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Immunology

Graft-versus-host-disease-associated lymphoid hypoplasia and B cell dysfunction is dependent upon donor T cell-mediated Fas-ligand function, but not perforin function

(bone marrow transplantation/cytotoxicity/immune dysfunction/engraftment/chimerism)

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ABSTRACT **Allogeneic bone marrow transplant recipients often exhibit a graft-versus-host-disease (GVHD)-associated immune deficiency that can be prolonged and lead to life-threatening infections. We have examined the role of donor T cell-mediated cytotoxic function in the development of GVHD-associated immune deficiency. A major histocompatibility complex-matched model of allogeneic bone marrow transplantation was employed in which lethally irradiated C3H.SW mice received a nonlethal dose of T cells from either perforin-deficient (B6-perforin 0/0), Fas-ligand (FasL)-defective (B6-*gld*), or normal (B6) allogeneic donor mice. T cell-depleted marrow from B6-Ly-5.1 congenic donor mice was transplanted along with the donor T cell populations to determine the effects of donor T cell-mediated cytotoxicity on engraftment. Our results demonstrate that recipients of perforin-deficient or normal allogeneic T cells exhibit profound lymphoid hypoplasia and severely reduced splenic proliferative responses to lipopolysaccharide *in vitro*. In contrast, GVHD-associated lymphoid hypoplasia is dramatically reduced and *in vitro* B cell function is intact in recipients of FasL-defective allogeneic T cells. Engraftment of myeloid and erythroid lineage cells occurs irrespective of donor T cell cytotoxic function. Although recipients of perforin-deficient or normal allogeneic T cells exhibited hematopoietic engraftment exclusively of donor origin, recipients of FasL-defective donor T cells exhibited significant mixed chimerism (Ly-5.1/Ly-5.2). Because only marrow of donor origin was transplanted, this finding suggests that Fas-mediated antirecipient cytotoxicity is required for clearance of residual hematopoietic stem cells of host origin that persist following lethal irradiation.**

Patients with chronic graft-versus-host disease (GVHD) after allogeneic bone marrow transplantation (BMT) often exhibit a persistent immune deficiency syndrome that increases susceptibility to bacterial, fungal, and viral opportunistic infections (1). These infectious complications constitute the most common cause of death among patients with chronic GVHD (2). GVHD-associated immune deficiency (GVHID) in clinical and experimental allogeneic BMT is characterized by marked lymphoid hypoplasia and functional defects in humoral and cell-mediated immunity (3, 4). GVHD-associated lymphoid hypoplasia was first described by Billingham and Brent (5) in neonatal mice transplanted with allogeneic spleen cells. Later work further characterized functional defects of antibody production in mice undergoing a graft-versus-host reaction (6, 7). Studies of spleen cell function from mice with chronic GVHD have demonstrated decreased B cell proliferative responses upon stimulation with bacterial lipopolysaccharide (LPS) *in vitro* (8, 9). In addition, numbers of colony forming units in the spleen and bone marrow of mice with chronic GVHD are severely reduced and the resulting depression of lymphopoiesis may contribute to the profound lymphoid hypoplasia characteristic of GVHID (10, 11).

Much evidence has accumulated in the literature which suggests that dysregulation of inflammatory cytokines such as interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α) is critical for the development of GVHID (12, 13). Relatively few studies in the literature have implicated antirecipient cell-mediated cytotoxicity as a principal mechanism underlying GVHID (14). Our laboratory has begun to examine the role of antirecipient specific cell-mediated cytotoxicity in the development of GVHID (15). We have employed a major histocompatibility complex (MHC)-matched model of BMT in which lethally irradiated mice receive nonlethal doses of T cells from perforin-deficient, Fas-ligand (FasL)-defective, or normal allogeneic donor mice. Transplantation of lower doses of MHC-matched allogeneic T cells induces a less severe form of GVHD that more closely resembles chronic GVHD (16). Utilization of nonlethal doses of donor T cells allows recipients to survive long enough for hematopoietic engraftment to occur. Transplantation of Ly-5.1 congenic marrow into lethally irradiated Ly-5.2 recipient mice enables the measurement of donor/host chimerism levels following marrow engraftment (17).

