Fas Ligand–mediated Killing by Intestinal Intraepithelial Lymphocytes
Participation in Intestinal Graft-versus-Host Disease

Tesa Lin,* Thomi Brunner,* Brian Tietz,* Jill Madsen,§ Emanuela Bonfoco,* Miriam Reaves,* Margaret Huflejt,‡ and Douglas R. Green*†
*Division of Cellular Immunology and †Division of Allergy, La Jolla Institute for Allergy and Immunology, San Diego, California 92121; and ‡Division of Pathology, Yale University School of Medicine, New Haven, Connecticut 06520-8023

Abstract
In vitro studies have demonstrated that intestinal intraepithelial lymphocytes (IEL) are constitutively cytotoxic; however, the mechanism and target of their cytotoxicity are unknown. Apoptosis of intestinal epithelial cells (IEC) and an increase in IEL numbers are classical signs of intestinal graft-versus-host disease (GVHD), although whether IEL can mediate IEC apoptosis directly in GVHD is unclear. Recent evidence suggests that target epithelial organ injury observed in GVHD is predominantly Fas-mediated; therefore, we investigated the possibility that IEL induce apoptosis of IEC through a Fas-mediated mechanism. Here, we demonstrate that the IEL isolated from normal mice readily display potent Fas ligand (FasL)-mediated killing activity after CD3 stimulation, and that IEC express Fas, suggesting that IEC are potential targets for FasL-mediated killing by IEL. In vitro, IEL isolated from GVHD mice have markedly increased FasL-mediated killing potential and are spontaneously cytolytic toward host-derived tumor cells predominantly through a Fas-mediated pathway. In vivo transfer of IEL isolated from GVHD mice increased significantly more IEC apoptosis in F1 wild-type mice than in Fas-defective F1lpr mice. Thus, these results demonstrate that FasL-mediated death of IEC by IEL is a major mechanism of IEC apoptosis seen in GVHD. (J. Clin. Invest. 1998. 101:570–577.) Key words: γδ T cells • graft-versus-host disease • Fas • Fas ligand • intestinal intraepithelial lymphocytes

Introduction
Murine intestinal intraepithelial lymphocytes (IEL) are a phenotypically diverse and complex T cell population which differs markedly from the T cell population found in the periphery (1, 2). Unlike peripheral T cells, a large percentage of murine IEL use the γδ T cell receptor (TCR). Furthermore, the vast majority of murine IEL, although clearly T cells, lack conventional T cell markers such as Thy 1, CD2, and CD5 (1, 3–5). These results have led several investigators to conclude that the majority of murine IEL are derived from a separate lineage, possibly through an extrathymic pathway (6–8).

Despite their phenotypic diversity, the majority of murine IEL express the cytotoxic CD8+ phenotype. Why IEL are skewed towards a CD8+ phenotype is unclear, but this characteristic appears to be evolutionarily conserved among several species (9–11). Functional in vitro studies using redirected cytolytic killing assays have revealed that murine IEL possess potent cytotoxicity. However, the biological significance of IEL cytotoxicity is unclear. The localization of IEL to areas constantly exposed to high microbiological content suggests that they may play a role in host defense; however, evidence supporting this hypothesis has not been conclusive (12–14).

In graft-versus-host disease (GVHD), donor cells recognize and eliminate host cells. In the intestine, an increase in the total number of IEL and apoptosis of intestinal epithelial cells (IEC) have been observed in GVHD (15, 16). Interestingly, direct evidence demonstrating that IEL can induce IEC apoptosis in GVHD has not been described. Several studies have suggested that the vast majority of IEL are anergic (3, 17, 18). In addition, studies in transgenic (Tg) mice which express a TCR that recognizes self antigen have revealed that in the presence of self antigen, Tg T cells are deleted in the thymus (negative selection), but for unclear reasons the IEL population contains an abundant number of these Tg T cells (19, 20). The lack of an obvious deleterious effect to the intestinal epithelium, despite the abundant presence of potentially autoreactive T cells, supports the argument that most IEL are anergic.

