Fas is not essential for lamina propria T lymphocyte homeostasis

David L. Boone,* Themistocles Dassopoulos,* Sophia Chai, Marcia Chien, James Lodolce, and Averil Ma

Department of Medicine, Inflammatory Bowel Disease Research Center and Committee on Immunology, The University of Chicago, Chicago, Illinois 60637

Submitted 30 August 2002; accepted in final form 9 April 2003


First published May 7, 2003; 10.1152/ajpgi.00373.2002.—IL-2 receptor α-deficient (IL2Rα−/−) mice spontaneously accumulate vast numbers of intestinal lamina propria (LP) T cells and develop bowel inflammation. The accumulation of T cells in IL2Rα−/− mice is thought to result, in part, from defective Fas-induced cell death. To understand the role of cell proliferation and death in regulating LP T cells in IL2Rα−/− mice, we have directly examined the proliferation and Fas sensitivity of wild-type, lpr/lpr, and IL2Rα−/− LP T cells. In wild-type mice, 5′-bromodeoxyuridine (BrdU) labeling and Fas susceptibility are greatest in CD44Hi LP T cells. Fas-deficient lpr/lpr mice have normal total numbers of LP T cells, despite an increased proportion of BrdU+ T cells. By contrast, IL2Rα−/− mice possess increased total numbers of LP T cells, despite normal proportions of BrdU+ LP T cells. Finally, wild-type and IL2Rα−/− LP T cells are equivalently Fas sensitive. These results demonstrate that LP T cells proliferate and are Fas sensitive. IL2Rα−/− mice accumulate a large number of these Fas-sensitive LP T cells and clearly differ from Fas-deficient lpr/lpr mice in this regard. Thus, our studies reveal these Fas-sensitive LP T cells and clearly differ from Fas-proportions of BrdU-labeled cells. In IL2Rα−/− mice, we have shown directly that Fas ligand (FasL) and suppress expression of FasL in vivo is the cytokine IL-2. Spontaneous bowel inflammation occurs in both IL-2-deficient (IL-2−/−) and IL-2 receptor α-deficient (IL-2Rα−/−) mice, indicating that IL-2 signals mediated through IL-2Rα are critical for downregulating these cells in vivo (26, 33). The inflammation in IL-2−/− mice is a T cell-dependent process (20) and depends on IL-12-mediated Th1 type cytokines (10). Moreover, several studies (14, 19, 22) have suggested that IL-2−/− T cells may not differentiate into cells that are fully susceptible to Fas-mediated programmed cell death (PCD). IL-2-induced T cell activation and proliferation may increase T cell expression of Fas ligand (FasL) and suppress expression of c-FLIP, an inhibitor of Fas receptor signals (24). Together, these studies suggest that the accumulation of activated T cells, which occurs in the absence of IL-2 signals, is associated with mutual induction of FasL expression and a relatively Fas-resistant state of activated T cells, at least compared with activated peripheral lymph node T cells in normal mice.

Although IL-2 receptor signals are likely associated with Fas sensitivity of activated T cells, it remains unclear whether IL-2 and Fas receptors play the same role in regulating PCD of LP T cells as they do in regulating peripheral lymph node (PLN) T cells. Although Fas has been suggested to play an important role in regulating the numbers of activated LPLs (2), the progressive accumulation of activated T cells (including stereotypical CD3+ B220+ T cells) seen peripheral lymph nodes and spleens of Fas-deficient lpr/lpr mice is not seen in intestines of these mice (1). Moreover, Fas-deficient lpr/lpr mice are not known to develop bowel inflammation similar to IL-2−/− and IL-2Rα−/− mice. Hence, the Fas receptor may play distinct roles from IL-2R in regulating the homeostasis of LP T cells.

* The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
To better understand the roles of Fas and IL-2 receptors in regulating LP T cells in vivo, we have used in vivo 5′-bromodeoxyuridine (BrdU) labeling to identify LP T cells that have passed through the S phase of the cell cycle. We have investigated the role of Fas-mediated PCD in regulating the homeostasis of these cells by studying the expression and functional competency of FasL/Fas signaling in normal LP T cells and by assessing cycling of LP T cells in lpr/lpr mice. Moreover, we have compared the homeostasis of LPL T cells in lpr/lpr vs. IL-2R\(\alpha\)−/− LPLs to respond to Fas signals. Our studies reveal novel insights into the homeostatic regulation of intestinal T cells, including critical distinctions between the roles of Fas and IL-2R signaling in regulating these cells.