The results of the present study demonstrate that recipients of perforin-deficient or wild-type allogeneic T cells exhibit profound lymphoid hypoplasia and severely reduced splenic proliferative responses to LPS *in vitro*. However, GVHD-associated lymphoid hypoplasia is dramatically reduced and *in vitro* B cell function remains intact in recipients of FasL-defective allogeneic T cells. Engraftment of myeloid and erythroid hematopoietic lineages appears to occur irrespective of the donor T cell cytotoxic function. Unexpectedly, spontaneous mixed chimerism developed in the recipients of FasL-defective donor T cells, while virtually no evidence of mixed chimerism was observed in recipients of perforin-deficient or wild-type donor T cells. This result suggests that Fas-mediated antirecipient cytotoxicity is required for clearance of residual hematopoietic stem cells of host origin that persist following lethal irradiation. The implications of these findings are dis-

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Abbreviations: GVHD, graft-versus-host disease; BMT, bone marrow transplantation; GVHID, GVHD-associated immune deficiency; LPS, lipopolysaccharide; IFN-γ, interferon γ; TNF-α, tumor necrosis factor α; FITC, fluorescein isothiocyanate; PE, phycoerythrin; MHC, major histocompatibility complex.

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cussed along with some of the currently accepted proposed mechanisms thought to underlie GVHID.

MATERIALS AND METHODS

Mice. C57BL/6J (H-2^b), B6Smn.C3H-*gld* (H-2^b), B6.SJL-*Cd45*^{a} *Pep-3*^{b}/BoyJ (H-2^b, Ly-5.1), and C3H.SW (H-2^b, Ly-5.2) mice were obtained from The Jackson Laboratory. Perforin-deficient C57BL/6 mice (B6-perforin 0/0) were originally obtained from D. Kägi and H. Hengartner (University of Zurich), and B. Ledermann and K. Bürki (Sandoz Pharma, Basel). B6-perforin 0/0 (H-2^b) mice were propagated at the University of Miami School of Medicine Specialized Animal Facility and were maintained in a pathogen-free colony until use.

BMT Model. Bone marrow cells were aspirated from the femurs and tibias of B6-Ly-5.1 donor mice. T cells were depleted from the bone marrow by incubation with anti-Thy1.2 mAb (30-H-12 culture supernatant) at 1:5 dilution and 4°C for 30 min followed by Low-Tox M complement (Accurate Chemicals) at 1:20 dilution and 37°C for 45 min. Spleen and lymph node cells were harvested from normal or cytotoxically defective donor mice, then pooled and treated with anti-B220 mAb (14.8 culture supernatant) at 1:2.5 dilution and 4°C for 30 min, followed by a secondary mouse-anti-rat mAb (18.5 ascites) at 1:50 and 4°C for an additional 30 min. The labeled cells were then treated with rabbit complement at 1:10 dilution and 37°C for 45 min to remove B cells and enrich for T cells. This procedure routinely enriches the T cell population to levels of purity between 75% and 80% as determined by flow cytometric analysis. Recipient mice were exposed to 900 cGy total body irradiation from a ^{60}Co source at a dose rate of 50 cGy/min 24 h before the BMT. The number of donor T cells (2.5×10^6) was selected based upon the ability of the wild-type inoculum to induce a mild form of GVHD that was nonlethal in the B6→C3H.SW donor-recipient strain combination. To ensure reproducible results, the number of $CD3^+$ T cells in the donor inoculum was precisely quantified by flow cytometry for each BMT. Precisely the same numbers of $CD3^+$ T cells from wild-type, perforin-deficient, or FasL-defective B6 donors were added to the T cell-depleted bone marrow cells (5×10^6) from B6-Ly-5.1 congenic donors and injected together into irradiated recipient mice intravenously via the lateral tail vein in a volume of 0.5 cc. The same number of T cells from syngeneic C3H.SW donors were added to the T cell-depleted bone marrow cells from C3H.SW (Ly-5.2) donors for the syngeneic negative control recipient group. Mice receiving transplants were distributed into groups containing four to eight mice per group in each experiment. Recipients were maintained on acidified water (pH 3.0) containing antibiotics (100 mg/liter neomycin sulfate, 10 mg/liter polymyxin B) from day −3 to day 14 post-BMT. Recipient mice were monitored for clinical signs of GVHD including weight loss, dermatitis, alopecia, diarrhea, hunched posture, and mortality. One or two representative mice were killed from each recipient group at various times post-BMT to harvest lymphohematopoietic tissues for functional and phenotypic analysis.