GVHD is mediated by the activity of two different mechanisms of cytotoxic T cell function, one dependent on perforin and one dependent on Fas (21). Recent studies suggest that both perforin-and Fas-mediated pathways are involved in the systemic signs of GVHD; however, target organ epithelial injury to the liver and skin appears to be especially restricted to Fas-mediated injury (22). These observations led us to investigate the role of Fas-mediated death in intestinal GVHD, specifically whether IEL participate directly in IEC apoptosis during GVHD through a Fas-mediated pathway.

Methods

Mice and induction of GVHD. C57BL/6 (H-2b), B6D2F1/J (C57BL/6 × DBA/2, H-2b/d), B6C3F1 (C57BL/6 × C3H/He, H-2b/d), C57BL/6

1. Abbreviations used in this paper: FasL, Fas ligand; FCM, flow cytometry analysis; GVHD, graft-versus-host disease; IEC, intestinal epithelial cell(s); IEL, intestinal intraepithelial lymphocyte(s); PE, phycoerythrin; TCR, T cell receptor; Tg, transgenic; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling.
Assays were done in triplicate.

The injection of 10^6 C57BL/6 spleen cells into the tail vein of either B6D2F1/J or B6C3HF1 mice. Unless stated otherwise, all mice were killed 3 wk after the induction of GVHD.

**Results**

**Determination of IEC apoptosis.** IEC apoptosis was detected in formalin-fixed sections of murine small intestine using the TUNEL assay (for terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling) to detect DNA fragmentation. Briefly, the small intestine was removed and incised longitudinally. The intestine was washed briefly in HBSS to remove fecal material and fixed overnight in 10% buffered formalin solution (Fisher Scientific Co., Pittsburgh, PA). Paraffin sections were deparaffinized with xylene and ethanol gradient. Tissue sections were then stained for TUNEL-positive cells following the manufacturer's suggested instructions (In Situ Death Detection kit, Boehringer Mannheim Biochemicals, Indianapolis, IN).

**Histologic evaluation of IEC apoptosis.** IEC apoptosis was determined to be >97% by flow cytometry (data not shown).

**Isolation of Thy 1⁺ and Thy 1⁻ IEL.** Thy 1⁺ IEL and Thy 1⁻ IEL were isolated by flow cytometry sorting (FACStar®). Purity was determined to be >97% by flow cytometry (data not shown).

**DNA fragmentation assay.** DNA fragmentation in L1210, L1210-Fas, and Fas-positive Jurkat cells as targets has been described previously (26, 27). Briefly, target cells (10^6/ml) were labeled with 5 μCi/ml [3H]thymidine for 2 h. Unincorporated [3H]thymidine was removed by two washes with HBSS. Target cells (2 × 10^6) were incubated with effector cells at various concentrations in flat-bottomed 96-well plates, coated previously with 3 μg/ml of anti-CD3, anti-TCR β, anti-TCR δ, or no antibody. After 12 h, cells were harvested using a Skatron Instruments cell harvester (Sterling, VA) and [3H]thymidine-labeled unfragmented DNA was calculated as follows: % DNA fragmentation = 100 (1 – cpm experimental group/cpm control group) ± SD.

**Antibodies.** Antibodies were obtained from the following suppliers: FITC-conjugated anti-TCR δ and nonconjugated anti-TCR δ (GL-3; PharMingen, San Diego, CA), FITC-conjugated and nonconjugated anti-TCR β (H57-597; PharMingen), phycoerythrin (PE)-conjugated anti-Thy 1.2 (PharMingen), biotin-conjugated anti-CD8β (Caltag Laboratories, Inc., Burlingame CA), biotin-conjugated anti-H-2K^b (PharMingen), FITC-conjugated anti-Cy3 (PharMingen), anti-Fas (Jo-2; PharMingen), PE-conjugated anti-hamster IgG (Caltag Laboratories, Inc.).