MATERIALS AND METHODS

Animals. Wild-type, IL-2R\(\alpha\)−/−, and Fas-deficient lpr/lpr mice, all on a C57BL/6J background, were purchased from Jackson Laboratory (Bar Harbor, ME) and were maintained in the University of Chicago animal facility under specific pathogen-free conditions.

Histology. Intestinal specimens were excised, fixed in buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. Inflammation was scored as described (7) in the proximate, mid-, and distal colon as follows: 1) leukocytic infiltration of the colon (0–3); 2) mucin depletion (0–2); 3) crypt abscesses (0–2); 4) epithelial erosion (0–2); 5) hyperemia (0–2); 6) mucosal thickness (1–3), and the average of the three sections was obtained. The index of the severity of disease ranged from 1 (no disease) to 15 (severe disease). All specimens were denoted, coded, and examined by the same person on 1 day to prevent observer biases.

Lymphocyte collection. LPL and PLN T cells were collected as described (20). Briefly, intestines were excised, cleared of adhering fat, and flushed with sodium bicarbonate free RPMI [supplemented with 10% FCS, β-mercaptoethanol, penicillin, and streptomycin (RPMI)]. Minced pieces of this intestine were then shaken in serum-free RPMI containing 1 mM EDTA to remove epithelial cells, washed, and then incubated (37°C, 30 min) in RPMI with 1 mg/ml collagenase B (Boehringer Mannheim) and 0.25 mg/ml deoxyribonuclease I (Sigma) in a rotary shaker. Pooled LPL from two sequential enzymatic digestions were collected, layered over a discontinuous gradient of 44 and 70% Percoll, and centrifuged at 800 \(g\) for 20 min. Lymphocyte-enriched fractions were collected at the interface between 44 and 70% Percoll. The enzymes used for isolating LPL did not affect the expression of any lymphocyte surface markers used in this study. Resting T lymphocytes were obtained by magnetic bead depletion of activated lymphocytes using anti-CD44 MAbs (PharMingen, San Diego, CA) and sheep anti-mouse IgG-coated Dynabeads M-450 (Dynal, Oslo, Norway). Activated T lymphocytes were obtained by culturing PLN T cells for 24 h in the presence of ConA (5 \(\mu\)g/ml), followed by 24 h in recombinant murine IL-2 (10 ng/ml; PharMingen).

Flow cytometric analysis of lymphocytes. Lymphocytes were incubated with FITC, phycoerythrin (PE), Cy-Chrome (CYC), or biotin-conjugated MAbs specific for murine CD3, CD4, CD8a, CD44, Fas (PharMingen) or FasL (kindly provided by J. Tschopp). Biotinylated MAbs were developed with CYC or allophycocyanin-conjugated streptavidin (PharMingen). Cells were analyzed with a FACScalibur flow cytometer using Cell Quest Software (Becton Dickinson, San Jose, CA).

FasL-mediated lymphocyte killing. Fas-induced killing of lymphocytes was assayed as described (8). Briefly, lymphocytes were cultured on 3T3 fibroblasts expressing either murine FasL (FasL fibroblasts) or an empty PSR\(\alpha\) vector (control fibroblasts) for 18 h, incubated with FITC-conjugated anti-Thy1.2 MAbs with or without anti-CD44 MAbs, resuspended in PBS containing propidium iodide (1.0 mg/ml), and analyzed by flow cytometry.

In vivo analysis of lymphocyte cell cycling. BrdU was introduced into mice by either twice-daily intraperitoneal injections (0.4 mg/injection) or by continuous feeding in drinking water (0.8 mg/ml) for 4 days. Lymphocyte cycling in vivo was determined by flow cytometric analysis of BrdU incorporation into T cells using modifications of previously described techniques (6, 32). Briefly, freshly isolated lymphocytes were incubated with MAbs specific for lymphocyte surface antigens, fixed, treated with DNase I, incubated with FITC-conjugated anti-BrdU antibody (20 \(\mu\)l/ml; Becton Dickenson), and analyzed by flow cytometry. For some studies, mice were thymectomized and allowed to recover for 4 wk. Thymectomized mice were fed BrdU water for 10–12 days and analyzed as above or switched to fresh water and “chased” for the indicated number of days.

