CD8+ Cells Are Not Necessary for Allograft Rejection or the Induction of Apoptosis in an Experimental Model of Small Intestinal Transplantation

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CD8$^+$ Cells Are Not Necessary for Allograft Rejection or the Induction of Apoptosis in an Experimental Model of Small Intestinal Transplantation

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Allospecific CTL can function as cellular effectors of solid organ graft rejection; however, the specific mechanisms of cell damage remain undetermined. In this study we examined the role of CD8$^+$ T cells in apoptosis and rejection of small intestinal allografts. ACI rat intestinal grafts transplanted into Lewis rat recipients showed apoptosis of epithelial crypt cells on day 3 posttransplantation as determined by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling staining. By day 7 numerous apoptotic crypt cells were detected in allografts, but were rarely observed in FK506-treated allograft recipients, isografts, or native intestine of allograft recipients. To further investigate the mechanism of rejection, recipient rats were depleted of CD8$^+$ cells by treatment with OX-8 mAbs the day before and the day after transplantation of rat intestinal allografts. Repletion of CD8$^+$ cells from allograft recipients did not alter the tempo or the histologic features of rejection compared with those in the control (IgG-treated) group. Moreover, there was no difference in the number of apoptotic crypt epithelial cells in the grafts of control and CD8-depleted rats. Reverse transcriptase-PCR analyses determined there were similar levels of transcripts for Fas, Fas ligand, perforin, and granzyme B in control and CD8-depleted allograft recipients. By Western blot it was determined that the levels of Fas ligand protein were increased in the CD8-depleted group compared with those in control and FK506-treated allograft recipients. These data suggest that CD8 cells are not required for tissue injury or apoptotic cell death in small intestine allograft rejection. The Journal of Immunology, 1998, 160: 3673–3680.

The requirement for CD8$^+$ T cells and contact-dependent cytotoxicity in the rejection of an allograft is controversial. Modulation of CD8$^+$ cells has lead to long term survival of heart allografts (1); however, in other studies, islets, skin, and heart allografts are rejected in CD8-depleted animals (2–4). In contrast, CD4$^+$ T cells appear to be essential for graft damage, since in most models, allograft rejection is abrogated in the absence of functional CD4$^+$ T cells (3, 5–8). The requirement for CD4$^+$ cells in allograft rejection has mainly been attributed to their ability to produce cytokines that facilitate the development of MHC class I-specific CD8$^+$ CTL and to promote delayed-type hypersensitivity. However, it is also possible that CD4$^+$ T cells may function as alloreactive CTL (9).

There are two distinct mechanisms by which CTL mediate target cell death: the perforin-dependent granule-exocytosis pathway and the Fas/Fas ligand (FasL)$^+$ pathway (10–13). CD8$^+$ CTL can kill by either of these two cytotoxic mechanisms, whereas CD4$^+$ CTL appear to mediate cytotoxicity primarily through the Fas-dependent pathway (14). Numerous reports have demonstrated up-regulation of transcripts for perforin, granzyme B, and FasL during allograft rejection (15–23). However, experiments with perforin knockout mice and gld and lpr mice suggest that neither the perforin nor the Fas pathway is essential for graft rejection (19, 24–26). Both perforin- and Fas-mediated pathways of cytotoxicity can result in target cell apoptosis. Furthermore, apoptosis can contribute to tissue damage during rejection of an allograft (27). We have previously determined that hepatocellular apoptosis in liver allografts parallels the pathologic and biochemical indicators of rejection (29). Similar studies have reported an increased incidence of apoptosis in rejecting cardiac, kidney, and intestinal allografts (29–31). However, in one experimental model of cardiac transplantation, only minimal apoptosis of cardiac myocytes was detected, although apoptotic cells were detected within the inflammatory infiltrate (32). Thus, further studies are necessary to determine the precise mechanism by which CTL kill alloreactive targets in vivo and the specific role of apoptosis in allograft rejection.

In this report we examine the role of CD8$^+$ cells and apoptosis in acute rejection in a high responder model of small intestinal transplantation. We demonstrate that apoptosis contributes to tissue damage during small intestinal allograft rejection. Furthermore, depletion of CD8$^+$ cells does not ameliorate rejection nor does it alter the extent of apoptosis in the allograft. These results demonstrate that CD8-independent pathways can mediate the apoptotic cell death that culminates in the rejection of an allograft.

