) to C57BL6 and NOD mice (data not shown).

Ex vivo treatment with sFasL

sFasL was produced according to a previous report (14) with modifications. In brief, CD8 signal sequence was attached to Pro137 of human FasL. A hybridoma producing K10 anti-FasL Ab (13) was injected i.p. into nude mice pretreated with Pritane (Sigma, St. Louis, MO). Ab was purified using a protein A-Sepharose (Pharmacia, Uppsala, Sweden) column. Bound IgG was eluted with 50 mM glycine-HCl, pH 2.5. The collected fraction was dialyzed against PBS and then filter-sterilized. Ab (1 mg) was injected i.p. three times a week into NOD mice before and after cotransfer. Control NOD mice were treated with the same dose of mouse IgG (Sigma) during the cotransfer experiments. Validity of the anti-FasL Ab preparation was confirmed by an almost complete abrogation of hepatitis after injection of 15 mg/kg Con A (Sigma) to C57BL6 and NOD mice (data not shown).

Flow cytometry

A total of 1–3 × 10⁶ lymphocytes were incubated with 10 μg/ml biotinylated anti-CD45RB Ab (PharMingen). They were then incubated with FITC-streptavidin (Vector, Burlingame, CA) and PE-anti-CD4 Ab (PharMingen) with or without 2.5 μg/ml propidium iodide (PI). Cells were gated by forward and side scatters, and further gated on CD4. CD45RBlow cells were defined as the dullest staining 20% of the untreated CD4⁺ cells (17). To count PI-stained cells, cells were gated on CD4 and CD45RB. For triple-colored analyses, Cy-Chrome-streptavidin (PharMingen), PE-anti-CD4 Ab, and FITC-anti-Fas Ab (PharMingen) were used for the second incubation. To examine the fraction of double-negative (CD4⁻ CD8⁻) B220⁺ cells in NOD-lpr/lpr cells, splenocytes were first incubated with anti-B220 Ab (PharMingen). Then, they were incubated with FITC-anti-rat IgG. After blocking any free binding site by adding purified rat Ig (PharMingen), cells were incubated with PE-anti-CD4 Ab and PE-anti-CD8 Ab (PharMingen).

Statistical analyses

The incidence of diabetes was plotted according to Kaplan-Meier method. The incidences were compared between the two groups using the log-rank test. Student’s t test was employed to compare mean values between the two groups. Values of p < 0.05 were regarded as statistically significant.

Results

NOD-lpr/lpr mice

NOD-lpr/lpr mice older than 3 mo had classical phenotypes of lpr mice such as generalized lymphadenopathy, splenomegaly, thymomegaly, nephritis, and increased CD4⁻ CD8⁻ B220⁺ cell fraction (>60%), while littermate NOD-lpr/w mice did not have such phenotypes (12). None of Fas-deficient female NOD-lpr/lpr mice (0/12) developed diabetes or insulitis up to 8 mo of age, while 60% (6/10) of control female NOD-lpr/lpr mice developed diabetes (p = 0.0001). None of the 12 NOD-lpr/lpr mice developed diabetes in 8 wk after adoptive transfer, while 73% (8/11) of the control NOD-lpr/w littermates developed diabetes during the same observation period, in three independent experiments showing similar results (Fig. 1B).

Adoptive transfer to NOD-lpr/lpr mice

Because the absence of diabetes and insulitis in NOD-lpr/lpr mice could have been due to an accumulation of unusual (double-negative) cells and distortion of lymphocyte development (8, 18), we transferred lymphocytes from diabetic NOD mice to 8- to 10-wk-old irradiated NOD-lpr/lpr mice. NOD-lpr/lpr mice were resistant to diabetes transfer, confirming previous results (1, 2). None of the 12 NOD-lpr/lpr mice developed diabetes in 8 wk after adoptive transfer, while 73% (8/11) of the control NOD-lpr/w littermates developed diabetes during the same observation period, in three independent experiments showing similar results (Fig. 1B). Pancreatic islets of NOD-lpr/lpr mice were either completely free of
insulitis or showed mild peri-insulitis after adoptive transfer, while NOD-lpr/w mice had florid insulitis and/or paucity of pancreatic islets because of extensive destruction after adoptive transfer (data not shown).