**Results**

IEL are potent inducers of Fas-mediated cell death. T cell cytotoxicity involves two major mechanisms, one dependent on perforin and the other on Fas ligand (FasL) (21). Using an assay that specifically quantifies FasL-mediated killing by activated murine T cells (26, 27), we analyzed the ability of IEL to kill Jurkat cells. Fig. 2 demonstrates that freshly isolated IEL do not spontaneously kill Jurkat cells, but when stimulated with plate-bound anti-CD3, IEL readily became very potent killers (Fig. 1). Significant killing of Jurkat cells was observed with E/T ratios as low as 0.3:1 (data not shown). Furthermore, CD3-stimulated IEL were five- to sixfold better at killing Jurkat cells than CD3-stimulated either spleen or lymph node cells. This ability of anti-CD3-stimulated IEL to kill Jurkat cells more efficiently than spleen or lymph node cells cannot be explained entirely by the higher percentage of CD3+ T cells found in the IEL population (80–90%), as lymph node cells have a comparable percentage of CD3+ T cells (70–75%; data not shown). Furthermore, when the E/T ratio for spleen and lymph node cells was corrected for the higher percentage of CD3+ cells found in the IEL population, IEL still induced significantly more Jurkat cell killing (data not shown).

To investigate the mechanism by which CD3-stimulated IEL mediated the killing of Jurkat cells, we added Fas-Fc, which blocks Fas and FasL interactions (27), to our assay. Fig. 2 demonstrates that Fas-Fc blocked completely the killing of Jurkat cells, whereas concanamycin A, which inhibits perforin-
mediated killing (28), had no apparent effect. Furthermore, IEL from gld mice, which have nonfunctional FasL, were unable to kill Jurkat cells (data not shown). Thus, in this assay, IEL appear to function effectively as killers via a FasL–Fas interaction.

IEL from gld mice consistently failed to kill Jurkat cells, indicating that the lack of FasL expression in these IEL is responsible for their inability to mediate killing. In contrast, IEL from wild-type mice were capable of killing Jurkat cells, suggesting that the presence of functional FasL is necessary for FasL-mediated killing.

FasL-mediated killing by IEL is primarily by Thy 1<sup>+</sup> TCR αβ and TCR γδ IEL. Because of the phenotypic complexity of the murine IEL population (1, 2), we attempted to determine which of the multiple IEL subsets were responsible for the Fas-mediated death of Jurkat cells. We sorted freshly isolated IEL into Thy 1<sup>+</sup> or Thy 1<sup>+</sup> population, and results shown are representative of three independent experiments.

IEL express Fas. If IEL are such potent FasL-mediated killers, what is their natural target? Because IEL are essentially surrounded by IEC, we rationalized that the latter are the most likely targets for FasL-mediated killing by IEL. FCM supports this hypothesis. IEL express very low levels of Fas, but IEC express moderate levels (Fig. 4). These results are consistent with recent observations that Fas is expressed on colon cancer epithelial cell lines (30, 31).

GVHD results in an increase in FasL-mediated killing by IEL which correlates with an infiltration of donor-derived Thy 1<sup>+</sup> TCR αβ CD4<sup>+</sup> CD8<sup>+</sup> IEL. The expression of Fas on IEC suggests that FasL-mediated killing by IEL, if unregulated, may play a role in the destruction of IEC in certain intestinal diseases. In this regard, we chose to examine the role of FasL-
mediated killing by IEL in GVHD, a disease characterized by an increase in the total number of IEL, as well as apoptosis of IEC. We used a model of murine acute GVHD in which parental spleen cells (C57BL/6, H-2b) are injected into F1 hosts (H-2b × H-2d) (15, 16, 32). In this model, donor-derived cells can be distinguished from host-derived cells by the lack of expression of host MHC H-2d. Consistent with previous studies (15), this model of GVHD resulted in a two- to threefold increase in total IEL number as well as IEC apoptosis (data not shown and Fig. 5). Similar findings were observed when C57BL/6 spleen cells were injected into B6C3HF1 (H-2b k) mice (data not shown).