Materials and Methods

Animals

Inbred male Lewis rats (RT1l) and ACI rats (RT1a), weighing 200 to 240 g, were purchased from Harlan (Indianapolis, IN). All animals were housed in...
accordance with institutional animal care policies and had access to water and standard laboratory chow ad libitum.

Small intestine transplantation

Heterotopic small intestine transplantation (SIT) was performed as previously described (33, 34). Donor and recipient surgeries were performed aseptically under anesthesia with isofluorane. The allogeneic combination consisted of ACI donors and Lewis recipients, while Lewis rats were both donors and recipients in the isogeneic transplants. Briefly, the whole length of donor small intestine from the ligaments of Treitz to the ileocecal valve was harvested, flushed intraluminally with cold (4°C) lactated Ringer’s solution containing 0.5% neomycin sulfate, and preserved at 4°C for 60 min. The graft was transplanted heterotopically with end-to-side, aorto-aortic, and portocaval anastomoses, respectively, using the technique described by Monchic and Russell (33). The proximal and distal ends of the graft were exteriorized as stomata to the right flank. The recipient’s own intestine was left intact. Both donor and recipients rats were fasted for 24 h before surgery. Some groups of transplanted animals were immunosuppressed with FK506 (Tacrolimus, Fujisawa, Osaka, Japan; 1.0 mg/kg/day, for 7 days). SIT recipients were examined daily for general condition and changes in body weight.

CD8 cell depletion

CD8+ cells were depleted in Lewis recipients by i.p. administration of 0.5 μl of OKT-8 mAb (2.4 mg/ml, Harlan-Serotec, Indianapolis, IN) on the day before and the day after SIT. Similar treatment of rats with the OK-8 mAb was found to successfully deplete CD8+ cells in experimental models of autoimmune disease (35). Control animals received the same amount of an isotype-matched (IgGl) mAb (MOPC21, Sigma Chemical, St. Louis, MO) via the same route at the same time points. Blood specimens were obtained, via the tail vein for flow cytometric analysis, before the administration of Ab. To check the efficiency of CD8+ cell depletion, blood samples and splenocytes were obtained at various time points (days 3–14) after SIT, and mononuclear cells were isolated by density gradient centrifugation for immunofluorescence and flow cytometric analyses.

Specimens

Groups (n = 3–5) were sacrificed on days 3, 7, and 14 after SIT. Proximal, mid, and distal portions of the small intestine graft and the native intestine were obtained. Half of each sample was frozen for further analysis, while the other half was fixed in 10% neutral buffered formalin. At harvest, the spleen was removed from those rats that underwent depletion of CD8+ cells (and controls) and was mechanically dispersed, and the mononuclear cells were isolated by density gradient centrifugation for subsequent flow cytometric analysis.

Flow cytometry

One million PBL or splenocytes were washed, pelleted, and incubated on ice for 1 h with either 10 μl of FITC-conjugated mouse anti-rat CD4 mAb and 10 μl of phycoerythrin-conjugated mouse anti-rat CD8 mAb (Harlan-Serotec) or isotype-matched control mAb. Cells (105) were analyzed on a FACScan flow cytometer using LYSIS II software (Becton Dickinson, Mountain View, CA). Gates were established using forward and side scatter to exclude dead cells and erythrocytes. Quadrants were set using cells labeled with the control Abs.

Histology

Tissue samples for histology were embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin. All samples were examined by a single, blinded, pathologist, using well-established criteria (36). Briefly, the histopathologic features examined included 1) villous changes, including height, blunting, mucin depletion, hemorrhage, and necrosis; 2) crypt epithelial injury, including apoptosis; 3) cryptitis; 4) inflammatory infiltrate, including eosinophilia; and 5) vasculitis. Graft rejection in this model was defined as the presence of an inflammatory infiltrate, crypt epithelial cell injury, and apoptosis. Specimens were graded for acute rejection using the following semiquantitative scheme: 0 = no evidence of rejection, 1 = mild acute rejection, 2 = moderate acute rejection, and 3 = severe acute rejection.

