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Differential Expression of Fas and Fas Ligand in Acute and Chronic Graft-Versus-Host Disease: Up-Regulation of Fas and Fas Ligand Requires CD8+ T Cell Activation and IFN-γ Production¹

Andrei Shustov,* Phuong Nguyen,* Fred Finkelman,† Keith B. Elkon,‡ and Charles S. Via2*

The parent-into-F₁ model of acute and chronic graft-vs-host disease (GVHD) was used as an example of in vivo cell-mediated or Ab-mediated responses, respectively, and the roles of Fas and Fas ligand (FasL) were investigated. Using both flow cytometry and PCR methodologies, we found that acute GVHD mice exhibited significant up-regulation of Fas and FasL, whereas Fas/FasL up-regulation in chronic GVHD mice was equal to or marginally greater than that in uninjected mice. Functional studies confirmed that Fas/FasL contributed to the anti-host CTL activity of splenocytes from acute GVHD mice, although a perforin-dependent pathway was also identified. Despite the presence of FasL on both donor CD4+ and CD8+ T cells in acute GVHD mice, depletion studies demonstrated that all the in vitro anti-host CTL activity resided in the CD8+ population. Furthermore, injection of CD8-depleted B6 spleen cells into F₁ mice blocked Fas/FasL up-regulation and IFN-γ production, resulting in chronic GVHD. Lastly, up-regulation of Fas/FasL in acute GVHD mice could be blocked by anti-IFN-γ mAb in vivo. Thus, in this in vivo model of alloantigen immune responsiveness, Fas/FasL up-regulation is critically dependent on Ag-specific (donor) CD8+ T cell activation and IFN-γ production. Donor CD4+ T cell activation in the absence of CD8+ T cell activation results in an autoantibody-mediated response, no significant Fas/FasL up-regulation, impaired elimination of autoreactive B cells, and persistent humoral autoimmunity. The Journal of Immunology, 1998, 161: 2848–2855.

Fas and Fas ligand (FasL)² play important roles in lymphocyte homeostasis, as evidenced by the development of lymphoproliferation and autoimmunity in lpr and gld mice (reviewed in Ref. 1) and humans with Fas mutations (2). Immune regulation by Fas/FasL is largely achieved in the peripheral immune system through induction of activation-induced cell death of T cells (1), B cells (3), and macrophages (4). FasL expression on activated T cells is believed to play an important role in the deletion of activated B cells that express Fas (5–7). Recent in vitro studies have shown that Fas-mediated killing is preferentially observed with Th1 clones but not with Th2 clones (5) and that IL-4 protects B cells from Fas-mediated apoptosis (8), raising the possibility that in vivo cell-mediated and Ab-mediated immune responses may exhibit differential Fas/FasL expression and function.

A useful model for studying in vivo Ag-driven responses is the P→F₁ model of graft-vs-host disease (GVHD). In this model, disease takes one of two forms: 1) acute GVHD, characterized by a severe reduction in host lymphocytes and a profound immunodeficiency; or 2) chronic GVHD, characterized by lymphoproliferation, autoantibody production, and a lupus-like disease. Acute GVHD results from the activation of both donor CD4+ and CD8+ T cell subsets in response to host alloantigen. A strong cell-mediated immune response ensues, resulting in the elimination of host lymphocytes in large part by donor anti-host CTL (9–11). In contrast, chronic GVHD results from the selective activation of alloreactive donor CD4+ T cells that provide cognate help to host B cells leading to activation and autoantibody production. In the absence of activated donor CD8+ T cells, anti-host CTL do not develop in chronic GVHD, and persistent autoantibody production by autoreactive B cells results in a lupus-like immune complex glomerulonephritis (12). Importantly, in both forms of GVHD, alloreactive donor T cells appear to undergo normal activation, expansion, cytokine production, and maturation into effector T cells. Thus, disease in this model results not from abnormal donor T cell function but, rather, from the activation of T cells specific for host alloantigens. Despite the pathologic outcomes observed, the model provides an opportunity to study normal in vivo Ag-driven CMI or Ab-mediated immune response.