Statistics. All data are derived from at least three independent experiments and are shown as the means ± SD. Where indicated, paired data sets were analyzed by Student’s t-test. Groups of data were analyzed by ANOVA followed by post hoc Tukey’s. In all cases, significance was inferred at \(P < 0.05\).

RESULTS

Cycling of PLN and LP T lymphocytes. LPLs are thought to proliferate poorly in vitro (15, 31). However, in vitro studies may neglect factors that normally support the proliferation of cells in vivo (15). Moreover, many LP T cells express high levels of the activation/memory marker CD44, which has been associated with a higher rate of proliferation in PLN T cells in vivo (32). We therefore determined the extent of T cell cycling in the lamina propria relative to the periphery. To detect cycling cells in vivo, we treated mice with BrdU for 4 days and then isolated and immunostained LP and PLN T cells. Flow cytometric analyses of these cells showed that the percentage of T lymphocytes that were BrdU\(^{+}\) was consistently higher in the LP T cell population than in the PLN population (10.1 ± 3.2 vs. 3.7 ± 1.1%; \(P < 0.001\); Fig. 1A). This was observed in mice fed BrdU water for a range of times from 2 to 5 days and was independent of the route of BrdU administration (feeding vs. twice-daily injections; data not shown). Consistent with prior work, the majority (70–80%) of LP T cells were CD44\(^{hi}\) memory phenotype cells (Fig. 1B). Tough and Sprent (32) demonstrated a correlation between activation state (CD44 surface expression) and cycling (BrdU incorporation) of PLN T cells. We therefore examined the relationship of CD44 expression to BrdU incorporation in LP T cells. The majority of the BrdU\(^{+}\) T cells in both the PLN and LPL compartments were CD44\(^{hi}\) (Fig. 1B). Further analysis of these populations revealed that a lower proportion of CD44\(^{hi}\) LP T cells incorporated BrdU than CD44\(^{hi}\) PLN T cells (10.5 ± 2.0 vs. 16.3 ± 5.1%; \(P < 0.01\), AJP-Gastrointest Liver Physiol • VOL 285 • AUGUST 2003 • www.ajpgi.org
although this amounted to many fewer cells in the PLN population because of a fewer number of CD44$^{Hi}$ cells.

Thymocytes incorporate BrdU at a high rate in vivo and, after several days of administration, begin to contribute significantly to the number of peripheral BrdU$^+$ T cells (32). We therefore studied T cell BrdU incorporation in thymectomized mice. After the recent thymic emigres were removed from the analysis, we administered BrdU for longer periods (10–12 days) to obtain higher proportions of BrdU$^+$ cells. The percentage of BrdU$^+$ T cells was again higher in LP T cells than in PLN T cells (32 ± 5.4 vs. 16.6 ± 4.8%; \( P < 0.05 \); Fig. 2A). On cessation of BrdU administration to thymectomized mice, the percentage of BrdU$^+$ T cells decreased in both the LPL and PLN compartments but at a faster rate in the LPL compartment (Fig. 2B), consistent with a more rapid turnover of T cells in the LPL population. The rate at which the percentage of BrdU$^+$ cells increases is the sum of their proliferation rate minus the death rate, whereas the rate of decay following cessation of BrdU treatment is sum of the death rate minus the proliferation rate of BrdU-labeled cells (4). Because substantial proliferation is required to dilute BrdU to undetectable levels in a given cell (4), it is unlikely that proliferation of BrdU$^+$ cells contributes to reduced percentages of BrdU$^+$ cells in a population. Instead, the loss of BrdU$^+$ reflects the loss of labeled cells from a population. Thus the LP T population contains significant numbers of cycling cells and is subject to negative homeostatic regulation to prevent T cell accumulation within this compartment.

**Fas-mediated cell death in LP T lymphocytes.** Because significant numbers of LP T cells are cycling in vivo, it is apparent that this population must be subject to negative homeostatic regulation. Because Fas-deficient mice accumulate T cells in the periphery but not in the lamina propria, we sought to determine whether LP T cells are insensitive to Fas-induced death or whether Fas-deficient LP T cells display reduced cycling in vivo. The latter possibility was considered in light of the observation that T cells deficient for the critical Fas death-signaling molecule FADD have defective proliferative responses to some stimuli (35). The cell surface expression of Fas is increased on activated T lymphocytes and is thought to correlate with, but not universally dictate sensitivity to FasL-mediated PCD (28). Because the majority of intestinal T lymphocytes display markers consistent with activation, we compared the level of Fas expression on LP T cells with activated and resting peripheral lymphocytes (Fig. 3). Intestinal T cells expressed elevated Fas levels, comparable with stimulated PLN T lymphocytes (Fig. 3A). Because FasL is dynamically regulated during T cell activation and must engage Fas to induce Fas-mediated PCD (28), we examined the expression of FasL on LP T cells. FasL expression on LP T cells was negligible, comparable with resting PLN T cells, and far below the levels found on activated PLN T cells (Fig. 3B). Thus LP T cells express high levels of Fas but not FasL.