TUNEL assay

The terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique was used to detect DNA fragmentation. Paraffin-embedded sections (4 μm) were cut and mounted on precoated glass slides. Sections were deparaffinized before digestion with 20 μg/ml proteinase K (Sigma). The TUNEL reaction was performed essentially as previously described (28), except that ApoTag ( Oncor, Gaithersburg, MD) reagents were used. Briefly, after quenching of the endogenous peroxidase, an equilibration buffer was added to each section, and the section was incubated for 30 min at room temperature. Sections were then incubated with the reaction mixture containing the terminal deoxynucleotidyl transferase enzyme for 1 h at 37°C in a humidified chamber. The reaction was terminated by a 30-min incubation in a stop/wash buffer (oncor). For visualization of incorporated digoxigenin-11-DUTP, sections were incubated with peroxidase-conjugated anti-digoxigenin for 30 min at room temperature. After extensive washing, the sections were incubated with the diaminobenzidine substrate for 2 to 5 min. The reaction was terminated by immersing the tissue in tap water. The sections were counterstained in methyl green (Vector Laboratories, Burlingame, CA) for 5 min at 60°C, dehydrated, and mounted. Negative controls were prepared for each section by substituting diH2O for the terminal transferase enzyme in the reaction mixture.

RNA isolation, cDNA preparation, and PCR

Total RNA was isolated from intestinal tissue using a guanidine isothiocyanate/pheno1 denaturing solution as previously described (34, 37). RNA integrity was confirmed by detection of the 28S and 18S RNA bands following agarose gel electrophoresis. One microgram of total RNA was used in cDNA synthesis with random hexamer oligonucleotides and avian myeloblastosis virus reverse transcriptase as previously described (34, 37). Amplification of rat Fas, FasL, perforin, granzyme B, and β-actin was accomplished using rat-specific oligonucleotide primers. PCR was performed essentially as detailed previously (34, 38) and was analyzed by gel electrophoresis. In some experiments, pre aliquoted PCR products were reannealed from the thermal cycling at five cycle intervals between cycles 35 and 50 for semiquantitative analysis. Preliminary experiments indicated that these cycles were within the linear range of amplification for FasL and granzyme B. The intensities of the PCR products were quantitated in densitometric units (DU) using an Image Analyzer (IS1000, a Innotech, San Leandro, CA). All values were normalized to the amount of β-actin detected in the corresponding sample at 25 cycles of amplification.

Western blot analysis

Lysates were prepared from intestinal tissue by homogenization in 5 vol of boiling lysis buffer (1.0% SDS and 1.0 μM sodium vanadate, Tris-HCl, pH 7.4) followed by microwaving for 15 s. Insoluble material was pelleted by centrifugation, and the cell-free supernatant was removed and frozen. Total protein was determined by the DC assay (Bio-Rad, Hercules, CA). Samples (10 μg/lane) were boiled in SDS sample buffer, and the proteins were separated by 10% SDS-PAGE. Separated proteins were transferred to nitrocellulose (Schleicher and Schuell, Keene, NH) and blocked overnight in blocking buffer (5% nonfat dry milk in PBS and 0.1% Tween-20). The membrane was then incubated with either a 1/1000 dilution of a mouse anti-human FasL mAb or a 1/500 dilution of mouse anti-rat Bcl-xl mAb (Transduction Laboratories, Lexington, KY) diluted in blocking buffer for 1 h at 37°C with constant agitation. The anti-human FasL mAb has previously been shown to recognize rat FasL. After extensive washing (0.5% Tween-20 in PBS), an optimal amount of anti-mouse Ig-horseradish peroxidase-conjugated Ab was added and incubated for 1 h. After multiple washes, detection was accomplished using the ECL substrate (Amersham, Arlington Heights, IL) according to the manufacturer’s instructions. Individual bands were quantitated using the IS1000 Image Analyzer. The results are expressed as arbitrary DU.

Results

Apoptosis in rejecting SIT allografts

Our previous studies of the histopathologic features of SIT suggested that apoptosis was an important component of allograft rejection (34). Hematoxylin-eosin-stained tissue sections demonstrated that crypt cell apoptosis was detected as early as postransplant day 3 in allografts. To further identify the apoptotic cells during SIT, we used the TUNEL assay on tissue sections from transplanted rats. TUNEL-positive cells, which also demonstrated morphologic changes consistent with apoptosis, were clearly detected in rejecting allografts by day 3 postransplant. There was a direct correlation between the number of apoptotic cells and the histopathologic progression of rejection. Whereas numerous apoptotic crypt cells were detected in allografts 7 days after transplantation (Fig. 1A), apoptotic crypt cells were rare in
isografts (Fig. 1B). Likewise, native intestine from allogeneic SIT recipients contained few cells undergoing apoptosis (Fig. 1C). In this model, at the time points examined, there was minimal apoptosis of the infiltrating cells within the allografts. Treatment of allograft recipients with the immunosuppressive drug, FK506 (D), ameliorated the histopathologic features of rejection, including apoptosis (Fig. 1D). Thus, in a high responder, allogeneic SIT model, apoptosis of crypt epithelial cells is a specific feature of graft rejection.