Immunophenotyping. Spleen, lymph node, thymus, bone marrow, and peritoneal macrophage cells were stained with fluorescein isothiocyanate (FITC)-conjugated or biotinylated antibodies and streptavidin-phycoerythrin (PE) (Fisher). mAbs used included anti-CD3 (145-2C11), anti-B220 (RA3-6B2), anti-CD19 (1D3), anti-CD43 (S7), anti-CD4 (RM4-5), and anti-CD8 (53-6.7) obtained from PharMingen, and anti-F4/80 from Accurate Chemicals. Anti-Ly-5.1 (A20) clone was provided by D. H. Sachs (Massachusetts General Hospital, Boston), and the anti-Ly-6G (1A8) clone was provided by T. R. Malek (University of Miami School of Medicine). For immunophenotyping, $0.5–1 \times 10^6$ cells were washed in fluorescence-activated cell sorter (FACS) buffer (PBS with 1% BSA/0.02% sodium azide), then incubated with the FITC-conjugated and biotinylated mAbs for 20 min on ice. Cells were again washed in FACS buffer and incubated with the appropriate secondary antibody. The stained cells were resuspended in FACS buffer at 1×10^6/ml and analyzed on a FACScan flow cytometer (Beckton Dickinson). Data were analyzed within a gate established using forward (180°) and side (90°) angle light scatter. Immunophenotypic data shown are analyses of lymphohematopoietic tissue from one or two recipient mice from each group at a given interval posttransplant, but are representative of the large number of mice tested (35/35 individual mice analyzed).

Lymphocyte Stimulation Assay. Recipient spleen cell populations were cultured at 1×10^5 per well in quadruplicate wells for 2 days in the presence of either culture medium alone or 50 mg/ml *Escherichia coli* LPS (Sigma) to induce polyclonal B cell activation. Proliferative responses were determined by measuring [^{3}H]thymidine incorporation following a 6- to 8-h pulse label period. All incorporation values were averaged and standard deviations for LPS-stimulated cultures ranged from 1.5% to 15.4%.

RESULTS

GVHD-Associated Lymphoid Hypoplasia Is Markedly Reduced in Spleens of Mice Receiving FasL-Defective Donor T Cells. Spleens were harvested from BMT recipients at various intervals between 35 and 80 days posttransplant. Numbers of spleen cells recovered are summarized in Table 1. C3H.SW mice receiving syngeneic T cells and T cell depleted C3H.SW (Ly-5.2) marrow did not develop signs of GVHD-associated lymphoid hypoplasia and had normal numbers of spleen cells.

Table 1. Spleen cell number, CD4/CD8 ratio, and LPS response 1–3 months after bone marrow transplant with cytotoxically defective allogeneic T cells

BM Transplant	Cells/spleen*	CD4/CD8 ratio†	LPS Response‡
C3H.SW → C3H.SW	$22.9 \times 10^6–100.0 \times 10^6$	5.1–5.6	169.4
B6 → C3H.SW	$0.3 \times 10^6–11.4 \times 10^6$	0.9–1.0	7.4
B6-perforin 0/0 → C3H.SW	$1.6 \times 10^6–11.9 \times 10^6$	1.0–1.1	5.3
B6-*gld* → C3H.SW	$25.0 \times 10^6–109.0 \times 10^6$	1.1–1.4	145.1

*Total numbers of splenocytes recovered from recipient spleens at 33–77 days after transplantation of MHC-matched allogeneic bone marrow and 2.5×10^6 cytotoxically defective donor T cells. Recovery numbers are expressed as number of spleen cells per mouse.

†CD4/CD8 ratios are calculated based on flow cytometric analysis of recipient spleen cells in at least three separate experiments for each group.

‡Values shown are the stimulation index values based on [^{3}H]thymidine incorporation data from two separate representative experiments. Incorporation values were averaged and standard deviations for LPS-stimulated cultures ranged from 1.5% to 15.4%. Similar results were observed in 11/12 separate lymphocyte stimulation assays.

Recipients of perforin-deficient or wild-type allogeneic T cells exhibited severe lymphoid atrophy characteristic of GVHID. In contrast, mice receiving FasL-defective allogeneic T cells did not exhibit significant hypoplasia (Table 1) in 9/9 mice tested. The spleen cell populations were stained for lymphoid and myeloid surface markers and analyzed by flow cytometry. Analysis of splenic T cell subsets in recipients of syngeneic T cells demonstrated the normal predominance of CD4+ T cells (Table 1). Mice receiving wild-type, perforin-deficient, or FasL-defective allogeneic T cells exhibited an altered CD4/CD8 ratio consistent with an ongoing graft-versus-host reaction (18). Mice receiving wild-type allogeneic T cells developed only mild transient weight loss and mild dermatitis, whereas recipients of perforin-deficient or FasL-defective allogeneic T cells did not develop clinically significant signs of GVHD during these studies.