Fas receptor is constitutively expressed and does not universally dictate sensitivity to Fas-mediated PCD. Instead, sensitivity to Fas changes during lymphocyte...
activation, possibly due to altered expression or function of death-signaling molecules (19, 28). Because FasL may induce distinct signals from anti-Fas agonist antibodies, we used fibroblasts expressing membrane-bound FasL to engage Fas receptors on LP T cells (8). Approximately 70% of thymocytes and 35% of activated PLN T cells underwent Fas-specific PCD when exposed overnight to FasL fibroblasts, confirming the sensitivity of these cells to Fas-mediated PCD (Table 1). In contrast, resting PLN T cells were largely resistant to Fas-mediated PCD (Table 1). Freshly isolated LP T cells die at a comparable rate to that of activated PLN T lymphocytes, indicating that LP T cells exist in a Fas-susceptible state in vivo (Table 1). Because peripheral T cell sensitivity to Fas-induced cell death is correlated with activation, we examined whether the degree of CD44 expression also correlated with Fas sensitivity of LP T cells. By assaying CD44 expression on LP T cells that had been incubated on either FasL-expressing or control fibroblasts, we found that CD44<sup>Hi</sup> LP T cells (compared with CD44<sup>Lo</sup> LP T cells) represent almost all of the Fas-sensitive T cells in this population (data not shown). This indicates that LP T cells, similar to activated peripheral T cells, express high levels of Fas and are sensitive to FasL-induced death. These in vitro studies are somewhat compromised by the substantial baseline death rates of T cells in culture overnight. Studies using both normal and lpr/lpr cells in our laboratory have found that as many as 60% of LPL and 20% of PLN T cells die from Fas-independent mechanisms in overnight cultures. This phenomenon, which may reflect alternate death pathways or general metabolic stress to the cells, highlights the importance of complementary in vivo approaches to the study of cell death in the intestinal mucosa.

MRL mice, which develop a lymphoproliferative disorder due to defective Fas receptor function, do not accumulate LP T cells (1). To confirm those findings in mice from matching genetic backgrounds, we analyzed the cellularity of PLN and lamina propria compartments from 6- to 12-wk-old lpr/lpr and control C57BL/6J mice. The number of lpr/lpr mouse PLN T cells was significantly greater than in normal littermates (lpr/lpr T cells = 275 ± 150% of normal; P < 0.01, n = 8). Flow cytometric analyses of these lymphocytes revealed that they included CD44<sup>Hi</sup> CD3<sup>+</sup> B220<sup>+</sup> T cells characteristic of lpr/lpr mice (data not shown). By contrast, there were no significant differences in the numbers of LP T cells between lpr/lpr and littermate controls (lpr/lpr T cells = 87 ± 39% of normal; P > 0.2, n = 5). The distribution of CD3<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup> T cells was normal (data not shown). In addition, CD3<sup>+</sup> B220<sup>+</sup> T cells were not observed in LPL populations from lpr/lpr mice, consistent with prior studies. To further examine the role of Fas in regulating LP T cell homeostasis, we performed BrdU labeling studies on lpr/lpr mice. A 3.2-fold (±1.0) higher percentage of lpr/lpr PLN T cells were BrdU<sup>+</sup> than normal. Similar to activated peripheral T cells, express high levels of Fas and are sensitive to FasL-induced death.