Rejection of SIT grafts after CD8 depletion

Since CD8\(^+\) T cells are thought to be the major lymphocyte population that mediates cytotoxicity in rejecting allografts, and because these cells are known to induce apoptosis, we depleted graft recipients of CD8\(^+\) cells. Groups of rats received either the OX-8 mAb or the same dose of isotype-matched control mAb on the day before and the day after transplant. PBL samples were obtained before transplant and 3, 7, and 14 days after SIT transplantation and were analyzed for the presence of CD4\(^+\) and CD8\(^+\) cells by flow cytometry. By 3 days after transplantation (2 days after the final injection of the OX-8 mAb), there was no evidence of CD8\(^+\) cells in the circulation of transplanted rats (Table I). CD8\(^+\) cells were effectively depleted through day 14. In contrast, there was no change in the levels of CD8\(^+\) cells in PBL from transplanted rats that received a similar dose of IgG1. Depletion of CD8\(^+\) cells had

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Table I.  **CD8\(^+\) depletion in transplanted Lewis rats**

<table>
<thead>
<tr>
<th></th>
<th>Pretransplant(^a)</th>
<th>IgG Treated</th>
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<tr>
<td></td>
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<td>Day 3</td>
</tr>
<tr>
<td><strong>Peripheral blood</strong></td>
<td></td>
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</tr>
<tr>
<td>CD4(^+)</td>
<td>50.7 ± 11.9(^b)</td>
<td>46.6 ± 5.0</td>
</tr>
<tr>
<td>CD8(^+)</td>
<td>12.0 ± 4.1</td>
<td>15.3 ± 2.9</td>
</tr>
<tr>
<td><strong>Splenocytes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4(^+)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD8(^+)</td>
<td>ND</td>
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\(^a\) Pretransplant, \(n = 10\); IgG treated, \(n = 3\), CD8 depleted day 3, \(n = 3\), CD8 depleted day 7, \(n = 7\), CD8 depleted day 14, \(n = 3\).

\(^b\) Data represent the percentage of positive cells. ND, not done.
no effect on CD4+ cells in the circulation. CD8+ cells were similarly depleted from the spleen after treatment with the OX-8 mAb (Table I).

Groups of CD8-depleted and control rats were sacrificed on days 3, 7, and 14 after SIT, and hematoxylin-eosin-stained sections of the graft and native intestine were examined for evidence of rejection. Signs of early rejection were evident by day 3 in the control group and to a slightly lesser extent in the CD8-depleted group (Table II). By day 7 after SIT, moderate to severe rejection, consisting of a characteristic mononuclear cellular infiltrate, blunted villi, epithelial necrosis with hemorrhage, and focal ulceration, was apparent in both groups (Fig. 2). There was no significant difference in the severity of rejection between the control and CD8-depleted groups on day 7 (Table II). In contrast, isografts, allografts from FK506-treated recipients, and the native intestine of allograft recipients demonstrated no evidence of rejection. By day 14, there was a complete sloughing of the epithelial cell layer, and allografts were rejected in both the control and CD8-depleted groups (data not shown). These data demonstrate that CD8+ cells are not necessary for rejection of SIT allografts.

CD8 depletion does not alter apoptosis of SIT allografts

Epithelial cells that display DNA fragmentation, as detected by the TUNEL assay, and morphology consistent with apoptosis were a prominent feature in the rejecting allograft (Fig. 3). Apoptotic cells, which were detected mainly in the crypts, were observed on day 7 in the allografts from both control and CD8-depleted rats. To

**FIGURE 2.** Rejection of small intestinal allografts. Hematoxylin- and eosin-stained sections from control (IgG-treated; A) and CD8-depleted (B) allograft recipients, 7 days after transplantation, show moderate to severe allograft rejection. An intense inflammatory cell infiltrate (closed arrows), epithelial cell damage, and crypt cell destruction (open arrows) are seen in both groups. Magnification, ×200.

**FIGURE 3.** Apoptosis of crypt epithelial cells occurs even in the absence of CD8. Tissue sections of small intestinal allografts, 7 days after transplantation, from the control group (A) and the CD8-depleted group (B) were analyzed for apoptosis by TUNEL. During rejection of a small intestinal allograft, apoptosis of crypt epithelial cells was readily apparent in both the control and CD8-depleted groups. Magnification, ×400.
from allograft recipients or the FK506-treated groups (Fig. 5). In contrast, there was a significant difference in the number of apoptotic cells detected between the control (IgG-treated) and CD-8-depleted groups.

