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Accumulation and Activation-Induced Release of Preformed Fas (CD95) Ligand During the Pathogenesis of Experimental Graft-Versus-Host Disease[1]

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Fas (CD95/APO-1) ligand (FasL)-mediated cytotoxicity has been implicated in tissue destruction in a variety of diseases, including acute graft-vs-host disease (GVHD). In this study, we have analyzed FasL expression and regulation during the course of experimental murine acute GVHD. Although activation-induced FasL-mediated cytotoxicity in control T cells was sensitive to the immunosuppressant cyclosporin A, we observed that functional FasL expression of GVHD T cells became increasingly cyclosporin A unresponsive. This was found to be the result of a massive in vivo accumulation and intracellular storage of FasL protein and its release in a transcription- and protein synthesis-independent manner. Immunohistochemistry analysis of FasL expression in situ revealed accumulation of FasL-expressing cells in the spleen, the liver, and small intestine, with a typical cytoplasmic and granular expression pattern. Thus, we conclude that the release of preformed FasL by infiltrating donor T cells may contribute to recipient tissue damage during the pathogenesis of acute GVHD. ***The Journal of Immunology,*** **2001, 167: 2936–2941.**

Allogenic bone marrow transplantations often result in graft-vs-host disease (GVHD),[3] which leads to severe tissue destruction and even to death of the affected patient. Donor T lymphocytes, originating from the transplanted bone marrow, recognize host Ag and develop into cytotoxic effector T cells. The two major cytotoxic mechanisms in T cell-mediated killing of target cells involve either the release of cytotoxic granule proteins, such as perforin and granzymes, or the expression of the apoptosis-inducing Fas (CD95/APO-1) ligand (FasL) (reviewed in Refs. 1 and 2). Both pathways appear to be important in the pathogenesis of systemic GVHD in experimental mouse systems. Inhibition of the FasL activity (3–8) or perforin (7) ameliorates certain aspects of GVHD-associated pathology, whereas inhibition of both cytotoxic effector mechanisms abrogates the development of lethal GVHD (9). One of the major target organs of GVHD-mediated tissue destruction is the intestinal mucosa. We have previously demonstrated that in GVHD the epithelial cell layer is infiltrated by donor $CD8\alpha\beta^+TCR\alpha\beta^+$ T cells, which show an increased functional expression of FasL upon restimulation (3). Resident recipient intraepithelial lymphocytes (IEL) of the $CD8\alpha\beta^+TCR\alpha\beta^+$, and $CD8\alpha\alpha^+TCR\alpha\beta^+$, and $TCR\gamma\delta^+$ phenotype become gradually replaced by cytotoxic donor T cells (3, 10). During intestinal GVHD, a dramatic increase in crypt cell and epithelial cell apoptosis is observed, possibly contributing to malabsorption of nutrients, weight loss, and lethal outcome of the disease. FasL appears to be the major cytotoxic effector mechanism by which these donor-derived IEL destroy the intestinal epithelium because induction of experimental acute GVHD in a Fas-deficient *lpr* mouse strain results in strongly reduced epithelial cell apoptosis (3).

FasL-mediated cytotoxicity is crucially involved in the pathogenesis of a wide range of diseases, including intestinal GVHD, experimental allergic encephalomyelitis, hepatitis, and others (reviewed in Refs. 2 and 11). Therefore, the regulation of FasL expression offers an attractive target for drug and therapy development. Recent years have revealed insight into the signaling events that regulate FasL transcription and function. Activation-induced FasL promoter activity is regulated by multiple transcription factors, including NF-AT, NF-κB, and early growth response gene (EGR)-2 and EGR-3 (12–15). Activation of NF-AT involves its dephosphorylation by the Ca^{2+}/calmodulin-activated phosphatase calcineurin, and is specifically blocked by the immunosuppressants cyclosporin A (CsA) and FK506 (reviewed in Refs. 16 and 17). Similarly, NF-κB may also be activated in a calcineurin-dependent manner (18). Although activation of EGR-2 and EGR-3 does not directly involve calcineurin activity, their own transcription requires the activity of this phosphatase and is thus blocked by CsA (14, 15). CsA is an immunosuppressant with wide applications in transplantation biology and treatment of pathologies mediated by excessive immune responses (reviewed in Refs. 16 and 17). At least in part, this immunosuppressant effect of CsA may be mediated by inhibition of activation-induced FasL expression (19–21).