We examined the FasL-mediated cytotoxicity of IEL at weekly intervals after the induction of GVHD. Beginning at week 2 of GVHD, FasL-mediated killing by the IEL of GVHD mice rose dramatically (Fig. 6). This increase in FasL-mediated killing by the IEL of GVHD mice was not due to a difference in the percentage of CD3^+ IEL, because the percentages were similar in both control and GVHD mice (data not shown). Instead, the increased killing activity was most likely due to a 10-fold increase in the percentage of Thy 1^+ IEL in GVHD mice (Fig. 7 A).

Phenotypic analysis by flow cytometry revealed that the vast majority of these Thy 1^+ IEL were donor-derived (H-2b^+), TCR αβ^+, CD4^+ , and CD8β^+ (Fig. 7, A and B). Thus, the elevated FasL-mediated killing by IEL observed in GVHD mice was most likely due to the infiltration of TCR αβ^+CD4^+CD8β^+ Thy 1^+ donor-derived IEL. These results are consistent with our finding that Thy 1^+ IEL are more potent at FasL-mediated killing than Thy 1^- IEL.

IEL from GVHD mice can mediate the killing of Jurkat cells through recognition of host antigen, and can spontaneously kill host-derived target cells in vitro primarily through a Fas-mediated pathway. In our model of GVHD, donor or parental C57BL/6 (B6) spleen cells were injected into host B6D2F1 (C57BL/6 × DBA/2) mice. In this model, C57BL/6 (donor)-derived spleen cells which infiltrate the host intestinal epithelium should recognize DBA/2 (host)-derived antigen. To test this hypothesis, we stimulated IEL from GVHD mice with DBA/2-derived L1210 cells (instead of anti-CD3). Fig. 8 demonstrates that L1210 cells readily stimulate IEL from GVHD (B6→B6D2F1) mice to mediate the killing of Jurkat cells. This stimulation appears to be specific, because L1210 cells did not stimulate IEL from non-GVHD (F1→F1) mice. Furthermore, in a model of GVHD where the host DBA/2 (H-2b^+) in the F1 mice is replaced with C3H (H-2k), L1210 cells were unable to stimulate IEL isolated from B6→B6C3F1 GVHD mice to mediate the killing of Jurkat cells. It is unlikely that IEL from B6→B6C3F1 GVHD mice were defective in FasL-mediated killing when compared with IEL from B6D2F1 mice, because in both combinations a similar level of infiltration by donor-derived Thy 1^+ CD8β IEL into the intestinal epithelium was observed (> 90%, data not shown). Furthermore, IEL isolated from either combination were equally potent in

---

**Figure 4.** FCM histogram analysis of Fas expression by IEL and IEC. Dashed lines, Control staining (hamster IgG followed by PE-conjugated anti–hamster IgG). Solid lines, Fas staining (anti-Fas followed by PE-conjugated anti–hamster IgG).

**Figure 5.** Injection of C57BL/6 spleen cells into B6D2F1 mice results in intestinal GVHD. Small intestine from B6→B6D2F1 GVHD mice was isolated 3 wk after injection and assessed for apoptosis by TUNEL staining. Arrows, Apoptotic cells found frequently in GVHD mice but infrequently in control (F1→F1) mice. Results shown are representative of three separate experiments.