To determine the specific pathway responsible for apoptosis in rejecting small intestinal allografts, transcripts for mediators of both the Fas pathway (Fas, FasL) and the granule exocytosis pathway (granzyme B, perforin) were analyzed from day 7 allografts of control (IgG-treated) and CD8-depleted groups by RT-PCR. There were no qualitative differences between the groups in the expression of these mediators. β-Actin was used as a control.

Mechanism of apoptosis

To determine the specific pathway responsible for apoptosis in rejecting small intestinal allografts, transcripts for mediators of both granule exocytosis (perforin and granzyme B) and Fas-based (Fas and FasL) pathways were analyzed by RT-PCR. Previous studies have demonstrated that both perforin and granzyme B are up-regulated during unmodified SIT rejection (22). Furthermore, we have determined that the number of transcripts for FasL are increased in rejecting small intestinal allografts, transcripts for mediators of both the Fas pathway (Fas, FasL) and the granule exocytosis pathway (granzyme B, perforin) were analyzed from day 7 allografts of control (IgG-treated) and CD8-depleted groups by RT-PCR. There were no qualitative differences between the groups in the expression of these mediators. β-Actin was used as a control.

Although we could not detect major differences at the level of gene transcription by a semiquantitative RT-PCR approach, we sought to determine whether the levels of FasL protein may differ between the groups. Cell-free lysates were prepared from the allografts of recipients given control Abs, recipients that were CD8 depleted, and recipients that were treated with FK506, and 10 μg of lysates were analyzed by Western blot. PAGE and Coomassie blue staining showed that all lanes had similar amounts of protein (data not shown). The 37-kDa FasL protein was detected in all allografts of control (IgG-treated) and CD8-depleted recipients of allografts. Data are shown from one allograft from each group but are representative of the three allografts analyzed.

There were differences in the levels of FasL and granzyme B mRNA between the control and CD8-depleted recipients of allografts, a semiquantitative RT-PCR procedure was used. There were no significant differences in the levels of transcripts for these mediators between the two groups (Fig. 5B). Thus, even in the absence of CD8" T cells, transcripts for mediators of both the granule exocytosis and Fas pathways were expressed at levels similar to those detected in unmodified SIT rejection.
CD8-INDEPENDENT APOPTOSIS IN ALLOGRAFT REJECTION

In this report we demonstrate that CD8$^+$ cells are not required for the rejection of small intestinal allografts. Moreover, we show for the first time that apoptosis can occur in rejecting allografts in the absence of CD8$^+$ cells. Our finding that CD8$^+$ cells are not essential for the rejection of small intestine allografts confirms and extends the results of previous studies in which rejection of MHC-disparate heart, liver, and skin grafts occurs in the absence of CD8$^+$ T cells. In some studies, however, depletion or modulation of CD8$^+$ T cells in allograft rejection. Taken together, these studies as well as the data reported herein call into question the role of CD8$^+$ CTL in allograft rejection.

CD8$^+$ CTL are thought to promote allograft damage through recognition of MHC class I alloantigens and cell-mediated cytotoxicity. Two well-described mechanisms of cell-mediated cytoxicity have been defined at the molecular level, the Ca$^{2+}$-dependent, granule exocytosis pathway, which involves perforin and the granzymes, and the Fas/FasL pathway, involving the direct interaction of FasL$^+$ CTL with Fas$^+$ target cells. Although the specific downstream events may differ, apoptotic cell death is the common end result in both pathways. In the current report we demonstrate in the ACI→Lewis model that apoptotic crypt epithelial cells are clearly evident in allografts in the very early stages of rejection. Similarly, in the Lewis→BN model of small intestinal transplantation, there is concordance between the early signs of rejection and the appearance of apoptotic enterocytes (31, 43). In both models, the number of apoptotic epithelial cells within the small intestinal allograft increases with the severity of rejection and eventually results in extensive cellular loss and complete rejection of the organ.