The present study was undertaken to determine whether differences in disease outcome in acute and chronic GVHD are associated with differences in Fas/FasL expression and, if so, whether differential cytokine production is important in mediating such an effect. In particular, we asked 1) whether the persistence of autoreactive B cells and humoral autoimmunity in chronic GVHD is associated with a reduced Fas/FasL up-regulation; 2) whether the
elimination of autoreactive B cells in acute GVHD is mediated by Fas-dependent anti-host CTL activity; and lastly 3) whether cytokines such as IFN-γ contribute to Fas/FasL up-regulation. Our results indicate that in acute GVHD, Fas/FasL play a major role in the elimination of autoreactive B cells and that the up-regulation of these molecules is critically dependent on IFN-γ production. In contrast, Fas/FasL up-regulation and IFN-γ production are significantly reduced in lupus-like chronic GVHD, leading to impaired elimination of autoreactive B cells and persistent autoantibody production.

Materials and Methods

Mice

C57BL/6 (B6), DBA/2 (DBA), and (C57BL/6 × DBA/2) (BDF1) male mice, 6 to 8 wk of age, were purchased from The Jackson Laboratories (Bar Harbor, ME).

Definition and induction of GVHD

Single cell suspensions were prepared in HBSS from the spleens of normal B6 or B6 parental donors. Cell suspensions were filtered through sterile nylon mesh, washed, and diluted to a concentration of 10^6 viable (trypan blue excluding) cells/ml. Acute GVHD was induced with 50 × 10^6 undepleted B6 spleen cells, and chronic GVHD was induced with either 90 × 10^6 DBA undepleted splenocytes or 50 × 10^6 C57B6 T cell-depleted B6 splenocytes. These donor cell inocula have been previously demonstrated to result in acute or chronic GVHD, respectively (10). Cell suspensions were injected i.v. into the tail vein of normal, unirradiated BDF1 recipients. Control mice consisted of uninfected age- and sex-matched F1 mice. The presence of acute GVHD was defined as a >50% reduction in host spleen cells and significant engraftment (>1 × 10^6) of donor CD4^+ and CD8^+ T cells at 14 days after parental cell transfer. The presence of chronic GVHD was defined as a >120% increase in host spleen cells and significant engraftment of only donor CD4^+ T cells. The effects of IFN-γ were blocked in vivo by the administration of neutralizing doses of anti-IFN-γ mAb, XMG-6 (13), given at 1 mg/mouse iv on days 0 and 7 after parental cell transfer. Control mAb (GL113) was administered at the same dose and schedule. Additional controls consisted of untreated acute GVHD mice and normal uninfected normal F1 mice.

Depletion of T cell subsets

Spleen cells were depleted of CD4^+ or CD8^+ T cells by treatment with anti-CD4, RL172 (14), or anti-CD8, 83-12-5 (15), mAb and complement as previously described (16). Flow cytometric analysis confirmed that this procedure resulted in <1% contaminating CD4^+ or CD8^+ T cells.

Detection of anti-host CTL activity ex vivo

Effector CTL activity was tested using freshly harvested splenocytes without an in vitro sensitization period. F1 splenocytes were tested for their ability to lyse either 1) 2-day LPS-stimulated blasts from DBA (H-2d) mice, or 2) Fas-positive or Fas-negative L1210 cells (H-2b, MHC class I positive, class II negative) (17) in 4-h ^51 Cr release assay as described previously (10). Effectors were tested in triplicate at four E:T cell ratios. The percent lysis was calculated according to the formula: ([cpm sample – cpm spontaneous]/[cpm maximum – cpm spontaneous]) × 100%. For studies using LPS-stimulated blasts, counts per minute spontaneous was replaced with counts per minute of splenocytes in normal F1 mice in the calculation of the cytotoxicity of experimental groups. Results are shown as the mean percent lysis ± SEM at a given E:T cell ratio for each treatment group.

RT-PCR

The coupled RT-PCR was used as previously described (18). RNase-free plastic and water were used throughout the assay. Tissues were homogenized in RNA-STAT-60 (Tel-Test, Friendswood, TX) at 50 mg of tissue/ml or 1 ml/10^6 cells. RNA samples were reverse transcribed with reverse transcriptase (M-MLV-RT, Life Technologies, Grand Island, NY); FasL- or IFN-γ-specific primers were used for amplification as previously described (18). For each gene product, the optimum number of cycles (that number of cycles that would achieve a detectable concentration that was well below saturating conditions) was determined experimentally. To verify that equal amounts of RNA were added in each RT-PCR reaction within an experiment, primers for the housekeeping gene, hypoxanthine phosphoribosyl transferase (HPRT), were used in each experiment. Gene expression was quantitated by densitometry for individual mice, normalized to each individual HPRT value, and group means were calculated.