---

IEL from GVHD mice can mediate the killing of Jurkat cells through recognition of host antigen, and can spontaneously kill host-derived target cells in vitro primarily through a Fas-mediated pathway. In our model of GVHD, donor or parental C57BL/6 (B6) spleen cells were injected into host B6D2F1 (C57BL/6 × DBA/2) mice. In this model, C57BL/6 (donor)-derived spleen cells which infiltrate the host intestinal epithelium should recognize DBA/2 (host)-derived antigen. To test this hypothesis, we stimulated IEL from GVHD mice with DBA/2-derived L1210 cells (instead of anti-CD3). Fig. 8 demonstrates that L1210 cells readily stimulate IEL from GVHD (B6→B6D2F1) mice to mediate the killing of Jurkat cells. This stimulation appears to be specific, because L1210 cells did not stimulate IEL from non-GVHD (F1→F1) mice. Furthermore, in a model of GVHD where the host DBA/2 (H-2b^+) in the F1 mice is replaced with C3H (H-2k), L1210 cells were unable to stimulate IEL isolated from B6→B6C3F1 GVHD mice to mediate the killing of Jurkat cells. It is unlikely that IEL from B6→B6C3F1 GVHD mice were defective in FasL-mediated killing when compared with IEL from B6D2F1 mice, because in both combinations a similar level of infiltration by donor-derived Thy 1^+ CD8β IEL into the intestinal epithelium was observed (> 90%, data not shown). Furthermore, IEL isolated from either combination were equally potent in
Lin et al.

...the CD3-stimulated killing of Jurkat cells (data not shown). Overall, these results suggest that donor-derived IEL infiltrating the intestinal epithelium recognize host-derived antigen and mediate the killing of bystander cells presumably through a Fas-mediated mechanism.

Although we observed a heavy infiltration of donor-derived IEL into the host intestinal epithelium during GVHD (Fig. 6, A and B), whether these IEL were capable of inducing host IEC injury directly is uncertain. Because freshly isolated IEC undergo a very high rate of spontaneous death in culture (our unpublished observations), we tested the ability of IEL from B6 (H-2b)→B6D2F1 (H-2b3d) GVHD mice to recognize and spontaneously kill DBA/2 (host)-derived L1210 and L1210-Fas tumor cells in vitro. L1210 cells express very low levels of Fas, whereas L1210-Fas cells express higher levels and are more sensitive to Fas-induced cell death (23). Fig. 9, A and B, demonstrates that IEL isolated from GVHD mice have relatively low spontaneous cytotoxicity toward L1210 cells, but they were significantly more cytotoxic toward L1210-Fas cells, suggesting that the killing in vitro of host-derived target cells by IEL involves primarily a Fas-mediated mechanism.

IEL from GVHD mice induce IEC apoptosis in vivo primarily through a Fas-mediated mechanism. Thus far, we have demonstrated that IEL isolated from GVHD mice have potent FasL-mediated cytotoxicity toward host-derived target cells in vitro. To test whether this also applies in vivo, we isolated GVHD IEL from B6→B6C3F1 mice 3 wk after induction of GVHD. The IEL were injected into wild-type B6C3F1 and B6C3F1-lpr mice (the latter have defective Fas expression). Although IEC apoptosis was observed in both cases, a significant decrease in IEC apoptosis was observed in B6C3F1-lpr mice compared with B6C3F1 mice (Figs. 10 and 11). These results suggest that IEL from GVHD mice induce IEC apoptosis primarily through a Fas-mediated pathway.

Discussion

Using an assay which had been shown initially to specifically measure FasL-mediated killing of Jurkat cells by activated T
cells (27), we have quantified the FasL-mediated killing potential of IEL. Our results demonstrate that unlike spleen and lymph node cells, IEL constitutively possess potent FasL-mediated cytotoxicity (Fig. 1). Furthermore, we have addressed directly the question of whether FasL-mediated cytotoxicity by IEL plays a role in the pathogenesis of intestinal GVHD.