Although transcriptional regulation of FasL expression is an important element in the control of T cell-mediated cytotoxicity, recent experimental evidence indicates that FasL activity may also be regulated at a posttranscriptional level. Chronically in vitro activated T cell blasts can store FasL protein in granule-like structures and release it in a protein synthesis-independent manner (22). In this study, we have analyzed the functional expression and regulation of FasL during the pathogenesis of experimental GVHD.

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[3] Abbreviations used in this paper: GVHD, graft-vs-host disease; CsA, cyclosporin A; EGR, early growth response gene; FasL, Fas ligand; IEL, intraepithelial lymphocyte.

We demonstrate in this study for the first time in a disease-related situation that GVHD T cells accumulate FasL protein in vivo and release it in a protein synthesis- and transcription-independent manner.

Materials and Methods

Induction of experimental acute GVHD

Experimental acute GVHD was induced as described previously (3). Briefly, spleen cells from C57BL/6 or C57BL/6 × DBA2 F_1 ($B6D2F_1$) mice (BRL, Fuellinsdorf, Switzerland) were isolated by dissociation of the spleen between frosted microscopy slides, followed by hypotonic lysis. GVHD was induced by injection of 10^8 C57BL/6 spleen cells into the tail vein of 7- to 10-wk-old $B6D2F_1$ recipients. Control mice received equal numbers of syngeneic $B6D2F_1$ spleen cells. After 1, 2, or 3 wk, mice were sacrificed, and spleen, small and large intestine, and liver were isolated. Some tissue samples were either embedded in Tissue Tek cryosection medium (Sakura Finetek, Zoeterwoude, The Netherlands) or fixed in 4% paraformaldehyde in PBS and paraffin embedded. Overall, these experiments were repeated >12 times with comparable results.

Isolation of T cells

IEL from small bowel were isolated as described before (3). Briefly, epithelial cells and IEL were dissociated in HEPES buffer (10 mM HEPES, pH 7.2, 25 mM $NaHCO_3$, 5.4 mM KCl, 0.3 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 137 mM NaCl, 5.6 mM D-glucose) containing 1 mM DTT and separated on a 40/70% Percoll gradient. The interphase containing enriched IEL (usually between 40 and 70% $CD8^+$ cells) was washed, resuspended in culture medium (RPMI 1640, 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μM 2-ME, 20 mM HEPES, pH 7.4.), and used for additional experiments.

Assessment of T cell subsets

After isolation, spleen cells or IEL from small intestine were washed in PBS, 1% calf serum, and 0.05% sodium azide (wash buffer), and stained with anti-CD8α, anti-CD8β, anti-TCRαβ, anti-TCRγδ, anti-CD3, anti-Thy-1.2, and anti-$H2^d$ (BD PharMingen, San Diego, CA). After two washes, cells were fixed in 1% paraformaldehyde in PBS and analyzed by flow cytometry on a FACScan using CellQuest software (BD Biosciences, San Jose, CA).

Assessment of FasL expression

Functional FasL expression was assessed as described previously (3, 20, 23). Briefly, splenic T cells or IEL were cocultured at different E:T ratio with 2×10^4/well [^{3}H]thymidine-labeled Jurkat E6 cells (Fas^+; American Type Culture Collection, Manassas, VA) on either anti-CD3-coated or untreated 96-well flat-bottom tissue culture plates. Inhibitors were added simultaneously at appropriate concentrations. After 16 h, unfragmented target cell DNA was harvested on glass fiber filters, and cytotoxicity was calculated as follows: percentage of DNA fragmentation = 100 × (1 − cpm experimental group/cpm control group). All assays were done in triplicates. Specificity of the assay (FasL-dependent killing) was confirmed by inhibition with Fas-Fc fusion protein or neutralizing anti-FasL (clone MFL3; BD PharMingen), as described previously (23) (see Fig. 2*C*).

Specific anti-$H2^d$ (host) cytotoxicity was assessed by coculturing [^{3}H]thymidine-labeled $H2^{d+}$ target cells (L1210-Fas) (3, 24) with splenic T cells or IEL at different E:T ratio. Target cell DNA fragmentation was calculated as described above.

