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Liver Damage by Infiltrating CD8+ T Cells Is Fas Dependent

Norman J. Kennedy, Jennifer Q. Russell, Nina Michail, and Ralph C. Budd

Ag stimulation of CD8+ lymphocytes in vivo results in their migration to various tissues as well as the activation of a cytolytic program involving perforin, TNF-α, and Fas ligand. The liver is one of the main sites for infiltration by activated CD8+ T cells, and this is followed by the death of hepatocytes. The contribution of the various cytolytic components to this process is unclear. Hepatocyte damage by CD8+ T cells was studied using the MHC class I-restricted OVA-specific TCR transgenic mouse (OT-1) to examine the contribution of Fas to hepatocyte death. Activated CD8+ T cells from both OT-1 and Fas-deficient OT-1/lpr mice migrated to the liver in similar numbers after OVA administration, but only in OT-1 mice was there evidence of significant hepatocyte damage histologically and by elevation of serum aspartate transaminase. These differences were not the result of inefficient induction of cytolytic activity in OT-1/lpr liver T cells, since they were as cytolytic in vitro as OT-1 liver T cells. This was supported by findings of similar high levels of message for perforin, TNF-α, and Fas ligand in liver lymphocytes from both mice. These findings demonstrate that following Ag activation, infiltrating liver CD8+ T lymphocytes induce hepatocyte damage in a Fas-dependent manner. The Journal of Immunology, 2001, 167: 6654–6662.

A c tivation of lymphocytes in vivo leads to their migration to a variety of nonlymphoid sites, including the lung, intestine, and liver (1, 2). This results in part from the up-regulation of lymphocyte surface molecules important to trafficking, such as the hyaluronate receptor CD44, LFA-1, and various chemokine receptors (3).

The fate of lymphocytes once they have extravasated into tissues is less well understood, as are the consequences to the tissues that are infiltrated. The liver is a useful model system in which to examine these events, as it contains a resident population of T cells that expresses an activated phenotype, including expression of CD44 (4). Following Ag stimulation, the number of lymphocytes infiltrating the liver increases enormously (1). We have previously observed that the administration of Ag to in vivo to TCR transgenic mice causes liver infiltration of Ag-specific CD8+ and CD8+ T cells, but only the CD8+ T cell subpopulation caused significant liver damage (5). Less apparent was the mechanism responsible for the liver damage.

Cytolytic T cells kill their targets in vitro primarily through perforin and Fas ligand (FasL)4 (6, 7). In vivo this may also involve additional effector molecules such as TNF-α (8, 9). Hepatocytes are exquisitely sensitive to Fas-induced death following in vivo administration of anti-Fas Ab (10). However, studies of lymphocyte-mediated liver injury have achieved various results regarding which pathways were critical for hepatocyte death. These differences depended somewhat on the lymphocyte activation method used. Studies using in vivo administration of Con A observed that hepatocytes were killed by either the perforin pathway (11) or required IFN-γ (12, 13), with little involvement of FasL or TNF-α. The findings in one study of a murine model of hepatitis B found little involvement of perforin or FasL in hepatocyte injury (14); however, conflicting data were reported by Kondo et al. (15), supporting a role for FasL. Still a third model used OVA-primed liposomes targeted to the liver and observed that adoptive transfer of OVA-specific Th1, but not Th2 CD4+ T cells induced liver injury that was mediated by IFN-γ and TNF-α, but not FasL (16). In none of these studies were liver lymphocytes actually purified and analyzed. It is thus unclear what was the actual level of cytolytic activity or expression of cytolytic mediators by the infiltrating T cells.

In the current studies, we have extended our earlier findings of hepatocyte injury mediated primarily by Ag-specific CD8+ T cells to further examine the specific method of cytolysis used by the liver-infiltrating T cells. For this purpose, we used the OT-1 mouse whose T cells recognize the OVA peptide (OVAp) (3), SIINFEKL, in the context of the MHC class I molecule H-2 Kd (17). Fas-deficient OT-1/lpr mice manifested markedly less hepatocyte damage despite similar CD8+ T cell activation, liver infiltration, and expression of cytolytic effector molecules. They also did not develop any accumulation of Ag-activated T cells in lymphoid tissues.