Inhibition of diabetes transfer by FasL constitutively expressed on lpr lymphocytes

Because these results apparently contradicted recent reports that anti-FasL Ab treatment did not inhibit diabetes in NOD mice and Fas-deficient neonatal pancreas was destroyed by autoreactive lymphocytes in diabetic NOD mice (5, 6), we studied if abnormal (double-negative) lymphocytes in NOD-lpr/lpr mice were interfering with the adoptive transfer experiments (Fig. 1B). We investigated whether abnormal lymphocytes from NOD-lpr/lpr mice were expressing FasL like lpr mice of different backgrounds (19, 20). RNase protection assays showed that lymphocytes from 4-mo-old NOD-lpr/lpr mice were constitutively expressing high level of FasL mRNA (Fig. 2A). Because such FasL-expressing cells might affect the outcome of adoptive transfer by inducing apoptosis on transferred lymphocytes, neonatal thymectomy of NOD-lpr/lpr mice was performed to eliminate or reduce the effects of such abnormal lymphoid cells. Neonatal thymectomized NOD-lpr/lpr mice still did not develop diabetes after adoptive transfer (n = 6). However, histological analysis revealed insulitis after adoptive transfer that was not observed in unmanipulated NOD-lpr/lpr mice consistently in four independent experiments, suggesting that Fas-FasL interaction is not necessary at least for insulitis. The average insulitis score was 0.74 ± 0.39 at 6–8 wk after adoptive transfer (mean ± SD, n = 6), which was significantly higher than that after adoptive transfer to unmanipulated NOD-lpr/lpr mice (0.02 ± 0.02, n = 6) (p < 0.01) (Fig. 2B). In the recipient NOD-lpr/lpr mice with previous neonatal thymectomy, exocrine pancreatitis or cellular depletion of the splenic white pulp was not observed, despite the clear evidence of insulitis, suggesting that the insulitis was a specific phenomenon and not a manifestation of generalized inflammatory responses such as graft-vs-host disease (GVHD).

To further prove that abnormal lymphoid cells in NOD-lpr/lpr mice inhibit transfer of autoimmune diabetes, lymphocytes from NOD-lpr/lpr mice were cotransferred to irradiated NOD mice together with splenocytes from diabetic NOD mice. Strikingly, none of the recipient NOD mice (n = 9) became diabetic in 8 wk after cotransfer, in three independent experiments showing similar results. In contrast, six of eight NOD mice (75%) became diabetic after cotransfer of lymphocytes from NOD-lpr/w mice in control experiments (Fig. 3A). Furthermore, administration of anti-FasL K10 Ab (13) (1 mg/injection, three times a week) reversed the inhibition of diabetes by cotransferred lymphocytes from NOD-lpr/lpr mice, indicating that abnormal FasL+ lymphocytes from NOD-lpr/lpr cells are responsible for the inhibition of diabetes transfer and sFasL is not an effector molecule in β cell apoptosis (Fig. 3A). Control Ab did not affect the abrogation of diabetes transfer by lymphocytes from NOD-lpr/lpr mice. Recipient NOD mice of NOD-lpr/lpr lymphocytes showed signs of GVHD such as lymphoid infiltration around the portal triad of the liver and cellular depletion of splenic white pulp (data not shown), which has been described in lymphocyte transfer from lpr mice to wild-type congenic mice (21). As expected, these signs of GVHD were almost completely abrogated by anti-FasL K10 Ab treatment, which is consistent with previous reports that FasL constitutively expressed on abnormal double-negative lymphocytes in lpr mice is responsible for GVHD-like syndrome (19, 20). Recipient mice of NOD-lpr/w lymphocytes did not show signs of GVHD as expected, further indicating genetic homogeneity between NOD mice and NOD-lpr/lpr (lpr/w) mice.

FIGURE 2. A, Expression of FasL in lymphocytes from NOD-lpr/lpr mice. Lymphocytes from NOD-lpr/lpr mice were constitutively expressing huge amounts of FasL transcript detected by RNase protection assays. Arrows indicate the size of the protected bands, which were shorter than that of the respective probes. B, Development of insulitis in neonatal thymectomized NOD-lpr/lpr mice after adoptive transfer (□, no insulitis; ■, peri-insulitis; ▴, insulitis with islet destruction < 50%; ▼, islet destruction > 50%). The mean insulitis score in the neonatal thymectomized recipients was significantly higher than that in unmanipulated mice.

Inhibition of diabetes transfer by ex vivo treatment with sFasL

Although these results suggested the possibility of FasL or FasL-expressing cells as a therapeutic agent for autoimmune diabetes, they cannot be directly employed because of the adverse effects of systemic FasL such as GVHD or hepatitis as observed in this study or other reports (22, 23). To exploit the effect of FasL on autoreactive lymphocytes without adverse effects of systemic FasL, we investigated if ex vivo pretreatment of splenocytes from diabetic
NOD mice with sFasL has similar inhibitory effects on diabetes transfer. Pretreatment of splenocytes with sFasL for 16 h decreased the incidence of diabetes after adoptive transfer from 75% (6/8, pretreated with control CHO K1 cell supernatant) to 22% (2/9) \((p < 0.01)\) (Fig. 3B), strongly indicating that sFasL could be a therapeutic or preventive agent against autoimmune diabetes. As expected, recipient mice of the lymphocytes treated ex vivo with sFasL did not show signs of GVHD (data not shown).