Although several studies have demonstrated previously that IEL have potent cytotoxic capability (33–35), all of these studies were performed using redirected cytolytic killing assays. The biological significance of this assay is unclear, because it is unlikely that such a mechanism occurs in vivo. In the redirected cytolytic killing assay, the Fc receptor present on the target cell presumably binds to the constant region of an antibody which recognizes a stimulatory molecule (usually

---

**Figure 9.** IEL from GVHD mice spontaneously kill host-derived target cells predominantly through a Fas-mediated mechanism. Microscopic and DNA fragmentation analysis of apoptosis of L1210 wild-type (wt) and L1210-Fas after 16 h of culture with IEL isolated from week 3 GVHD mice. (A) IEL from GVHD mice were cultured at a 10:1 E/T ratio. Black arrowheads, Healthy L1210/L1210-Fas cells. White arrowheads, Fragmented (apoptotic) cells. Minimal apoptosis of L1210wt and L1210-Fas cells was observed when cultured with IEL isolated from control mice (data not shown). (B) IEL from week 3 GVHD mice were cultured with 3H-labeled L1210wt (white bars) and L1210-Fas target cells (black bars) at a 7:1 E/T ratio. In each experiment, IEL were pooled from two mice. N.D., Not done.

---

**Figure 10.** IEL from GVHD mice induce intestinal IEC apoptosis in vivo primarily through a Fas-mediated mechanism. IEL isolated from week 3 GVHD mice (B6→B6C3F1) were injected into B6C3F1lpr and B6C3F1 wild-type mice. Arrows, Apoptotic IEC as identified by TUNEL staining. Results shown are representative of three independent experiments.
anti-TCR) present on cytotoxic T cells. Thus, the antibody–
target cell complex serves as a template for the stimulation of
cytotoxic effector cells, resulting in the killing of the target cell.
The exact mechanism by which effector cells kill target cells in
this assay is unclear. It is likely that both perforin- and Fas-
mediated mechanisms are involved, because IEL isolated from
either gld mice or perforin-deficient mice have both been
shown to possess redirected cytotoxicity (34).

Two previous studies have addressed the role of FasL-
mediated killing by IEL (34, 36). Guy-Grand et al. demonstrated
that IEL from both gld mice (defective FasL) and perforin-deficient mice are capable of redirected cytolytic killing
(34). However, it is unclear from their study whether other cyto-
totoxic mediators, such as TNF, can also play a role. Furthermore,
from their results it is difficult to extrapolate the relative
importance of FasL-mediated killing in the IEL of normal
mice, because the lack of perforin in the perforin-knockout
mice may result in a compensatory upregulation of FasL-medi-
ated killing. In the second study, Gelfanov et al. concluded that
both TCR αβ CD8αβ and TCR αβ CD8αεα IEL have FasL-mediated killing activity (36). However, in their study,
IEL were stimulated extensively in vitro for 9 d with anti-TCR
αβ, anti-CD28, and IL-2, followed by a 3-h incubation with
PMA and A23187. Hence, it is unclear whether FasL-mediated
killing is a constitutive function present in most freshly
isolated IEL or a function acquired by a small subset of IEL
after extensive stimulation and expansion in vitro. In the same
study, lymph node cells stimulated identically were also capable
of FasL-mediated killing. We have also observed that
spleen cells treated in vitro with PMA and ionomycin or con-
canamycin A over several days were also capable of FasL-
mediated killing (our unpublished observations). Finally, Gelfa-

Figure 11. Quantification of
IEC apoptosis after in vivo
transfer of GVHD IEL
(B6→B6C3F1) IEL into
B6C3F1 wild-type and
B6C3F1/lpr mice. Mice were
examined 2 wk after in vivo
transfer. Results shown are the
average number of TUNEL-
positive cells (see Fig. 10) seen
per 10 high power fields (HPF,
×40) from three separate experi-
ments.