Materials and Methods

Mice

Strains of C57BL/6, C57BL/6-lpr, and transgenic OT-1 and OT-1/lpr mice were bred at the animal facilities of the University of Vermont College of Medicine. Original breeding pairs of C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). OT-1 mice bear a transgenic TCR that recognizes chicken OVApeptide 257–264 restricted to class I MHC, Kd, and were provided by F. Carbone (Monash University Medical School, Victoria, Australia) and M. Bevan (University of Washington, Seattle, WA). OT-1 mice were maintained by breeding TCR transgenic males to normal C57BL/6 females. Offspring were screened for the clonotype TCR using anti-Vα2 mAb. Breeding OT-1 mice with C57BL/6/lpr mice created OT-1/lpr mice. Offspring were screened for the lpr mutation by PCR, as previously described (18), and for the clonotype TCR using anti-Vα2 mAb.

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Abs, cell preparations, and flow cytometry

Monoclonal anti-murine CD8α conjugated to Red613 was purchased from Life Technologies (Gaithersburg, MD). Monoclonal anti-murine CD4 conjugated to Tricolor or PE was purchased from Caltag Laboratories (Burlingame, CA). Monoclonal anti-murine Vα2 conjugated to PE, monochlonal anti-murine CD69 conjugated to PE, monochlonal anti-murine CD80 (B7.1) conjugated to FITC, monoclonal anti-murine CD60 (B7.2) conjugated to PE, and monoclonal anti-murine H-2 Kk conjugated to biotin were purchased from BD PharMingen (San Diego, CA).

Single cell suspensions were made by homogenizing tissues in RPMI 1640 medium (Life Technologies) supplemented with 5% bovine calf serum (BCS; HyClone Laboratories, Logan, UT). For flow cytometry, 750,000 cells were incubated in 0.1 ml PBS containing 0.5% BSA Fraction V, 0.001% (w/v) sodium azide (PBS-azide) (Sigma, St. Louis, MO). The Abs listed above (3 μg/ml) at 4°C for 30 min. After washing with PBS-azide, cells were fixed in 1% methanol-free formaldehyde (Ted Pella, Reading, CA) in PBS-azide. Samples were stored at 4°C until they were analyzed with a Coulter Elite flow cytometer calibrated using DNA check beads (Coulter, Hialeah, FL).

OVA peptide and treatment of TCR transgenic mice

Peptide to chicken OVA 257–264 (SIINFEKL) (OVAp) was produced at Macromolecular Resources (Colorado State University, Fort Collins, CO). Mice received two i.p. injections 24 h apart of 190 μl 100 μM peptide solution in PBS or PBS alone. Tissues were harvested 2, 3, 5, or 7 days after the first injection of peptide.

Isolation of liver lymphocytes

Mice were euthanized, and the peritoneal cavity was opened and the portal sinus and centrifuged using Microtainer separator tubes (BD Biosciences, Franklin Lakes, NJ). Serum was collected according to the manufacturer’s directions. Serum aspartate transaminase levels were determined by colorimetric assay conducted by the chemistry laboratory at Fletcher Allen Medical Center (Burlington, VT).

Semi-quantitative PCR

A total of 5 × 10^6 liver lymphocytes was lysed in Ultraspec RNA reagent (Biotecx Laboratories, Houston, TX), and RNA was prepared according to the manufacturer’s directions. Oligo(dT) priming and reverse transcriptase were used to prepare cDNA from RNA samples. PCR amplifications were performed as follows: 94°C for 3 min, 55°C × 1 min, 72°C × 1 min (35 cycles). Primers used for amplification of perforin (22), FasL (23), TNF-α, and hypoxanthine phosphoribosyltransferase (HPRT) (24) have been described previously. PCR reactions were separated on 1.5% agarose gels, and gels were stained with ethidium bromide (Sigma) and 0.6% H2O2 were added at room temperature for 20 min. Sections were mounted using GVA mounting solution (Zymed Laboratories) and photographed with ASA 100 Kodak Gold 135 mm film.