Flow cytometric analysis and engraftment studies

Spleen cells were prepared as previously described (10). Following incubation with anti-murine Fcy receptor mAb, 2.4G2 (19), for 10 min, cells were stained with saturating concentrations of FITC-conjugated, biotin-conjugated, or PE-conjugated mAb against CD4, CD8, B220, H-2K^b, Fas (CD95), and FasL purchased from Becton Dickinson (Mountain View, CA) or PharMingen (San Diego, CA). Two- and three-color flow cytometric analyses were performed using a FACSScan flow cytometer (Becton Dickinson). Lymphocytes were gated by forward and side scatter, and fluorescence data were collected for 10,000 cells. Studies of donor T cells were performed on 5,000 gated cells that were CD4^+ or CD8^+ and did not stain positively for MHC class I of the uninfected parent. Monocyte populations were excluded on the basis of forward and side scatter.

Statistical analysis

Results for group means were compared by Student’s t test.

Results

Fasl mRNA is increased in acute compared with chronic GVHD

To determine the level of FasL gene expression in acute and chronic GVHD mice, splenic mRNA was isolated and amplified by RT-PCR as described in Materials and Methods. On day 10 after GVHD induction (Fig. 1), mice with acute GVHD had significantly higher mean FasL expression compared with normal or chronic GVHD mice (p < 0.05). Mean densitometric ratios of FasL/HPRT expression were approximately eightfold higher in spleen cells obtained from mice with acute GVHD (mean = 0.249 ± 0.06) compared with those from mice with chronic GVHD (mean = 0.029 ± 0.01; p = 0.02) or those from normal F1 mice (mean = 0.029; p = 0.04). Mean values for chronic GVHD did not differ significantly from normal F1 values. Similar results were observed on day 14 after parental cell transfer and have been confirmed in a total of three independent experiments at both time points. Kinetic studies revealed that increased Fasl mRNA expression was detected as early as day 5 after parental cell transfer in acute, but not chronic, GVHD (data not shown).

Cell surface expression of Fasl is increased in acute GVHD compared with chronic GVHD

B6 and DBA mice express different allelics of FasL (20). To verify that the mAb used for these studies could detect Fasl on DBA T cells, splenocytes were activated with anti-CD3 mAb, and Fasl expression was monitored by flow cytometry. An approximately twofold increase in Fasl surface expression compared with that
for freshly isolated splenocytes was observed for CD4^+ and CD8^+ T cells from both B6 and DBA splenocytes (data not shown), indicating that both B6 and DBA splenocytes are capable of up-regulating FasL following activation in vitro and that the mAb used was capable of detecting FasL on both parental donors.

To determine whether the increased FasL mRNA seen in acute GVHD mice was associated with an increase in FasL surface protein expression, flow cytometric staining of FasL was performed on splenocytes from acute or chronic GVHD mice at 10 and 14 days after parental cell transfer. As shown in Figure 2 and Table I, acute GVHD mice exhibited consistent and statistically significant increases in FasL expression on both donor CD4^+ and CD8^+ T cells compared with chronic GVHD mice, naive un.injected donor cells, or control uninjected host (F1) T cells. Interestingly, acute GVHD mice also exhibited increased FasL expression on host CD4^+ and CD8^+ T cells compared with host T cells from either chronic GVHD or control mice (Fig. 3 and Table I). In contrast, FasL expression on both donor and host T cells from chronic GVHD was not consistently increased over that in the controls, although in one experiment using a brighter staining anti-FasL mAb, a small but statistically significant increase in FasL expression was observed compared with the control value (Table I, Expt. 3).

**Cell surface expression of Fas is also increased in acute compared with chronic GVHD**

If Fas/FasL play a role in mediating the elimination of host splenocytes in acute GVHD, it might be expected that the increase in donor T cell FasL expression seen in acute GVHD would be accompanied by increased expression of Fas in host lymphocytes. Compared with either chronic GVHD mice or control F1 mice, the increase in FasL expression on donor T cells was associated with a statistically significant increase in Fas expression on host T cells in acute GVHD mice (Table I). In contrast, Fas expression on both donor and host T cells from chronic GVHD was not consistently increased over that in the controls.