To assess the functional capability of the splenic B cells *in vitro*, spleen cells were cultured for 2 days in the presence of LPS, and proliferative responses were measured by [^{3}H]thymidine incorporation. Stimulation index values from two representative proliferation assays is presented in Table 1. The results of several proliferation assays (11/12) have routinely demonstrated that LPS responses are intact in recipients of FasL-defective allogeneic T cells, whereas LPS responses are virtually absent in recipients of perforin-deficient or wild-type allogeneic T cells.

The spleen cells were also stained for the B cell marker CD19 and the donor hematopoietic lineage marker Ly-5.1. Recipients of perforin-deficient or wild-type allogeneic T cells exhibited hypocellularity within the lymphoid gate by light scatter and virtually no CD19$^+$ B cells by immunofluorescence (Fig. 1). In contrast, splenic B cell content in recipients of FasL-defective allogeneic T cells was similar to that of the syngeneic control recipient mice (Fig. 1 *d* and *h*). Interestingly, a significant proportion of the CD19$^+$ spleen cells were of host origin (Ly-5.2$^+$) in mice receiving FasL-defective donor T cells despite the fact that only bone marrow of donor origin (Ly-5.1$^+$) was transplanted and that the recipient mice were myoablated by lethal irradiation prior to the transplant (Fig. 1*h*).

Analysis of spleen cells within the myeloid gate identified essentially normal levels of myeloid lineage engraftment in all four recipient groups (Fig. 1). Notably, significant levels of mixed chimerism were observed only in the recipients of FasL-defective allogeneic T cells (Fig. 1*l*), while recipients of perforin-deficient or wild-type donor T cells exhibited myeloid cell engraftment almost exclusively of donor origin (Fig. 1 *j* and *k*). This same pattern of mixed chimerism was completely stable for more than 120 days posttransplant for all hematopoietic lineages examined (data not shown).

GVHD-Associated Thymic Atrophy Is Markedly Reduced in Mice Receiving FasL-Defective Donor T Cells. Thymi were harvested from BMT recipients at various time intervals 1–2 months posttransplant. Numbers of thymocytes recovered from these recipient mice are summarized in Table 2. Mice receiving perforin-deficient or wild-type allogeneic T cells exhibited severe thymic atrophy. In contrast, significant numbers of thymocytes were always recovered from recipients of FasL-defective allogeneic T cells (Table 2).

Phenotypic analysis of thymocytes was performed with mAbs against the T cell marker CD3 and the hematopoietic lineage marker Ly-5.1. Subpopulations of CD3$^-$, CD3^{lo}, and CD3^{hi} thymocytes are present in all four recipient groups (Fig. 2). Notably, significant mixed chimerism is present only in recipients of FasL-defective allogeneic T cells (Fig. 2*h*), whereas the thymocytes of mice receiving perforin-deficient or