In some experiments, FasL protein expression was also assessed by Western blotting. Splenic T cells or isolated IEL were counted and washed to remove serum proteins, and 2×10^6 cells were lysed in 50 μl reducing protein sample buffer (50 mM Tris, pH 6.8, 100 mM DTT, 2% SDS, 0.1% bromophenol blue, 10% glycerol). Total cell lysates were separated by SDS-PAGE (12% gel) and transferred to polyvinylidene difluoride membrane (Macherey & Nagel, Oensingen, Switzerland). Membranes were blocked with 5% nonfat dry milk in TBS. FasL was detected using a polyclonal rabbit anti-mouse FasL (N20, recognizing the intracellular portion of FasL; Santa Cruz Biotechnology, Santa Cruz, CA; 1:500) and secondary swine anti-rabbit HRP conjugate (1:2000; DAKO, Glostrup, Denmark), and visualized by ECL (Amersham, Arlington Heights, IL). To control for equal protein loading, blots were then stripped and reprobed with an anti-actin Ab (Amersham).

In vivo expression of FasL was assessed by immunohistochemistry. Cryostat sections (5 μm) from control or GVHD tissue on polylysine-coated slides were fixed and permeabilized in acetone for 10 min and then air dried. Sections were rehydrated and blocked for unspecific binding with TBS, containing 1% casein, 5% goat serum, and 0.1% NaN_3, for 30 min at room temperature. Slides were then incubated with either hamster anti-mouse FasL (clone FLIM58, 20 μg/ml; MBL, Labforce, Nunningen, Switzerland) or hamster isotype control in blocking solution for 1 h at room temperature. After two washes in TBS, sections were incubated with anti-hamster Ig FITC conjugate (anti-hamster mixture, 20 μg/ml; BD PharMingen) for 1 h at room temperature in the dark. Slides were then washed twice in TBS and embedded in 80% glycerol in PBS containing 1 mg/ml *p*-phenylene-diamine (Sigma, St. Louis, MO). FasL expression was then analyzed by confocal microscopy on a Bio-Rad 300 confocal microscope (Bio-Rad, Glattbrugg, Switzerland). The same settings were used to analyze sections stained with isotype control. Experiments and analysis were repeated three times.

Fas and FasL mRNA expression were detected by RT-PCR. Briefly, IEL, spleen cells, or T cell blasts were either left untreated or stimulated for 5 h with plate-bound anti-CD3. RNA was isolated, and RT-PCR for Fas, FasL, and actin was performed as described previously (25). Amplified specific bands were analyzed densitometrically, and the ratio between actin and FasL signal in the anti-CD3 and anti-CD3 plus CSA-treated groups was calculated. Experiments were repeated more than five times.

Measurement of IL-2 synthesis

Isolated spleen cells from control or GVHD mice were resuspended at 4×10^6 cells/ml in complete medium. Cells were then stimulated with plate-bound anti-CD3 Ab in the presence or absence of 200 ng/ml CsA for 16 h. Cell-free supernatant was harvested, and IL-2 production was analyzed by ELISA (matched anti-IL-2 Ab pairs; BD PharMingen).

Statistical analysis

The relative inhibition of activation-induced functional FasL expression upon treatment with CsA in control groups or GVHD-affected groups was subjected to a univariate ANOVA. The relative inhibition was calculated as follows: 100 × (1 − percentage of DNA fragmentation (stimulated plus CsA)/percentage of DNA fragmentation (stimulated)). In the linear model, the main effects and all two-way effects of following factors were included: E:T ratio, experiments (spleen, n = 5; IEL, n = 4), and disease status (control, GVHD). The statistical results obtained were analyzed for equality of error variances, fit of model, and residual distribution.

Results

Functional FasL expression by GVHD T cells is insensitive to inhibition by CsA

We and others have previously demonstrated that FasL is crucially involved in the pathogenesis of acute GVHD, in particular in intestinal epithelial cell damage (3). In this study, we therefore analyzed the regulation of FasL expression during acute experimental GVHD. Functional FasL expression was assessed by the ability of ex vivo stimulated T cells to kill Fas-sensitive target cells (3, 23). In T cell hybridomas and normal T cells, FasL expression is under strict transcriptional control of NF-AT, and is thus inhibited by CsA and FK506 (12, 19–21). In agreement with our previous findings (20), we observed that activation-induced (anti-CD3) functional FasL expression was effectively blocked in splenic T cells and IEL isolated from control mice. Surprisingly, however, CsA only very inefficiently blocked activation-induced cell surface expression of FasL in T cells isolated from GVHD mice (Fig. 1*A*). Identical results were obtained when Ag-specific cytotoxicity against $H2^{d+}$ target cells was assessed (Fig. 1*B*). Similarly, donor T cells isolated from spleen and small intestine were equally CsA insensitive (Fig. 1, *A* and *B*). The cytotoxicity observed was still specifically mediated through cell surface expression of FasL, because soluble Fas fusion protein (Fas-Fc) completely blocked target cell killing upon effector T cell stimulation by anti-CD3 (Fig. 1*C*). Similarly, Ag-specific ($H2^d$) cytotoxicity of alloreactive T cells was insensitive to CsA, but almost completely blocked upon neutralization of FasL (Fig. 1*C*). Statistical analysis of the data revealed that the difference between the degree of inhibition of activation-induced functional FasL expression by CsA in control