**Table I. Fas and FasL expression are increased in acute GVHD mice compared to chronic GVHD**

<table>
<thead>
<tr>
<th>Group</th>
<th>Donor CD4</th>
<th>Donor CD8</th>
<th>Host CD4</th>
<th>Host CD8</th>
<th>B220</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Expt. 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>6.7</td>
<td>8.2</td>
<td>6.8</td>
<td></td>
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<tr>
<td>Acute GVHD</td>
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<td>12.4**</td>
<td>11.4***</td>
<td>12.3***</td>
<td>7.7*</td>
</tr>
<tr>
<td>Chronic GVHD</td>
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<td>6.8</td>
<td>7.1</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>Expt. 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>NT</td>
<td>4.5</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>Acute GVHD</td>
<td>6.6**</td>
<td>6.5**</td>
<td>5.7*</td>
<td>9.6*</td>
<td></td>
</tr>
<tr>
<td>Chronic GVHD</td>
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<td>5.4*</td>
<td>4.8</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>Expt. 3</td>
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<td>21.2*</td>
<td>27.0*</td>
<td>44.4**</td>
<td></td>
</tr>
<tr>
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<td>18.2**</td>
<td>13.6**</td>
<td>17.7*</td>
<td></td>
</tr>
</tbody>
</table>

*p values in the acute GVHD upper row denote comparison to chronic GVHD.

*p values in the acute GVHD lower row denote comparison to control F1.

**Acute and chronic GVHD were induced as described in Materials and Methods.** Splenocytes were analyzed by flow cytometry for Fas and FasL expression at either 10 days (Expts. 1 and 3) or 14 days (Expt. 2) after parental cell transfer. Commercially obtained anti-FasL mAb was used in Expts. 1 and 2. A polyclonal rabbit anti-mouse anti-FasL mAb was used in Expt. 3. Values represent average mcf ± SE, n = 3 mice/group for Expts. 1 and 3, n = 4 mice/group for Expt. 2. Groups were compared by t test, and p values denoted as *p < 0.05; **p < 0.01; ***p < 0.005. Values for a single naive (uninjected) donor CD4^+ and CD8^+ T cell are also shown in Exp. 1 and were comparable to those of uninjected normal hosts.
acute GVHD mice exhibited consistent and statistically significant increases in Fas expression on both donor and host lymphocytes, with the largest degree of up-regulation seen on host B cells and host CD8\(^{+}\) T cells (Figs. 4 and 5 and Table I). Fas expression on lymphocytes from chronic GVHD mice was in some instances greater than that in control mice, however; the chronic GVHD mcf values were consistently lower than those observed in acute GVHD mice (Table I).

Anti-host CTL kill by both a Fas-dependent and a Fas-independent (perforin-mediated) mechanism

A major difference between acute and chronic GVHD is the potent anti-host CTL activity in acute GVHD (21). Since FasL expression on T cells is up-regulated in acute, but not chronic, GVHD, it is possible that FasL contributes to anti-host CTL activity and the elimination of host lymphocytes. To examine this possibility, spleen cells from acute GVHD mice were tested for their ability to lyse Fas-positive and Fas-negative H-2\(^{d}\) targets. As shown in Figure 6, anti-host-CTL killed both Fas-positive and Fas-negative targets; however, the killing of Fas-positive targets was much greater than that of Fas-negative targets. These findings suggest that both a Fas-dependent and a Fas-independent pathway mediate anti-host CTL activity in acute GVHD. In a second experiment (Fig. 7), anti-host CTL activity was assessed in the presence and the absence of concanamycin A (CMA), a perforin pathway inhibitor (22). Treatment with CMA reduced anti-host CTL activity on Fas-positive host targets by approximately 50% and reduced killing of Fas-negative targets to background levels, confirming that CTL use both perforin and Fas pathways in acute GVHD.

CD8\(^{+}\) T cells are the major effectors of FasL-mediated anti-host cytotoxicity

Up-regulation of FasL on both CD4\(^{+}\) and CD8\(^{+}\) donor T cells in acute GVHD mice suggests that both T cell subsets contribute to anti-host CTL activity. Previous studies of anti-host CTL activity have demonstrated lysis of MHC class I-positive, class II-negative host targets, consistent with the presence of CD8\(^{+}\) CTL (21). To determine whether donor CD4\(^{+}\) CTL also contribute to the killing of host lymphocytes in acute GVHD, anti-host CTL activity was determined using acute GVHD splenocytes depleted of either CD4\(^{+}\) or CD8\(^{+}\) T cells and tested on LPS-stimulated blasts (Fas positive, MHC class I and class II positive), L1210\(^{+}\) cells (Fas positive, MHC class I positive), or L1210\(^{-}\) cells (Fas negative, MHC class I positive; Fig. 8). If MHC class II-restricted CD4\(^{+}\) CTL are present, CD8 depletion should not abolish all killing on host LPS-stimulated blasts. Alternatively, if CD4\(^{+}\) CTL are present that kill through a Fas-dependent mechanism, CD8 depletion should eliminate all killing on Fas-negative targets but not on Fas-positive targets. As shown in Figure 8, the substantial killing...
of LPS-stimulated blasts and L1210-positive and -negative targets seen for undepleted acute GVHD splenocytes was entirely eliminated by CD8 depletion. Furthermore, depletion of CD4 T cells resulted in enhanced killing of all targets, consistent with the increased concentration of effector (CD8+) CTL. While these results do not totally exclude the presence of CD4+ CTL in vivo, they do not support a major role for such cells in the elimination of host lymphocytes in acute GVHD mice.