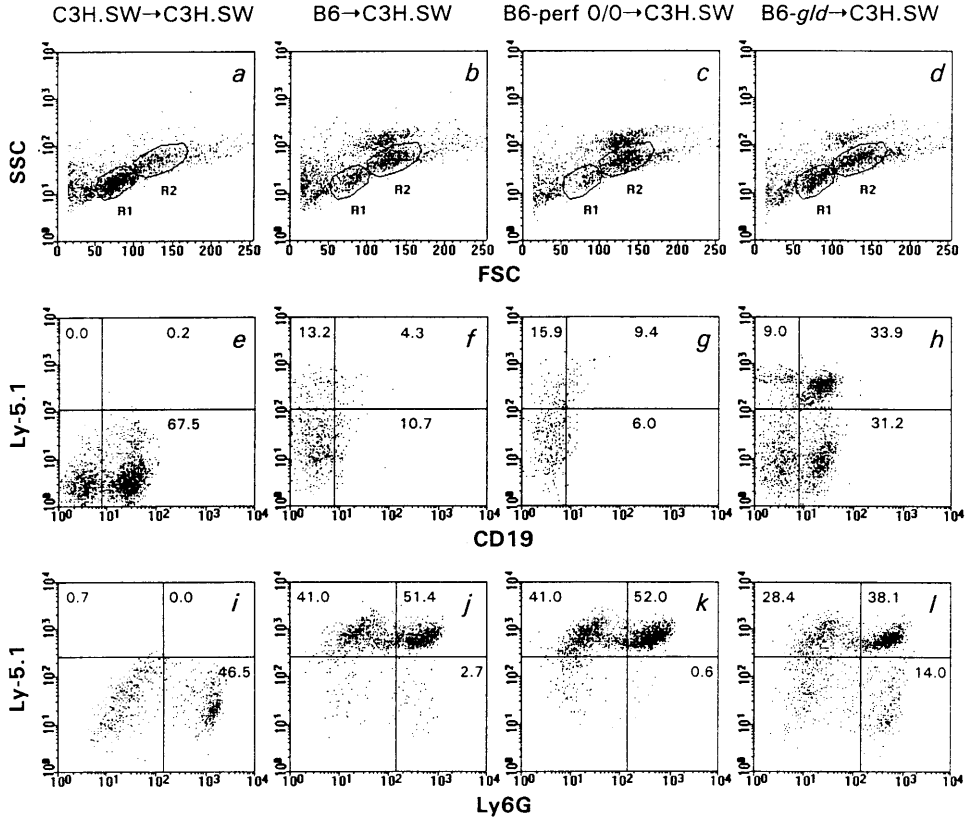

FIG. 1. Light scatter (*a–d*) and immunofluorescent analysis (*e–l*) of recipient spleen cells 39 days after BMT with 2.5×10^6 syngeneic, wild-type allogeneic, perforin-deficient allogeneic, or FasL-defective allogeneic T cells. Splenocytes were stained with anti-CD19–FITC (FL1) and anti-Ly-5.1–biotin plus avidin–PE (FL2) and analyzed within the lymphocyte gate region (R1). Splenocytes were also stained with anti-Ly6G–FITC (FL1) and anti-Ly-5.1–biotin plus avidin-PE (FL2) and analyzed within the granulocyte/monocyte gate region (R2).

Table 2. Thymus cell number 1 month after bone marrow transplant with cytotoxically defective allogeneic T cells

BM Transplant	Cells/thymus*
C3H.SW → C3H.SW	22.0×10^6–35.8×10^6
B6 → C3H.SW	0.0×10^6–2.6×10^6
B6-perforin 0/0 → C3H.SW	0.0×10^6–8.1×10^6
B6-*gld* → C3H.SW	15.4×10^6–28.8×10^6

*Total numbers of thymocytes recovered from recipient thymi at 33–39 days after transplantation of MHC-matched allogeneic bone marrow and 2.5×10^6 cytotoxically defective donor T cells. Recovery numbers are expressed as number of thymus cells per mouse.

wild-type T cells are virtually completely replaced by cells of donor origin (Fig. 2 *f* and *g*).

GVHD-Associated Lymphoid Hypoplasia Is Markedly Reduced in the Bone Marrow of Mice Receiving FasL-Defective Donor T Cells. Bone marrow was harvested from BMT recipients 1–3 months posttransplant and analyzed by light scatter and immunofluorescence. In normal mouse bone marrow, the lymphoid population consists predominantly of $CD19^+$ B cells and B cell progenitors (19). C3H.SW mice receiving syngeneic T cells and T cell depleted C3H.SW (Ly-5.2) marrow exhibited a normal marrow lymphoid population with the majority of the gated cells staining positive for CD19 (Fig. 3 *a* and *e*). Analysis of marrow from recipients of perforin-deficient or wild-type allogeneic T cells routinely demonstrated the presence of severe lymphoid hypoplasia and the absence of virtually all $CD19^+$ B lineage cells (Fig. 3). In contrast, mice receiving FasL-defective allogeneic T cells did not exhibit evidence of lymphoid hypoplasia, and a significant population of $CD19^+$ cells was always observed in their marrow (Fig. 3 *d* and *h*). Significant mixed chimerism was routinely observed in the recipients of FasL-defective donor T cells, but was never observed in the recipients of perforin-deficient or wild-type allogeneic T cells.

Bone marrow cells were also stained for myeloid lineage markers and analyzed by flow cytometry. As shown in Fig. 3, engraftment of myeloid lineage cells was essentially normal in all four recipient groups. Significant mixed chimerism was observed in the recipients of FasL-defective donor T cells (Fig. 3*l*), whereas mice receiving perforin-deficient or wild-type donor T cells exhibited virtually complete donor chimerism (Fig. 3 *j* and *k*). A population of more granular cells with elevated side angle light scatter properties was often present in the spleen and marrow of mice receiving perforin-deficient, FasL-defective, or wild-type allogeneic T cells.