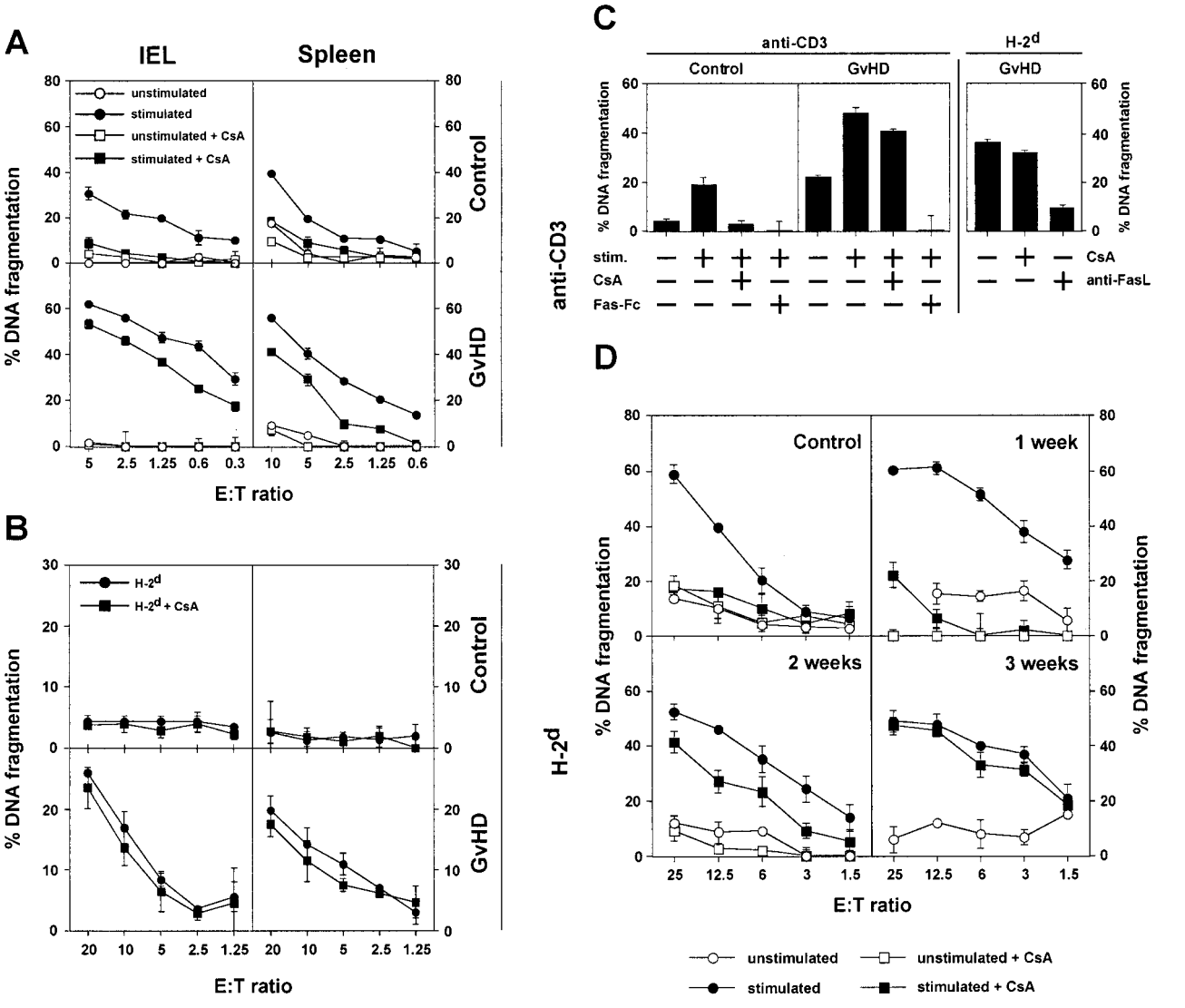

FIGURE 1. Funtional FasL expression in GVHD T cells is CsA insensitive. IEL and spleen cells from control ($B6D2F_1$) or GVHD animals were isolated 2 wk post disease induction, and functional FasL was analyzed as described in *Materials and Methods*. Effector cells were stimulated by plate-bound anti-CD3 (*A*) or $H2^{d+}$ target cells (*B*), and FasL expression was blocked by addition of 200 ng/ml CsA. Target cell DNA fragmentation (mean values of triplicates ± SD) at different E:T ratios is shown. Experiments were repeated three times with identical results. *C*, Functional FasL expression in control and GVHD splenic T cells upon stimulation by anti-CD3 or $H2^{d+}$ target cells, and its inhibition by CsA (200 ng/ml) and/or interference with Fas-FasL interaction by Fas-Fc (20 μg/ml) or anti-FasL (20 μg/ml) was assessed at an E:T ratio of 20:1. Target cell DNA fragmentation (mean values of triplicates ± SD) is shown. *D*, Spleen cells from control animals or GVHD animals after 1, 2, or 3 wk post disease induction were isolated, and CsA sensitivity of functional FasL expression was analyzed as described above. Target cell DNA fragmentation (mean values of triplicates ± SD) is shown.

vs GVHD-affected groups (spleen cells and IEL) was highly significant ($p < 0.001$). Similarly, no significant difference was found between the cytotoxicity of $H2^d$-stimulated GVHD T cells (spleen cells and IEL), and $H2^d$-stimulated/CsA-treated GVHD T cells. Thus, inhibition of FasL transcription by CsA does not inhibit activation-induced functional cell surface expression of FasL in GVHD T cells.

This CsA insensitivity of activation-induced cell surface expression of FasL gradually increased during disease progression. One week post disease induction, splenic T cells already showed a primed phenotype and killed Fas^+ target cells more efficiently than $B6D2F_1$ control T cells. Yet, GVHD T cells were found fully responsive to CsA treatment at this stage. However, functional FasL expression by GVHD splenic T cells was only partially blocked by CsA at 2 wk after disease induction, and no inhibition was apparent after 3 wk (Fig. 1*D*).

Activation-induced FasL transcription in GVHD T cells remains CsA sensitive

The observed CsA insensitivity of functional FasL expression in GVHD T cells, but not control T cells, may have different underlying reasons, including altered signal transduction pathways (26) or transcription-independent cell surface expression of preformed protein (22, 27). We first analyzed whether NF-AT-dependent transcription in GVHD T cells is still blocked by CsA. A well-known transcriptional target of NF-AT is the cytokine IL-2. Fig. 2*A* shows that activation of control cells as well as GVHD T cells resulted in strong induction of IL-2 synthesis, which was efficiently blocked by addition of CsA on both cases.

We then further tested whether activation-induced FasL transcription in control T cells or GVHD T cells was equally CsA sensitive. Spleen cells from control mice or GVHD mice were