Fas/FasL up-regulation is linked to activation of donor CD8+ T cells and IFN-γ production

It has been previously shown that DBA CD8+ T cells have impaired generation of anti-host CTL in vivo and a reduced allogeueic precursor CTL frequency in vitro compared with H-2-identical B10.D2 mice (21, 23). Although increased production of IL-4 and IL-10 is seen in both acute and chronic GVHD mice, only acute GVHD mice exhibit activation of donor CD8+ T cells and increased IFN-γ production (23). To test whether these observations could be linked to Fas/FasL expression, we compared Fas/FasL expression in F1 mice receiving total (B63F1) or CD8-depleted B6 donor cells (B6 CD8 depl3F1). Chronic GVHD is typically observed in this latter P3F1 combination (21). As shown in Figure 9, B6 CD8 depl3F1 mice exhibit a loss of anti-host CTL activity on either P815 targets (Fas dull, MHC class I positive) or on LPS-stimulated blasts (Fas positive, MHC class I and II positive). It should be noted that the level of Fas expression on LPS-stimulated blasts is similar to that seen on host B cells in acute GVHD (an approximately fourfold increase in mcf compared with
that in freshly isolated splenocytes). This loss of functional CTL activity was associated with a marked reduction in FasL and IFN-γ mRNA expression (Fig. 10). Flow cytometric staining of B6 CD8 deple→F1 splenocytes (Fig. 11) confirmed very low expression of FasL on donor CD4+ T cells and of Fas on host B cells (compared with undepleted B6→F1 mice), reminiscent of that observed in DBA→F1 chronic GVHD mice (Fig. 2). To determine whether IFN-γ was necessary for Fas/FasL up-regulation, a neutralizing anti-IFN-γ mAb was administered in vivo to acute GVHD mice beginning at the time of cell transfer and at weekly intervals thereafter. As shown in Figure 12, in vivo inhibition of IFN-γ significantly reduced expression of FasL on donor CD4+ and CD8+ T cells and expression of Fas on host B cells compared with those in untreated acute GVHD mice.

Discussion

The initial steps in both acute and chronic GVHD are 1) activation of donor CD4+ T cells in response to allogeneic MHC class II molecules on the F1 host cells (12, 24); 2) production of cytokines such as IL-2, IL-4, and IL-10 (21, 23); and 3) activation of host B cells resulting in autoantibody production and lymphoproliferation detectable at 10 days after parental cell transfer (23). In acute GVHD, the activation of donor CD8+ T cells is associated with IFN-γ production (by both donor CD4+ and CD8+ T cells) and the development of donor anti-host CTL (23, 25), which rapidly eliminate F1 lymphocytes such that by 2 wk after parental cell transfer, acute GVHD mice exhibit lymphopenia and a profound immunodeficiency. Because host B cells are eliminated, autoimmunity in acute GVHD is transient. In contrast, the activation of donor CD4+ T cells, but not CD8+ T cells, in chronic GVHD is associated with no detectable IFN-γ production and no detectable donor anti-host CTL activity. As a result, autoreactive B cells are not eliminated, and lymphoproliferation with autoantibody production continues, resulting in the eventual development of a lupus-like immune complex glomerulonephritis after several months (12).

We have previously shown that acute GVHD was attenuated when FasL mutant C3H/gld mice were used as donors (26), suggesting an important role for FasL in the elimination of host cells. The present study directly demonstrates that Fas/FasL up-regulation occurs in acute, but not chronic, GVHD, requires activation of donor CD8+ T cells, and can be blocked almost entirely by anti-IFN-γ mAb treatment in vivo. These results strongly argue that IFN-γ plays a major role in the in vivo up-regulation of Fas/FasL in an alloantigen-driven response. Our inability to achieve complete inhibition of Fas/FasL up-regulation with anti-IFN-γ mAb suggests either failure to neutralize all IFN-γ in vivo or that other cytokines contribute to up-regulation of Fas/FasL in vivo.