DISCUSSION

The results of the present study demonstrate that the absence of FasL-dependent cell-mediated cytotoxic function in the donor T cell population markedly diminishes the development of GVHD-associated lymphoid hypoplasia and B cell immune deficiency. However, the absence of perforin-mediated cytotoxic function in the donor T cells does not diminish the severity of lymphoid hypoplasia or B cell dysfunction observed in GVHID. We conclude that Fas-mediated cytotoxic effector function by the donor T cell population is either directly or indirectly involved in the mechanisms underlying GVHD-associated lymphoid hypoplasia. Furthermore, we propose that the Fas-dependent T cell-mediated suppression of lymphocyte genesis in the marrow is primarily responsible for the persistent B cell immune deficiency and thymic atrophy observed in chronic GVHD.

The finding of a critical role for FasL-dependent cell-mediated cytotoxicity in GVHID is not inconsistent with the previously identified essential role of inflammatory cytokines such as IFN-γ or TNF-α in GVHID (12, 13). In a model of chronic GVHD employing sublethal irradiation and MHC-matched allogeneic BMT, Klimpel *et al.* (12) found that mice given anti-IFN-γ mAb had no lymphoid hypoplasia and near normal gross and histologic appearance of their thymus, spleen, and lymph node tissue. In addition, Wall and Sheehan (13) found that neutralizing anti-TNF-α mAb reversed the ability of splenocytes from animals with GVHD to suppress proliferative responses of normal splenocytes to mitogens *in vitro*. It has also been shown that serum levels of IFN-γ and TNF-α cytokines are elevated in patients following clinical allogeneic BMT (20–22). In light of such results and based on the present findings, we propose a model in which dysregulation and overproduction of these cytokines contributes to up-regulation of Fas expression on lymphocyte progenitor populations in the marrow post-BMT, resulting in the enhancement of their susceptibility to Fas-induced apoptosis. In the absence of elevated levels of IFN-γ or TNF-α, hematopoietic progenitor populations in the marrow are not likely to be as exquisitely susceptible to Fas-mediated deletion (23, 24). Alternatively, lymphoid lineage-committed progenitor cells

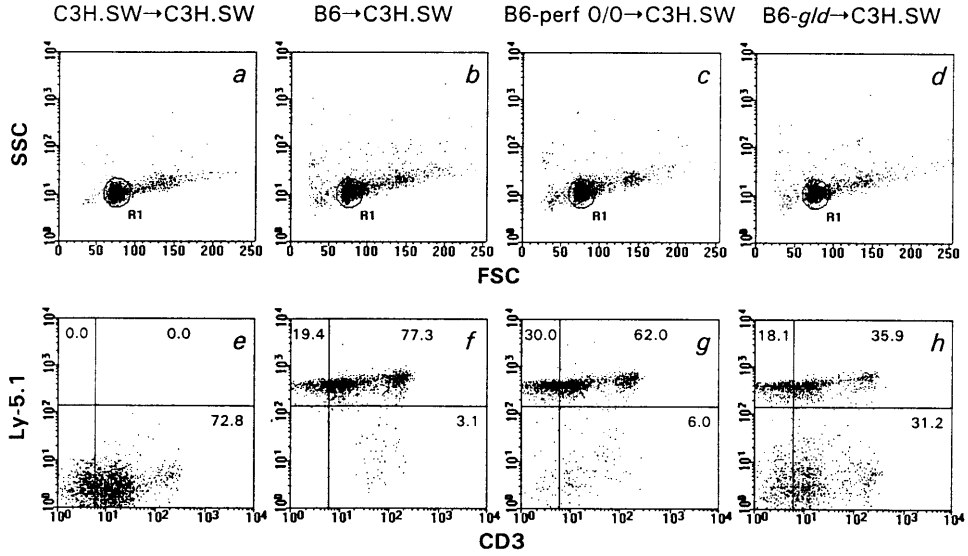

FIG. 2. Light scatter (*a–d*) and immunofluorescent analysis (*e–h*) of recipient thymus cells 39 days after BMT with 2.5×10^6 syngeneic, wild-type allogeneic, perforin-deficient allogeneic, or FasL-defective allogeneic T cells. Thymocytes were stained with anti-CD3–FITC (FL1) and anti-Ly-5.1–biotin plus avidin–PE (FL2) and analyzed within the thymocyte gate region (R1).