**sFasL kills “memory” cells among peripheral lymphocytes**

Because these results suggested that sFasL functionally affects peripheral lymphocytes in contrast to previous reports that agonistic anti-Fas Ab did not induce apoptosis on unmanipulated murine or human peripheral lymphocytes (24-27), we next studied what kind of effect the sFasL treatment exerts on splenocytes from NOD mice. Staining with Hoechst 33258 showed no significant increase in the number of apoptotic cells after incubation with sFasL, compared with incubation with control CHO K1 supernatant (data not shown). We thought that sFasL might induce apoptosis on a small subset of splenocytes. Because previous reports showed that CD45RO⁺RBlow “memory” cells among CD4⁺ T cells expressed high level of Fas (25, 28), we examined the change in percentage of such cells after treatment with sFasL. Treatment of lymphocytes from diabetic \((n = 3)\) or nondiabetic NOD mice \((n = 4)\) with sFasL for 16 h decreased the portion of CD45RBlow cells among CD4⁺ T cells from 16.4 ± 2.7% (treated with control CHO K1 supernatant) to 10.2 ± 3.0% (mean ± SD, \(n = 7, p < 0.01\)) (Fig. 4A). The same treatment also increased the portion of PI-stained dead cells among CD4⁺CD45RBlow cells from 12.7 ± 5.5% (treated with CHO K1 supernatant) to 32.2 ± 12.1% (mean ± SD, \(n = 7, p < 0.01\)) (Fig. 4A). CD45RBlow⁺CD4⁺ T lymphocytes from diabetic and nondiabetic NOD mice were equally susceptible to treatment with sFasL (data not shown). Those from diabetes-resistant mouse strains such as C57BL/6 or ICR were also susceptible to the sFasL treatment (data not shown). Triple-colored flow cytometry showed that the decrease in the number of CD4⁺CD45RBlow cells after sFasL treatment was due to the death (and decrease) of Fas⁺CD45RBlo cells as expected \((7.3 ± 3.2%\) among CD4⁺ cells after treatment with control CHO K1 supernatant vs 3.6 ± 1.9% after treatment with sFasL; \(n = 8, p < 0.05\)) (Fig. 4, C and D). The portion of Fas⁺CD45RBhi cells among CD4⁺ cells was not significantly decreased by sFasL treatment \((19.0 ± 11.3%\) among CD4⁺ cells after treatment with control CHO K1 supernatant vs 14.8 ± 7.9% after treatment with sFasL; \(n = 8, p > 0.1\)). Treatment with 1 μg/ml agonistic anti-Fas Ab (Jo2) and 30 μg/ml cycloheximide also significantly decreased the fraction of CD45RBlow cells among CD4⁺ cells from 23.8 ± 2.2% (treated with cycloheximide alone) to 13.4 ± 4.1% \((n = 5, p < 0.01)\) (Fig. 4E).

**Discussion**

We unequivocally demonstrated that FasL is not an effector molecule in β islet cell death. The inability to transfer diabetes to NOD-lpr/lpr mice was due to abnormal FasL⁺ lymphoid cells in NOD-lpr/lpr mice that could exert apoptosis on adoptive transferred lymphocytes and inhibit the development of diabetes. Because we hypothesized that abnormal FasL⁺ cells affect diabetes transfer, we used 4- to 5-mo-old NOD-lpr/lpr mice for cotransfer experiments or RNase protection assays to ensure sufficient FasL expression on abnormal (double-negative) lymphocytes. In other experiments such as adoptive transfer to NOD-lpr/lpr mice (Fig. 1B) or neonatal thymectomized NOD-lpr/lpr mice (Fig. 2B), 8- to 10-wk-old NOD-lpr/lpr mice were used to minimize artificial effects arising from lymphoproliferation characteristic of lpr mice. Although we did not elucidate which cells were expressing FasL and could not exclude the effects of cells other than double-negative cells, FasL seems to be responsible for the inhibition of diabetes transfer regardless of the cell type(s) expressing it. A decrease in the number of such cells by neonatal thymectomy facilitated the development of insulitis, consistent with our hypothesis. Failure to transfer diabetes to neonatal thymectomized NOD-lpr/lpr mice could be due to the residual αβ⁺, γδ⁺ T cells, NK cells, or B cells expressing FasL. Furthermore, cotransfer of abnormal FasL⁺ cells from NOD-lpr/lpr mice completely inhibited diabetes transfer probably by exerting apoptosis on autoreactive lymphocytes, which is consistent with a previous report showing a shortened life span of transferred lymphocytes in NOD-lpr/lpr mice (5). GVHD observed in the recipient mice of the cotransfer was due to abnormal FasL on lymphocytes from NOD-lpr/lpr mice, consistent with the critical role of FasL as an effector molecule in GVHD (29). GVHD was not due to incomplete backcrossing in the production of NOD-lpr/lpr mice because no significant proliferative response was observed in MLC between NOD lymphocytes and NOD-lpr/lpr (or -lpr/w) lymphocytes (data not shown) and no such GVHD was observed in control recipient NOD mice to which lymphocytes from NOD-lpr/w mice were infused. Finally, diabetes was
restored by anti-FasL Ab treatment in recipient mice into which FasL+ lymphocytes from NOD-lpr/lpr mice and lymphocytes from diabetic NOD mice were cotransferred. This result ultimately proves that FasL is not an effector molecule in β cell death and lymphocytes from NOD-lpr/lpr mice induce apoptosis on cotransferred lymphocytes through Fas-FasL interaction. FasL might be an effector molecule only for a small number of CD8+ T lymphocytes that initially infiltrate pancreatic islets (4) but definitely not for the majority of diabetogenic lymphocytes because diabetes was actually provoked by blocking Fas-FasL interaction.