Based on the correspondence between Fas/FasL expression in vivo and functional analysis in vitro, our results also suggest that the up-regulation of Fas/FasL plays a quantitatively significant role in anti-host CTL activity. However, donor CTL also kill host cells via the perforin pathway, as demonstrated by the reduction in cytotoxicity of CD8+ T cells exposed to a perforin pathway-blocking agent, concanamycin. These results are similar to those obtained in another model of GVHD using irradiated recipients and bone marrow transplantation. In this model, anti-host CTL were significantly attenuated with either Fas- or perforin-deficient donors (27, 28). Although CD4+ CTL clones generated in vitro have been shown to kill exclusively by the Fas pathway (29), and we observed FasL up-regulation on both donor CD4+ and CD8+ T cells in the P→F1 model, all the anti-host CTL activity (both Fas-dependent and Fas independent) could be accounted for by donor CD8+ CTL. This result is surprising considering that IFN-γ-producing CD4+ T cells are present in acute GVHD (23), and this functional subset expresses FasL.

Donor CD8+ T cells contribute to acute GVHD development not only through their role as CTL effectors, but also through their ability to promote IFN-γ production (23). Our results indicate that IFN-γ production, in turn, promotes the elimination of host cells by making donor CTLs better killers through the up-regulation of FasL and making host cells better targets through the up-regulation of Fas. Few studies have examined cytokine regulation of FasL expression in vivo. In a recent study using cloned T cells, it was reported that IFN-γ-inducing factor (IL-18) and IL-12 enhanced the FasL-mediated cytotoxicity of Th1 cells, whereas IFN-γ, TNF-α, and IFN-γ had no effect (30). However, IFN-γ has previously been shown to increase Fas expression on naive or unprimed cord lymphocytes, and the Fas-encoding gene has an IFN-γ-responsive element in its promoter (31, 32). The reciprocal, although weaker, up-regulation of Fas on donor T cells and of FasL on host T cells is consistent with a host anti-donor CTL response described by others (33), which, because of its lesser magnitude, is unable to prevent the stronger donor anti-host response.

Our results have important implications for the in vivo regulation of cell-mediated and antibody-mediated immune responses. In the P→F1 model, both donor and host immune responses are normal before cell transfer, and no radiation of the recipient is involved. Disease in this model results not from abnormal functioning of the immune response but, rather, from the targeting of a normal immune response to host alloantigens. Thus, acute and chronic GVHD can be viewed as a paradigm for Ag-driven in vivo
cell-mediated or Ab-mediated immune response, respectively. The data in the present study suggest that Fas/FasL up-regulation may be a feature of cell mediated Ag-driven responses, particularly when IFN-γ is produced, but not of Ab-mediated responses.

Lastly, our results have implications for the development of humoral autoimmunity. Escape of autoreactive B cells with subsequent autoantibody production is felt to be a possible mechanism involved in the development of humoral autoimmune diseases such as lupus (5, 34). Fas and FasL are important in the elimination of autoreactive B cells, as mice deficient in either of these molecules (e.g., lpr or gld, respectively) exhibit humoral autoimmunity. It has been postulated that autoreactive B cells are normally kept in check by Fas/FasL-mediated killing, particularly by CD4+ T cells. Fas and FasL are important in the elimination of autoreactive B cells, as mice deficient in either of these molecules (e.g., lpr or gld, respectively) exhibit humoral autoimmunity. It has been postulated that autoreactive B cells are normally kept in check by Fas/FasL-mediated killing, particularly by CD4+ T cells (5, 6, 29), and that up-regulation of Fas on B cells is essential for their elimination (35, 36). However, our results indicate that activation of autoreactive B cells in the absence of IFN-γ may prevent their elimination through the down-regulation of Fas/FasL. Not only are CTL-killing mechanisms impaired through reduced FasL expression, but B cells are less susceptible to elimination due to reduced Fas expression (37). We have previously shown that the administration of the IFN-γ-promoting cytokine, IL-12, to chronic GVHD mice can result in the elimination of autoreactive B cells by converting disease to acute GVHD (38). However, this effect was IFN-γ independent. We are currently testing the feasibility of inducing Fas/FasL up-regulation and autoreactive B cell elimination by IFN-γ administration to mice undergoing chronic lupus-like GVHD.

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References