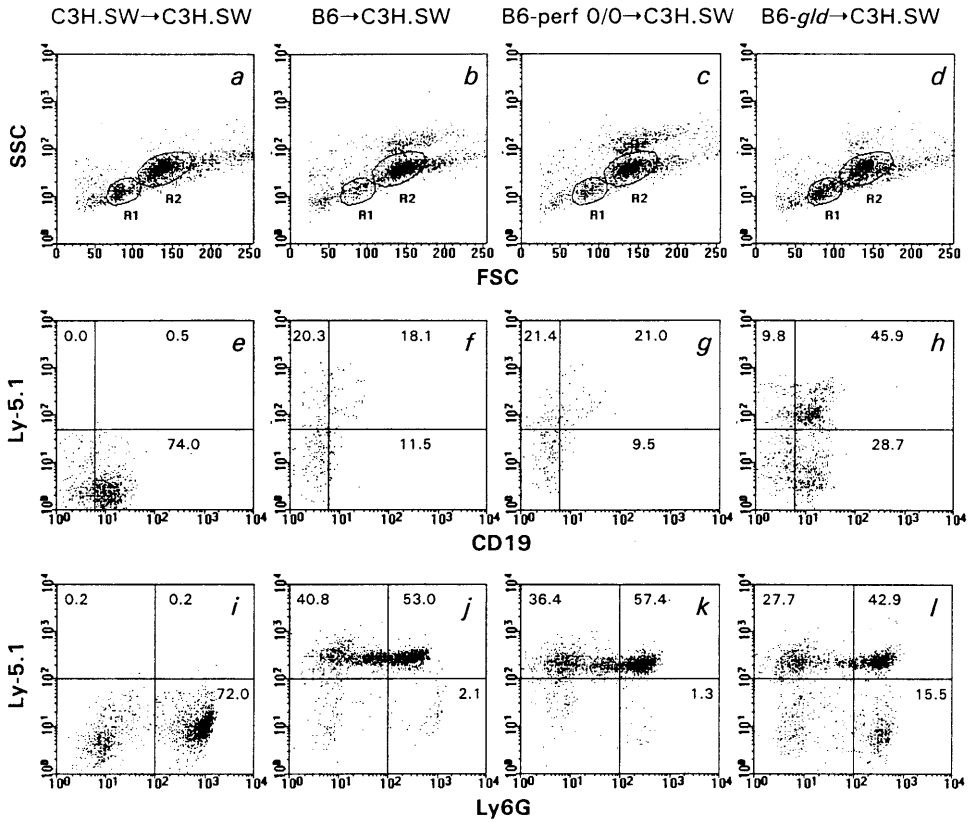

FIG. 3. Light scatter (*a–d*) and immunofluorescent analysis (*e–l*) of recipient bone marrow cells 39 days after BMT with 2.5×10^6 syngeneic, wild-type allogeneic, perforin-deficient allogeneic, or FasL-defective allogeneic T cells. Marrow cells were stained with anti-CD19–FITC (FL1) and anti-Ly-5.1–biotin plus avidin–PE (FL2), and analyzed within the lymphocyte gate region (R1). Marrow cells were also stained with anti-Ly6G–FITC (FL1) and anti-Ly-5.1–biotin plus avidin–PE (FL2), and analyzed within the granulocyte/monocyte gate region (R2).

may be constitutively susceptible to Fas-induced apoptosis even without exposure to elevated levels of IFN-γ or TNF-α.

We speculate that because early B lineage committed progenitor cells arising from the donor hematopoietic stem cells express Fas, such cell populations are vulnerable to induction of apoptosis by interaction with FasL (25). Expression of FasL on alloactivated donor T cell populations which abnormally encounter B lineage progenitor cells in the marrow could lead to self-reactive deletion of the donor origin B cell progenitors. The alloactivated T cells may gain access to the marrow either by abnormal circulation or by becoming localized in the marrow during an anti-recipient-specific inflammatory process (26). Abnormal re-entry of circulating T cells or inflammation in the recipient thymus may lead to a similar dysregulated deletional process for thymocyte progenitors (27, 28). Notably, it has been shown recently that immature $CD4^+CD8^+$ thymocytes are specifically sensitive to anti-Fas antibody-induced apoptosis (29). Based on our findings concerning lymphoid atrophy in GVHD, we propose that FasL-dependent T cell-mediated cytotoxic activity could also induce substantial damage to stromal cells in the recipient bone marrow or to epithelial cells in the recipient thymus contributing to further impairment of normal lymphopoiesis in both compartments (30).