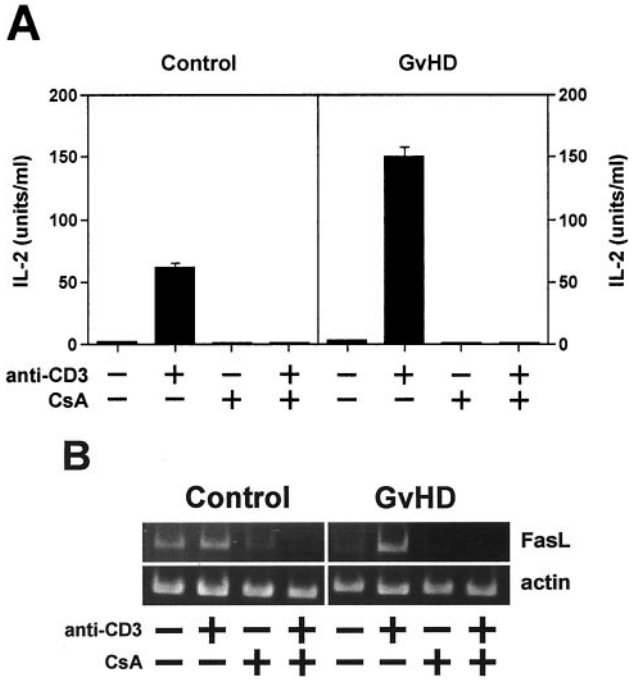

FIGURE 2. FasL transcription in GVHD T cells is CsA sensitive. *A*, Splenic T cells from control or GVHD animals were isolated 2 wk post disease induction. Anti-CD3-induced IL-2 synthesis by control or GVHD spleen cells and its inhibition by CsA were assessed by ELISA. Mean values of triplicates ± SD are shown. *B*, Splenic T cells from control or GVHD animals were stimulated by plate-bound anti-CD3 in the absence or presence of 200 ng/ml CsA. Fas, FasL, and actin expression were assessed by RT-PCR. Ratio of FasL vs actin signal was analyzed densitometrically. Control spleen cells = 0.119 (anti-CD3) vs 0.0063 (anti-CD3 plus CsA); GVHD spleen cells = 0.117 (anti-CD3) vs 0.0063 (anti-CD3 plus CsA).

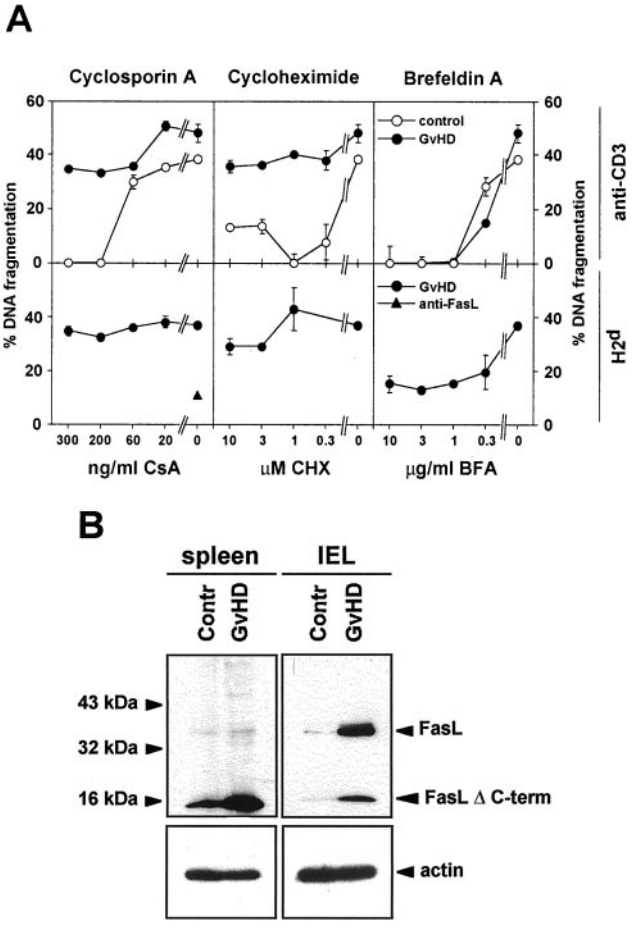

FIGURE 3. GVHD T cells express and release preformed FasL. *A*, Splenic T cells from control animals or GVHD animals were isolated 2 wk after GVHD induction, and functional FasL expression upon ex vivo stimulation with either anti-CD3 or $H2^{d+}$ targets was assessed at an E:T ratio of 20:1. Functional FasL expression was blocked by increasing concentrations of CsA, cycloheximide (CHX), and brefeldin A. $H2^{d}$-specific cytotoxicity was also blocked with 20 μg/ml neutralizing anti-FasL Ab. Mean values of triplicates ± SD are shown. *B*, FasL expression in splenic T cells or IEL isolated from control or GVHD animals was detected by Western blot. Arrows indicate full-length FasL and its cleavage product (FasL Δ c-term). Actin levels on the same blot show equal protein loading.

therefore isolated and restimulated ex vivo in the presence or absence of CsA. FasL expression was analyzed by RT-PCR and compared with Fas and actin expression. As reported previously for T cell hybridomas (20), we observed (slight) induction of FasL mRNA expression upon activation of control T cells, which was significantly reduced upon CsA treatment (Fig. 2*B*). In contrast, activation-induced Fas expression was not altered by CsA (data not shown). In GVHD T cells, FasL expression was strongly induced upon restimulation, reflecting the in vivo primed stage of T cells, and significantly reduced upon CsA treatment (Fig. 2*B*). Therefore, FasL transcription, in contrast to FasL cell surface expression, in GVHD T cells appears to be fully sensitive to the inhibitory action of CsA.