A significant decrease in the incidence of diabetes after ex vivo treatment of lymphocytes with sFasL suggests the possibility of a new treatment modality without the adverse effects of systemic FasL. A <100% inhibition of diabetes by sFasL pretreatment of splenocytes could be due to a weaker activity of sFasL compared with membrane FasL that can reportedly kill even naive lymphocytes (30). In an effort to investigate the effect of sFasL on peripheral lymphocytes that was evident in our ex vivo treatment model, we observed that “memory” T lymphocytes were selectively killed by sFasL. These results are in contrast to previous reports that agonistic anti-Fas Ab did not exert apoptosis on unmanipulated peripheral lymphocytes and Fas on peripheral lymphocytes is nonfunctional (24–26). sFasL produced by Suda et al. exerted cytotoxicity on peripheral lymphocytes including naive T cells; however, their sFasL was an artificially processed multimer acting like membrane FasL (27, 30). The previous inability to detect apoptotic peripheral lymphocyte fraction after anti-Fas Ab treatment seems to be due to a low frequency of apoptotic CD4+CD8low cells that escaped detection while using unfraccionated total peripheral lymphocytes. The apoptotic population observed in our study would include autoreactive diabetogenic lymphocytes whose ablation leads to the abrogation of diabetes. Our observations are consistent with a successful transfer of diabetes by CD45RBlowCD4+ cells but not by CD45RBhighCD4+ cells (31). Recent papers suggested that memory cells could be divided into CD45RBlow and CD45RBhigh subsets. According to them, only the former cells (mostly Fas+) are able to respond rapidly and short-lived, representing “preactivated” lymphocytes, while the latter “memory revertants” (mostly Fas−) are quiescent and long-lived, persisting without Ag (32, 33). Their theory may
explain why memory cells express Fas and are vulnerable to Fas-mediated apoptosis, contrary to the general notion that memory cells should live long. Our ex vivo treatment with sFasL seems to kill potentially dangerous "preactivated" cells with Fas up-regulation and may not affect "true" CD45RB\textsuperscript{high} memory T cells without Fas up-regulation.

We conclude that FasL is not an effector molecule in autoimmune β cell destruction and NOD-lpr/lpr mice are resistant to diabetes transfer because FasL constitutively expressed on their abnormal lymphocytes exerts cytotoxicity on diabetogenic lymphocytes, resolving the contradiction between previous papers. We also demonstrated that memory T cells are susceptible to sFasL or anti-Fas Ab. Physiological functions of sFasL in human beings are not known, while its pathological role has been reported (14, 34). sFasL might be involved in the deletion of potentially hazardous "memory" cells in vivo in human beings. More importantly, our data suggest the possibility that FasL could be used as a therapeutic agent for a variety of autoimmune disorders in which T lymphocytes are the major effector cells, such as autoimmune diabetes and experimental allergic encephalomyelitis. FasL has been expressed intraarticularly to ameliorate collagen-induced arthritis (35). Although administration of FasL\textsuperscript{−/−} cells cannot be considered as a therapeutic tool, ex vivo treatment of lymphocytes with sFasL or agonistic anti-Fas Ab after lymphopheresis could be employed to ameliorate full-blown clinical symptoms and signs of autoimmune diseases.

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