Detection of apoptosis by TUNEL

To analyze apoptotic cells by flow cytometry, the TUNEL assay was used as described (19, 20). Lymphocyte populations were initially incubated in complete medium at 37°C for 4 h. Cells were initially stained for expression of CD4, CD8, and then fixed for 15 min in 1% formaldehyde. Cell membranes were then permeabilized for 15 min using 70% ethanol at 4°C. Samples were incubated at 37°C for 1 h in 50 μl containing 10 U TdT and 0.5 nm d-UTP-biotin (Roche Diagnostics, Indianapolis, IN). Specimens were washed twice with PBS/1% BSA and incubated with 1/50 dilution of streptavidin-TRITC (Caltag Laboratories) at 4°C for 30 min. Cells were washed twice and analyzed by flow cytometry. Negative controls were consist of staining of cells with the same protocol, but in the absence of d-UTP-biotin.

A modification of Gavrieli et al. (21) was used to measure apoptosis in situ. Tissue samples were fixed in 10% neutral buffered Formalin for 24 h and embedded in paraffin, and 5-μm sections were cut and mounted on slides and heated at 60°C overnight. Slides were hydrated by washing twice in 100% ethanol, then 5 min each in 96%, 90%, and 80% ethanol, and finally twice in double-distilled water (ddH2O). Nuclear proteins were rinsed with 25 μg/ml proteinase K (Sigma) in 10 mM Tris, 5 mM CaCl2, pH 7.4, at room temperature for 15 min, followed by washing in ddH2O. Endogenous peroxidase was inactivated by submerging the slides in 0.6% H2O2 at room temperature for 15 min. Sections were rinsed twice in ddH2O and treated with 30 mM Tris-HCl, pH 7.2, 140 mM sodium cacodylate, 4 mM MgCl2, 0.1 mM DTT, after which, positive control slides were treated with 10 μg/ml DNase I (Sigma) at room temperature for 15 min. All slides were washed three times in ddH2O before the addition of TdT buffer (30 mM Tris-HCl, pH 7.2, 140 mM sodium cacodylate, 1 mM CoCl2). Nick end labeling of DNA was performed by incubating slides with 30 U TdT (Roche Diagnostics) in 100 μl TdT buffer/0.05% BSA at 37°C for 60 min. TdT was omitted from negative control slides. The reaction was terminated by submerging slides in TB buffer (300 mM NaCl, 30 mM sodium citrate) at room temperature for 20 min. Sections were rinsed with ddH2O, covered with 2% BSA in TBS (20 mM Tris-HCl, 150 mM NaCl, 2 mM CaCl2, pH 7.4), and incubated with 100 μl streptavidin-peroxidase (Zymed Laboratories, San Francisco, CA) at room temperature for 20 min. Following three washes of TBS, 90 μl 1:20 aminoethylcarbazole (Zymed Laboratories) and 0.6% H2O2 were added at room temperature for 20 min. Sections were mounted using GVA mounting solution (Zymed Laboratories) and photographed with ASA 100 Kodak Gold 135 mm film.

Cell surface expression of FasL

A total of 0.5 × 10^6 lymph node or liver lymphocytes was stained for cell surface expression of FasL using the Enzymatic Amplification Staining Kit (EAS Kit; Flow-Amp Systems, Cleveland, OH). Cells were washed twice with staining buffer (PBS, pH 7.4, 1% BSA, 1% FBS), then incubated at 4°C for 20 min with 5 μl of either isotype control hamster IgG-biotin or hamster anti-murine FasL-biotin (BD PharMingen). After two washes with staining buffer, all samples were incubated with 1/50 dilution of streptavidin-HRP secondary reagent (EAS kit) at 4°C for 20 min. Cells were subsequently washed twice with staining buffer, then once with PBS, pH 7.4. Cells were reacted with a 1/20 dilution of amplifier solution (EAS kit) at room temperature for 20 min, followed by two washes with staining buffer. Cells were then stained with directly conjugated Vα2-FITC (BD PharMingen) simultaneously with streptavidin-PE (Caltag Laboratories) and incubated at 4°C for 20 min. Following two washes with staining buffer, cells were fixed in methanol-free 1% formaldehyde/PBS and stored at 4°C until analyzed by flow cytometry.
Results