Our results suggest that both TCR γδ and TCR αβ IEL are readily capable of FasL-mediated killing. To our knowledge,
this is the first report demonstrating that TCR γδ IEL are ca-

anti-TCR) present on cytotoxic T cells. Thus, the antibody–
target cell complex serves as a template for the stimulation of
cytotoxic effector cells, resulting in the killing of the target cell.
The exact mechanism by which effector cells kill target cells in
this assay is unclear. It is likely that both perforin- and Fas-
mediated mechanisms are involved, because IEL isolated from
either gld mice or perforin-deficient mice have both been
shown to possess redirected cytotoxicity (34).

Two previous studies have addressed the role of FasL-
mediated killing by IEL (34, 36). Guy-Grand et al. demonstrated
that IEL from both gld mice (defective FasL) and perforin-deficient mice are capable of redirected cytolytic killing
(34). However, it is unclear from their study whether other cyto-
toxic mediators, such as TNF, can also play a role. Furthermore,
from their results it is difficult to extrapolate the relative
importance of FasL-mediated killing in the IEL of normal
mice, because the lack of perforin in the perforin-knockout
mice may result in a compensatory upregulation of FasL-medi-
ated killing. In the second study, Gelfanov et al. concluded that
both TCR αβ CD8αβ and TCR αβ CD8αεα IEL have FasL-mediated killing activity (36). However, in their study,
IEL were stimulated extensively in vitro for 9 d with anti-TCR
αβ, anti-CD28, and IL-2, followed by a 3-h incubation with
PMA and A23187. Hence, it is unclear whether FasL-mediated
killing is a constitutive function present in most freshly
isolated IEL or a function acquired by a small subset of IEL
after extensive stimulation and expansion in vitro. In the same
study, lymph node cells stimulated identically were also capable
of FasL-mediated killing. We have also observed that
spleen cells treated in vitro with PMA and ionomycin or con-
canamycin A over several days were also capable of FasL-
mediated killing (our unpublished observations). Finally, Gelfa-

Figure 11. Quantification of
IEC apoptosis after in vivo
transfer of GVHD IEL
(B6→B6C3F1) IEL into
B6C3F1 wild-type and
B6C3F1/lpr mice. Mice were
examined 2 wk after in vivo
transfer. Results shown are the
average number of TUNEL-
positive cells (see Fig. 10) seen
per 10 high power fields (HPF,
×40) from three separate experi-
ments.

Our results suggest that both TCR γδ and TCR αβ IEL are readily capable of FasL-mediated killing. To our knowledge,
this is the first report demonstrating that TCR γδ IEL are ca-

anti-TCR) present on cytotoxic T cells. Thus, the antibody–
target cell complex serves as a template for the stimulation of
cytotoxic effector cells, resulting in the killing of the target cell.
The exact mechanism by which effector cells kill target cells in
this assay is unclear. It is likely that both perforin- and Fas-
mediated mechanisms are involved, because IEL isolated from
either gld mice or perforin-deficient mice have both been
shown to possess redirected cytotoxicity (34).

Two previous studies have addressed the role of FasL-
mediated killing by IEL (34, 36). Guy-Grand et al. demonstrated
that IEL from both gld mice (defective FasL) and perforin-deficient mice are capable of redirected cytolytic killing
(34). However, it is unclear from their study whether other cyto-
toxic mediators, such as TNF, can also play a role. Furthermore,
from their results it is difficult to extrapolate the relative
importance of FasL-mediated killing in the IEL of normal
mice, because the lack of perforin in the perforin-knockout
mice may result in a compensatory upregulation of FasL-medi-
ated killing. In the second study, Gelfanov et al. concluded that
both TCR αβ CD8αβ and TCR αβ CD8αεα IEL have FasL-mediated killing activity (36). However, in their study,
IEL were stimulated extensively in vitro for 9 d with anti-TCR
αβ, anti-CD28, and IL-2, followed by a 3-h incubation with
PMA and A23187. Hence, it is unclear whether FasL-mediated
killing is a constitutive function present in most freshly
isolated IEL or a function acquired by a small subset of IEL
after extensive stimulation and expansion in vitro. In the same
study, lymph node cells stimulated identically were also capable
of FasL-mediated killing. We have also observed that
spleen cells treated in vitro with PMA and ionomycin or con-
canamycin A over several days were also capable of FasL-
mediated killing (our unpublished observations). Finally, Gelfa-