The most significant finding to emerge from our study is that depletion of CD8$^+$ CTL in recipients of small intestinal allografts has no effect on the incidence or the number of apoptotic graft epithelial cells. The mechanism by which apoptosis is induced in the CD8-depleted animals is not clear. Perforin is expressed predominantly by CD8$^+$ T cells, NK cells, and γδ T cells, although under certain conditions CD4$^+$ T cells do express perforin (14). In our studies the expression of perforin is similar during unmodified rejection and during rejection in the absence of CD8$^+$ cells. We have not yet determined the phenotype of the perforin-expressing cell. Nevertheless, our findings do not rule out a role for perforin expression by CD8$^+$ cells in unmodified rejection. Indeed, it remains possible that perforin is expressed by CD8$^+$ cells in unmodified rejection and by a different cell type in CD8-independent rejection. The abundance of γδ T cells in the small intestine suggests that these cells should also be explored as a possible source of perforin. Numerous reports have determined that there is up-regulation of transcripts for perforin, granzyme A, and granzyme B in both clinical and experimental models of allograft rejection (15, 17, 18, 20, 21, 23). For example, during small intestine allograft rejection, specific increases in mRNA for both granzyme B and perforin have been demonstrated (22). Other studies have established the presence of perforin- or granzyme-producing cells within the inflammatory infiltrate during allograft rejection (16, 44). Although we detected increased levels of FasL transcripts in rejecting small intestinal allografts compared with isografts, we did not detect any differences in FasL mRNA between the CD8-depleted and control groups. It is possible that our semiquantitative RT-PCR approach may not be sensitive enough to reveal two- to threefold differences in FasL mRNA that may have been revealed had a competitive PCR strategy been used. By immunoblotting, however, we observed increased levels of FasL in allografts obtained from CD8-depleted recipients relative to those in allografts from unmodified hosts. One interpretation of this finding is that in the absence CD8$^+$ CTL, CD4$^+$ T cells may mediate apoptosis through the Fas/FasL pathway, since it has been suggested that the majority of CD4$^+$ CTL activity is through the Fas pathway (45). Additional experiments will specifically address the role of CD4$^+$ T cells in the small intestinal allograft model.

Other plausible explanations, apart from the contribution of putative CD4$^+$ CTL, should be considered. It is possible, for example, that small numbers of CD8$^+$ T cells, which we could not detect by flow cytometry, remain after treatment with the OX-8 mAb and mediate apoptosis and graft rejection. This seems unlikely, as it would be expected that if suboptimal numbers of CD8$^+$ CTL were mediating apoptosis, rejection would be delayed compared with that in controls. However, we observed no difference in the tempo or the severity of rejection in control and CD8-depleted recipients. It is also possible that alloreactive CD8$^+$ CTL were successfully depleted from the periphery and spleen but remained in the graft. Although we did not directly isolate T cells from the allograft, we did thoroughly analyze the graft-associated lymph nodes (which were of donor origin, and transplanted along with the allograft). By flow cytometry we did detect a small number of CD8$^+$ T cells in the graft-associated lymph nodes, but these were all of donor origin (data not shown).

CD4$^+$ T cells and cytokines have been suggested to participate directly in the rejection of an allograft (46, 47). Indeed, VanBuskirk et al. reported that both cytolytic and noncytolytic CD4$^+$ T cells promote acute cardiac allograft rejection when adoptively transferred into cardiac allograft-bearing SCID mice (46). TNF-α has been proposed as a candidate Th1-type cytokine that can promote allograft rejection due to its broad range of effects, including activation of macrophages. More relevant to the current study is that TNF can induce apoptosis of target cells through TNF receptor 1 (48). TNF-α is up-regulated in rejecting small intestinal allografts (34, 49), although there is no difference in the expression of TNF-α between allografts from CD8-depleted and control recipients (data not shown). Additional experiments will address the possibility that TNF-α has a direct role in inducing apoptosis of small intestinal allografts.
TGF-β1 has been shown to induce apoptosis of hepatocytes (50–53), and we have previously determined a direct correlation between intragraft TGF-β1 gene expression and apoptosis in a rat model of liver allograft rejection (28). Other macrophage-derived cytokines may also have direct and indirect roles, perhaps involving nitric oxide, in the induction of apoptosis of allografts. A recent report has suggested that activated macrophages are responsible for apoptosis of allografted Meth A tumor cells (25). These macrophages induce apoptosis through a unique Ca²⁺-dependent mechanism distinct from both the perforin and Fas/Fasl pathways.

Apoptosis is a component of the tissue damage observed in the rejection of an allograft. We demonstrate, for the first time, that CD8⁺ CTL are not necessary for the induction of apoptosis in an allograft. Allograft rejection has been reported to occur in a CD8⁻/⁻ manner in many model systems, and we demonstrate that the small intestinal allograft is no exception. Further, these data indicate that cells other than CD8⁺ can develop perforin-mediated cytotoxic activity (54). These findings have important implications regarding our understanding of the process of apoptosis in allograft rejection and in the development of novel therapeutics for transplantation.

References