Hepatocyte damage is more pronounced in OT-1 than OT-1 lpr mice following in vivo OVAp

Lymph node and spleen composition and cell numbers were very similar between the OT-1 and OT-1 lpr mice used in these studies, as was the proliferative capacity to OVAp (data not shown). Following administration of OVAp, the livers of OT-1 mice typically appeared very pale and friable by day 2, which was never observed in the livers of OT-1 lpr mice. Furthermore, one of the three OT-1 mice for each time point died on day 2 or 3 in each of the three experiments. By contrast, no OT-1 lpr mice died. Paralleling these

FIGURE 1. Similar kinetics of accumulation and decline of liver lymphocytes in OT-1 and OT-1 lpr mice administered OVAp. OT-1 (■) and OT-1 lpr mice ( □) received two 190-μl doses of either PBS or 100 μM OVAp administered i.p. 24 h apart. On the day indicated following the first injection, lymphocytes were isolated from liver, lymph node, and spleen and assessed for the number of Vα2+ CD8+ and Vα2+ CD4+ CD8− cells. Shown are the means (± SD) from three experiments, each analyzing two or three mice per time point.

FIGURE 2. Despite comparable liver infiltration of lymphocytes after OVAp administration, hepatocyte damage is more pronounced in OT-1 mice than OT-1 lpr mice. Mice received either PBS (A and D) or OVAp (B, C, E, and F), as described in Fig. 1. Livers from OT-1 (A–C) and OT-1 lpr mice (D–F) were taken on day 2 or day 3 after the first injection and stained with H&E. B and E, Show the degree of lymphocyte infiltration in the periporal region, which was similar in both groups of mice. C, Reveals extensive hepatocyte damage in OT-1 livers consisting of initial loss of granularity and membrane clarity (left half, filled arrow), progressing to complete loss of cellular definition (right half, open arrow), which was not observed in OT-1 lpr mice (F). Magnification, ×400.
findings, OVAp administration induced extensive infiltration of Va2+ CD8+ lymphocytes into the livers of both OT-1 and OT-1/lpr mice by day 2 (Fig. 1). Importantly, no difference was observed in either the kinetics of the OVAp response or the number of Va2+ CD8+ infiltrating lymphocytes in the livers of the two types of mice. In addition, the rates of decline of liver lymphocytes after day 2 were essentially identical (Fig. 2).

A very similar pattern of Va2+ CD8+ expansion and contraction was observed in the lymph nodes of both types of mice, except for an isolated increase in OT-1 mice on day 3 that was not observed at other time points, nor in the spleen cells. These findings were consistent over three experiments and highlight that the kinetics of loss of OT-1 T cells was no different on the lpr background. We (25) and others (1, 26) have observed the appearance of CD4+8+ T cells following activation of CD8+ T cells. Although these cells increased in number following OVAp, they did not accumulate in OT-1/lpr mice (Fig. 1, lower panels).

Fig. 2 shows H&E staining of liver sections before and after OVAp treatment. Livers from OT-1 (Fig. 2A) or OT-1/lpr (Fig. 2D) mice that received PBS showed a normal morphology of hepatocytes characterized by distinct membranes and vacuolar appearance of the cytoplasm. There were also few lymphocytes within the sinusoids or perportal regions of the liver. However, by day 2 after OVAp, large numbers of lymphocytes infiltrated the livers of both OT-1 and OT-1/lpr mice. Infiltrating lymphocytes in OT-1 livers were seen clustered initially in the perportal regions (Fig. 2B), followed shortly by migration into the parenchyma by day 3 (Fig. 2C). A similar degree of perportal infiltrates (Fig. 2E) and parenchymal invasion (Fig. 2F) was visible in OVAp-treated OT-1/lpr mice. Concomitant with lymphocyte infiltration, hepatocyte damage was discernable in livers from primarily OT-1 mice given OVAp. Damaged hepatocytes stained more darkly and displayed a hazy ground-glass morphology with few intracellular vacuoles and indistinct cellular membranes (Fig. 2C, left half). Additionally, areas of more extensive liver damage were frequently observed by pink staining of the cytoplasm with nearly complete loss of cellular detail (Fig. 2C, right half). Markedly less damage was noted in livers from OT-1/lpr mice given OVAp. In this case, hepatocytes clearly displayed vacuolar cytoplasm and distinct membranes, despite similar degrees of lymphocytic infiltration (Fig. 2E).