Figure 11. Quantification of
IEC apoptosis after in vivo
transfer of GVHD IEL
(B6→B6C3F1) IEL into
B6C3F1 wild-type and
B6C3F1/lpr mice. Mice were
examined 2 wk after in vivo
transfer. Results shown are the
average number of TUNEL-
positive cells (see Fig. 10) seen
per 10 high power fields (HPF,
×40) from three separate experi-
ments.

Our results suggest that both TCR γδ and TCR αβ IEL are readily capable of FasL-mediated killing. To our knowledge,
this is the first report demonstrating that TCR γδ IEL are ca-

ant-TCR) present on cytotoxic T cells. Thus, the antibody–
target cell complex serves as a template for the stimulation of
cytotoxic effector cells, resulting in the killing of the target cell.
The exact mechanism by which effector cells kill target cells in
this assay is unclear. It is likely that both perforin- and Fas-
mediated mechanisms are involved, because IEL isolated from
either gld mice or perforin-deficient mice have both been
shown to possess redirected cytotoxicity (34).

Two previous studies have addressed the role of FasL-
mediated killing by IEL (34, 36). Guy-Grand et al. demonstrated
that IEL from both gld mice (defective FasL) and perforin-deficient mice are capable of redirected cytolytic killing
(34). However, it is unclear from their study whether other cyto-
toxic mediators, such as TNF, can also play a role. Furthermore,
from their results it is difficult to extrapolate the relative
importance of FasL-mediated killing in the IEL of normal
mice, because the lack of perforin in the perforin-knockout
mice may result in a compensatory upregulation of FasL-medi-
ated killing. In the second study, Gelfanov et al. concluded that
both TCR αβ CD8αβ and TCR αβ CD8αεα IEL have FasL-mediated killing activity (36). However, in their study,
IEL were stimulated extensively in vitro for 9 d with anti-TCR
αβ, anti-CD28, and IL-2, followed by a 3-h incubation with
PMA and A23187. Hence, it is unclear whether FasL-mediated
killing is a constitutive function present in most freshly
isolated IEL or a function acquired by a small subset of IEL
after extensive stimulation and expansion in vitro. In the same
study, lymph node cells stimulated identically were also capable
of FasL-mediated killing. We have also observed that
spleen cells treated in vitro with PMA and ionomycin or con-
canamycin A over several days were also capable of FasL-
mediated killing (our unpublished observations). Finally, Gelfa-

Figure 11. Quantification of
IEC apoptosis after in vivo
transfer of GVHD IEL
(B6→B6C3F1) IEL into
B6C3F1 wild-type and
B6C3F1/lpr mice. Mice were
examined 2 wk after in vivo
transfer. Results shown are the
average number of TUNEL-
positive cells (see Fig. 10) seen
per 10 high power fields (HPF,
×40) from three separate experi-
ments.

Our results suggest that both TCR γδ and TCR αβ IEL are readily capable of FasL-mediated killing. To our knowledge,
this is the first report demonstrating that TCR γδ IEL are ca-
mechanism in the pathogenesis of other intestinal diseases where total IEL numbers are also increased, such as celiac disease or milk and soy protein enteropathy (38, 39).

Acknowledgments

The authors would like to thank Dr. W. Olsen for words of encouragement.

This research was supported by National Institutes of Health Clinical Investigator Award DK02445-01 and Glaxo Digestive Disease Basic Research Award to T. Lin, and by National Institutes of Health grant GM-52735 to D.R. Green. This paper is publication 222 from the La Jolla Institute for Allergy and Immunology.

References