Table 1. Percent Fas-specific killing of T lymphocytes

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percent Fas-specific killing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal thymocytes</td>
<td>66.5 ± 6.5*</td>
</tr>
<tr>
<td>Activated PLN</td>
<td>33.0 ± 11.0*</td>
</tr>
<tr>
<td>Resting PLN</td>
<td>6.5 ± 1.25</td>
</tr>
<tr>
<td>NL LPL</td>
<td>46.5 ± 12.0*</td>
</tr>
<tr>
<td>IL-2R&lt;sup&gt;-/--&lt;/sup&gt; LPL</td>
<td>58.2 ± 7.5*</td>
</tr>
</tbody>
</table>

Values are means ± SD. Fas-specific killing was determined by comparing the number of Thy1.2<sup>+</sup> PI<sup>-</sup> cells collected after 18-h exposure to control vs. Fas ligand (FasL)-expressing fibroblasts (no. of Thy1.2<sup>+</sup> PI<sup>-</sup> cells on control fibroblasts − no. of Thy1.2<sup>+</sup> PI<sup>-</sup> cells on FasL fibroblasts) × 100 = % Fas-specific killing. *P < 0.05 compared with resting PLN.
mice. This suggests that different mechanisms might underlie the accumulation of T cells in the PLN vs. the intestinal lamina propria of IL-2Rα−/− mice.

To better understand why IL-2R signals maintain normal numbers of LP T cells and because prior studies suggested that IL-2R signals predispose activated PLN T cells to undergo Fas-mediated PCD (18, 28), we asked whether IL-2 deficiency reduces the sensitivity of LP T cells to undergo Fas-mediated PCD. Freshly harvested LP T cells from IL-2Rα−/− and control mice were incubated with FasL-expressing fibroblasts over-

Table 2. Proliferation rates of CD4 and CD8 T lymphocytes of Normal and IL-2Rα−/− mice

<table>
<thead>
<tr>
<th></th>
<th>PLN</th>
<th></th>
<th></th>
<th>LPL</th>
<th>% BrdU</th>
<th>% BrdU</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total number</td>
<td>CD4</td>
<td>CD8</td>
<td>Total number</td>
<td>CD4</td>
<td>CD8</td>
</tr>
<tr>
<td>Normal</td>
<td>(10⁶)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.2 ± 0.3</td>
<td>1.85 ± 0.35</td>
<td>1.35 ± 0.25</td>
<td>1.85 ± 0.19</td>
<td>10.2 ± 1.27</td>
<td>7.55 ± 2.15</td>
</tr>
<tr>
<td>IL-2Rα−/−</td>
<td>39.8 ± 8.5*</td>
<td>11.1 ± 2.1*</td>
<td>2.63 ± 0.82</td>
<td>12.06 ± 6.48*</td>
<td>9.35 ± 1.87</td>
<td>6.0 ± 1.07</td>
</tr>
</tbody>
</table>

Values are means ± SD. Mice were fed 5'-bromodeoxyuridine (BrdU) in drinking water for 3–4 days. of each group. *P < 0.05 vs. Normal. IL-2Rα−/−, IL-2 receptor α-deficient.
night. Comparable Fas-specific killing was observed in LP T cells freshly harvested from all strains (Table 1). Thus IL-2R signals are not necessary for the acquisition of Fas sensitivity by LP T cells.

**DISCUSSION**

The inflammation that occurs in IL-2Rα−/− mice is thought to be due, in part, to the reduced ability of IL-2Rα−/− T cells to undergo Fas-induced cell death (24). This relationship between IL-2 and Fas is supported by the observation that peripheral lymphadenopathy occurs in IL-2−/−, IL-2Rα−/−, and Fas-deficient mice. However, spontaneous IBD occurs in IL-2−/− and IL-2Rα−/− but not Fas-deficient mice. We therefore examined the possibility that Fas and/or IL-2 play roles in the homeostatic regulation of LP T cells that are distinct from their roles in peripheral T cell homeostasis. In this report, we show that although LP T cells are Fas sensitive, Fas signals contribute only minimally to their basal homeostatic regulation, possibly because of a paucity of FasL expression in activated LP T cells. We also show that LP T cells are cycling in lpr/lpr mice and also in IL-2Rα−/− mice but that these cells accumulate only in the intestinal lamina propria of the latter. Finally, we demonstrate that FasL sensitivity is not diminished in IL-2Rα−/− LP T cells, suggesting that the accumulation of T lymphocytes in IL-2−/− animals is not due to a diminution of their sensitivity to Fas killing.