GVHD T cells store and release preformed FasL protein

Recent reports have suggested that FasL-mediated cytotoxicity may not only be regulated at a transcriptional, but also at a post-translational level (22, 27, 28). We thus wanted to investigate whether a transcription- and protein synthesis-independent release of preformed FasL was the underlying reason for the CsA-insensitive functional FasL expression by GVHD T cells. Fig. 3*A* shows that anti-CD3-induced FasL expression in control $B6D2F_1$ T cells was efficiently blocked by the protein synthesis inhibitor cycloheximide. However, this inhibitor had only a minimal inhibitory effect, even at high concentrations, on functional FasL expression in GVHD T cells. This lack of inhibition of functional FasL expression was paralleled by the inability of CsA (Fig. 3*A*) and the transcription inhibitor actinomycin D (data not shown) to block cell surface expression of FasL. In contrast, brefeldin A, which inhibits intracellular protein transport, completely blocked FasL surfacing in both control and GVHD T cells (Fig. 3*A*). Identical results were obtained in parallel experiments when Ag-specific ($H2^{d}$), FasL-dependent cytotoxicity was analyzed (Fig. 3*A*). Whereas CsA and cycloheximide failed to block target cell killing by alloreactive T cells, brefeldin A and neutralizing anti-FasL efficiently inhibited donor T cell cytotoxicity.

These data indicate that FasL protein accumulates in GVHD T cells and is released upon restimulation in a protein synthesis-independent manner. This issue was further investigated by detection of FasL protein by Western blot. In particular in IEL from control animals, FasL protein was hardly detectable (Fig. 3*B*). In marked contrast, high levels of FasL were detected in IEL isolated from GVHD animals. Thus, cell-associated FasL expression was strongly increased during GVHD. Interestingly, only a marginal increase in full-length FasL protein (40 kDa) was observed in GVHD spleen cells; however, massive accumulation of a smaller 14-kDa band became apparent in both GVHD spleen cells and IEL, most likely representing the cell-associated transmembrane-intracellular stem, left behind upon proteolytic cleavage of transmembrane FasL by metalloproteases (29–31).

Intracellular granular localization of FasL protein in GVHD-affected tissue

Because our results described above were only indirect proofs that FasL accumulates in T cells during the pathogenesis of GVHD, we directly wanted to assess FasL expression and subcellular localization in situ. Tissue sections from control or GVHD animals were therefore stained with isotype control Ab or anti-FasL, fluorescence-labeled secondary reagents, and expression was detected by confocal microscopy. No unspecific binding of the isotype control was detected in either control or GVHD tissue (Fig. 4*B*), and only minimal anti-FasL staining was observed in control tissue (data not shown). In contrast, massive FasL expression was detected in spleen, liver, and small intestine of GVHD animals, thus confirming our above-described findings (Fig. 4, *A* and *C–E*, and data not shown). Analysis of subcellular localization further revealed a mostly cytoplasmic expression pattern of FasL protein with a frequent granule-like pattern (Fig. 4, *C–E)*. Thus, this study represents the first demonstration of intracellular granular accumulation of FasL protein in situ in a disease-related situation.

Discussion

FasL is an apoptosis-inducing member of the TNF family, with important implications in immune homeostasis, immune privilege, as well as the pathogenesis of a variety of diseases (reviewed in Refs. 11, 32, and 33). The expression of FasL is crucially regulated by a variety of transcription factors, and the signal transduction pathways leading to the activation of these transcriptional regulators represent attractive therapeutic targets to regulate FasL-mediated cytotoxicity. Although until recently it was believed that FasL is only regulated on a transcriptional level, it has been recently demonstrated that activated T cells, but presumably also other cell types, can store preformed FasL in granule-like structures and release it in a protein synthesis-independent way (22). While most of these studies to date have used in vitro systems (22, 28), we demonstrate in this study for the first time in vivo accumulation and activation-induced release of preformed FasL during the pathogenesis of a T cell-mediated disease.