We have found that *in vitro* LPS function is intact in our BMT model whenever significant numbers of mature B cells are present in the peripheral lymphoid compartment. This finding suggests that B cell dysfunction in chronic GVHD may be the result of B cell deficiency or T helper cell dysfunction rather than an intrinsic functional defect in the B cell population (11, 31). Garvy *et al.* (32) have suggested that the B cell deficiency in minor GVHD appears to arise from an interruption either at the pro-B cell stage or earlier in the B-lymphopoietic pathway. In preliminary phenotypic and functional studies of recipient marrow, we have confirmed that pro-B cell ($CD43^+B220^+$) populations are markedly decreased and colony formation in response to recombinant mouse interleukin 7 is impaired in recipients of perforin-deficient and normal allogeneic T cells. In contrast, pro-B cells are present in relatively normal numbers, and interleukin 7-responsive colony-forming units is intact in recipients of FasL-defective allogeneic T cells (data not shown).

Engraftment and hematopoiesis of myeloid and erythroid lineage cells remains intact in the recipient mice irrespective of donor T cell-mediated perforin or FasL cytotoxic function. This finding is consistent with the previous report that erythroid and myeloid hematopoiesis remains relatively intact in murine models of chronic GVHD (10). Accordingly, we speculate that committed myeloid and erythroid lineage progenitor cells in the marrow are not as vulnerable to FasL or perforin-mediated killing compared with lymphoid lineage progenitors. Interestingly, a population of large highly granular cells is apparent upon light scatter analysis of spleen and marrow tissues in recipients of perforin-deficient, FasL-defective, and wild-type allogeneic T cells. Immunofluorescent analysis of this population demonstrates that these cells are $CD3^-$ and $CD19^-$, but are $Ly6G^+$. This population may represent a previously uncharacterized myeloid cell population that is expanded during the graft-versus-host reaction.

We have consistently observed the spontaneous development of multilineage mixed chimerism in recipients of FasL-defective donor T cells. This is remarkable because only marrow of donor origin ($Ly\text{-}5.1^+$) was transplanted into lethally irradiated $Ly\text{-}5.2^+$ recipient mice. The observation of persistent multilineage reconstitution by hematopoietic cells of both donor and host origin can only mean that hematopoietic

stem cells (HSC) of host origin persist following lethal irradiation. The finding that significant mixed chimerism develops only in the absence of donor T cell-mediated FasL function suggests that the residual host HSC are somehow deleted or inhibited by a FasL-dependent mechanism. Recently, it has been demonstrated that while $CD34^+$ cells in freshly isolated bone marrow do not express Fas, the expression of Fas and susceptibility to anti-Fas antibody-induced apoptosis by these cells is markedly up-regulated following exposure to IFN-γ or TNF-α (23, 24). We conclude that the expression of functional FasL by wild-type or perforin-deficient alloreactive donor T cells is both necessary and sufficient to delete residual host HSC that persist following lethal total body irradiation. Therefore, the inability of FasL-defective donor T cells to delete the postirradiation residual host HSC may lead to the establishment of persistent mixed chimerism even without the addition of host origin marrow.

In our model system, it is not possible to assess the level of donor/host chimerism of erythroid lineage cells because the Ly-5 gene product is not expressed on erythroid lineage cells (33). However, preliminary data from this laboratory has found that $F4/80^+$ thioglycollate-elicited peritoneal macrophage populations are of mixed origin in recipients of FasL-defective allogeneic T cells, whereas these populations are exclusively of donor origin in recipients of perforin-deficient or wild-type allogeneic T cells. The spontaneous mixed chimerism that develops in recipients of FasL-defective donor T cells may have potential advantages for the recipient because it has been suggested that mixed allogeneic chimerism promotes a state of donor-specific tolerance for solid organ allografts (34).

In conclusion, the results of this investigation have identified a critical role for FasL-dependent donor T cell-mediated cytotoxicity in the development of GVHD-associated lymphoid hypoplasia and B cell immune dysfunction. Studies are currently underway to examine the susceptibility of lymphoid progenitor populations to Fas-mediated killing *in vitro*. Our laboratory is also attempting to further characterize the status of humoral immunity in recipients of FasL-defective T cells. Finally, the current findings suggest that therapeutic modalities that successfully interfere with FasL-mediated killing *in vivo* might significantly improve the posttransplant immune status of patients following clinical allogeneic BMT.

Note Added in Proof. A recent paper by Via *et al.* (35) reported reduced depletion of host lymphocytes in acute GVHD following transfer of C3H.gld spleen cells in a parent into F_1 GVH model.

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