Our data showing that the intestinal LP T cell population includes a significant proportion of cycling cells that rapidly turn over in vivo suggest that this population must be subject to negative homeostatic regulation, because the size of the LP compartment remains relatively constant. We therefore investigated the possible role of Fas-mediated cell death as a mechanism for negative homeostatic regulation of LP T cells. Although LP T cells expressed high levels of Fas and were sensitive to FasL-induced killing ex vivo, there was no increase in the number of LPLs of lpr/lpr mice compared with littermate controls in the same genetic background. This confirms prior studies showing no intestinal inflammation in lpr/lpr mice (1). Unlike activated peripheral T cells, the activated T cells of the gut do not display increased FasL expression. Thus Fas may not play a significant role in intestinal T cell homeostasis because LP T cells do not upregulate FasL. To dispel the possibility that lpr/lpr LPLs do not accumulate owing to a lack of cycling in the gut, we demonstrated that these cells indeed do incorporate BrdU in vivo and that the rate of cycling was somewhat higher than that of control littersmates. The slightly higher percentage of BrdU+ cells seen in lpr/lpr LP T cells suggests that Fas deficiency allows a greater proportion of proliferating LP T cells to survive in vivo, even in the C57BL/6J background. However, the maintenance of normal LP T cell numbers in lpr/lpr mice argues that alternative cell death mechanisms compensate for the absence of Fas-mediated PCD in these mice. Such pathways may include those mediated by other death-inducing receptors. TNF receptors are unlikely to play this role, because TNF−/− and TNFR1−/− and TNFR2−/− mice do not develop intestinal inflammation (11, 25) and do not have increased numbers of LP T cells (D. Boone, unpublished observations). It is possible that LP T cell homeostasis does not require the active induction of cell death but instead is accomplished through “death by neglect.” This might occur if LP T cells are inappropriately driven to cycle by bystander mechanisms or bacterial induction of monocyte/macrophage cytokine elaboration and then die as a result of no TCR ligation with their cognate antigen. Although T cells die by neglect in the thymus, a similar process has not yet been described in the gut. These findings do not preclude a more significant role for Fas-mediated PCD during grossly inflammatory states. For example, FasL expression in intestinal tissues may be induced as a result of systemic staphylococcal enterotoxin exposure and may play a role in the death of T cells in the intestine following their peripheral expansion (3).

IL-2 receptor signaling primes activated T cells for Fas-mediated PCD, and some studies have suggested that a failure of FasL induction and Fas signaling on activated T cells may be central to the pathogenesis of autoimmunity in IL-2−/− mice (24). In contrast to lpr/lpr mice, both IL-2−/− and IL-2Rα−/− mice accumulate activated LPL T cells, suggesting that IL-2 receptor signaling may be more important than Fas receptor signals for the negative maintenance of LP T cell homeostasis. Our finding that IL-2Rα−/− LP T cells are highly sensitive to FasL-induced death supports the idea that defects in T cell Fas sensitivity are not the cause of IBD seen in IL-2Rα−/− mice. Although IL-2Rα−/− LP T cells do not cycle more rapidly than wild-type LP T cells suggests that defective cell death processes may underlie the IBD of IL-2Rα−/− mice. In addition, we observed a higher rate of BrdU incorporation in the PLN of IL-2Rα−/− mice and cannot discount the possibility that the increased cell numbers in the LP of these mice was a consequence of the peripheral expansion and recruitment of T cells into the gut. In either case, novel IL-2-dependent death molecules that regulate the LP T cell population may yet be discovered.

In addition, the role of regulatory cells in IBD has recently received renewed interest (13, 29). One such population, identified phenotypically as CD4+ CD25+ regulatory cells, has been implicated in the SCID transfer model of colitis. These CD25+ cells produce significant immunoregulatory transforming growth factor-β (TGF-β) and can prevent colitis in some models of IBD (30). Mice with targeted disruption of TGF-β signaling in T cells develop intestinal inflammation (17, 27). Significantly, Ehrhardt et al. (10) showed that treatment of mice with colitis-inducing trinitrophenol-keyhole limpet hemocyanin causes significant TGF-β production in wild type but not IL-2−/− mice. The absence of these TGF-β-producing regulatory cells rather than defective Fas signaling may underlie the IBD seen in IL-2Rα−/− mice.
DISCLOSURES

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants RO1-DK-52751 and DDRC-DK-42086, National Cancer Institute Center Grant CA-14599, National Sciences and Engineering Research Council Canada postdoctoral fellowship (to D. L. Boone), Crohn’s and Colitis Foundation (to D. L. Boone), and the Gastrointestinal Research Foundation.

REFERENCES