Intracellular storage of preformed FasL and its protein synthesis-independent release may allow a more rapid and efficient target cell killing (22, 34). If FasL would be only under transcriptional control, FasL-mediated cytotoxicity would be extremely time consuming, and therefore most likely quite inefficient. Thus, similar to granzyme B- and perforin-mediated killing, the release of preformed FasL to the cell surface, and thus rapid induction of target cell apoptosis allows a "hit and go" strategy of T cell-mediated cytotoxicity. However, to ensure Ag-specific cytotoxicity and avoid unspecific bystander killing, cell surface expression of FasL must be tightly regulated. We have observed that unactivated GVHD T cells do not cause significant target cell killing, thus indicating that FasL is only very transiently expressed on the cell surface. Most likely, down-modulation of transmembrane FasL by metalloprotease cleavage, and thus limitation of its cytotoxic activity may represent an important regulatory mechanism (29–31). This suggestion is supported by our observation that most of the FasL detected by Western blot in splenic GVHD T cells was in its truncated form, and that ex vivo isolated GVHD T cells show little bystander killing of Fas^+ targets without restimulation (Figs. 1 and 2).

The sustained stimulation of donor T cells by alloantigens most likely represents the underlying trigger for FasL transcription and accumulation of the preformed protein in intracellular compartments during GVHD. Although the activation-induced release was found to be CsA-insensitive, CsA-sensitive transcription factors, such as NF-AT, NF-κB, and EGR-2 and EGR-3, are most likely crucially involved in activation-induced FasL transcription during GVHD. However, we cannot exclude that other (possibly even CsA-insensitive) mechanisms may contribute to FasL transcription in vivo. For example, the sustained activation and proliferation of donor T cells lead to elevated expression of c-Myc, a transcription

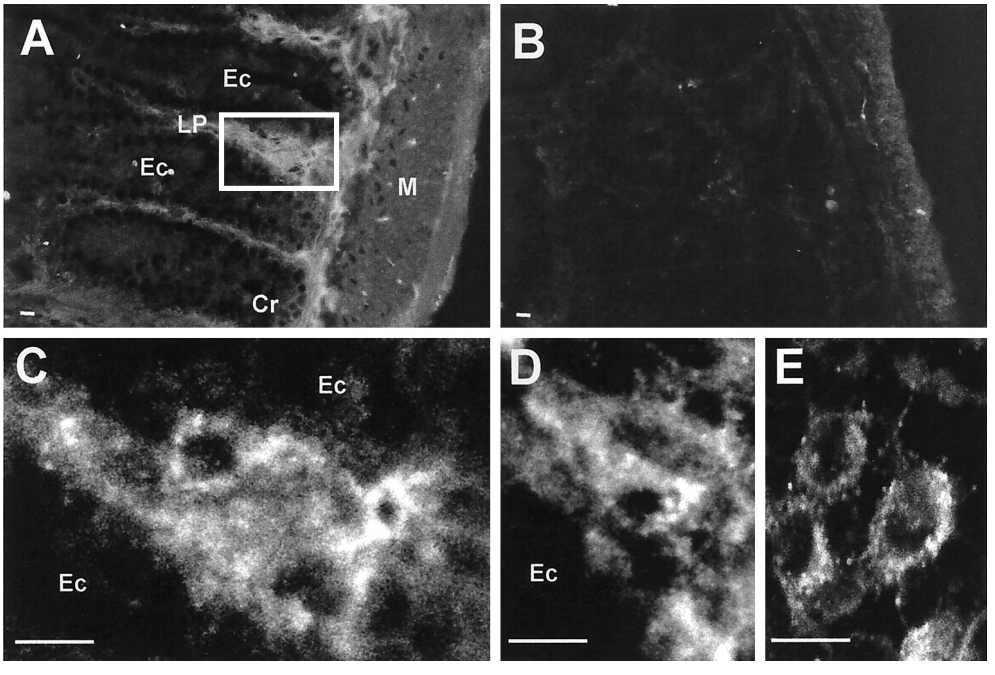

FIGURE 4. Immunolocalization of FasL in GVHD-affected tissue. Tissue sections from small intestine (*A–D*) or spleen (*E*) from GVHD animals 2 wk post disease induction were stained with anti-FasL Ab (*A*, *C*, *D*, and *E*) or isotype control (*B*) and secondary fluorescence-labeled reagents. Fluorescence was analyzed by confocal microscopy. *C*, Higher magnification of the section depicted in *A*. Bar length, 10 μm. Cr, Crypt cells; Ec, epithelial cells; LP, lamina propria; M, muscle layer.

factor known to drive FasL promoter activity (25, 35–37). In addition, enhanced expression of inflammatory cytokines, such as TNF-α, may cause a continuous activation of NF-κB, and thus further enhance FasL transcription (13, 26, 38). We conclude that FasL-mediated cytotoxicity is regulated at transcriptional and post-translational levels, which may allow multiple strategies of therapeutic interference.

Acknowledgments

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