Differences in hepatocyte damage were even more evident when liver sections are stained for nicked DNA using the TUNEL method. Three and five days after the initial OVAp injection, extensive hepatocyte death was visible in OT-1 mice (Fig. 3A–C). However, livers from OT-1/lpr mice given OVAp displayed only minimal damage (Fig. 3D–F). Serum levels of the hepatocyte enzyme aspartate transaminase (AST) also confirmed the differences in liver injury. Mice treated with PBS manifested normal basal AST values (88–200 U/L). Following OVAp administration, however, serum AST values rose dramatically at day 2 in OT-1 mice, but only slightly in OT-1/lpr mice (Fig. 4). The AST values for OT-1 mice at day 2 were actually an underestimate, as the serum tested gave a reading beyond the 750 U/L upper limit of the linear range of the assay. Despite this, the difference in AST values between the OT-1 and OT-1/lpr mice on day 2 was highly significant (p < 0.0007, paired Student’s t test). Additionally, whereas the difference in AST values on day 2 from OT-1 mice given OVAp vs PBS was significant (p < 0.0006), the same comparison in OT-1/lpr mice did not reach statistical significance.

The lack of hepatocyte damage in OT-1/lpr mice given OVAp, despite similar liver lymphocyte infiltration, is consistent with the fact that these mice lack surface expression of Fas. In this model, CD8+ T cells are activated by OVAp to become effector CTL expressing FasL. A portion of the cells migrates to the liver and induces hepatocyte death. Implicit in this model is the ability of OT-1/lpr T cells to fully activate in response to OVAp to express cytolytic molecules. This issue was examined further by assessing
the functional state of CD8+ cells in the liver as well as their expression of the cytolytic effector molecules TNF-α, perforin, and FasL.

Liver lymphocytes manifest similar phenotypes and rates of cell death in OT-1 and OT-1 lpr mice

Activated T cells undergo apoptosis by a process known as activation-induced cell death. A phenotypic change that often occurs concomitant with the entry of T cells in apoptosis is the surface expression of CD44 and CD8 by flow cytometry. Cells were also simultaneously assessed for death by TUNEL assay and expression of B220. Numbers in quadrants represent the percentage of Vα2+ CD8+ cells that are either B220+ or TUNEL+ . Shown are the findings from OT-1 mice in one of three experiments. Summary of OT-1 and OT-1 lpr results is shown in Table I.

Table I. Appearance of dying TUNEL+ B220+ cells lymphocytes after OVAp administrationa

<table>
<thead>
<tr>
<th>Condition</th>
<th>Spleen</th>
<th>Liver</th>
<th>PBS day 3</th>
<th>B220+</th>
<th>TUNEL+</th>
<th>PBS day 3</th>
<th>B220+</th>
<th>TUNEL+</th>
<th>PBS day 3</th>
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<th>PBS day 3</th>
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<tr>
<td>OVA day 3</td>
<td>38</td>
<td>48</td>
<td>45</td>
<td>43</td>
<td>29</td>
<td>33</td>
<td>81</td>
<td>57</td>
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<td>81</td>
<td>57</td>
<td>29</td>
<td>33</td>
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<tr>
<td>OVA day 7</td>
<td>24</td>
<td>27</td>
<td>45</td>
<td>43</td>
<td>24</td>
<td>27</td>
<td>58</td>
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a Mice received injections i.p. of either PBS or OVAp and on the days indicated postinjection, splenocytes and liver lymphocytes were isolated and analyzed for the proportion of Vα2+ CD8+ cells that expressed either surface B220 or nicked DNA by the TUNEL assay. Similar results were observed in two additional experiments.
exogenous OVAp Ag for cytolyis of the Hepa 1.6 targets was supported by the ability of liver lymphocytes to also efficiently lyse allogeneic P815 (H-2d) targets (Fig. 6B). OT-1 T cells do not manifest natural cross-reactivity to H-2b. This suggested that liver lymphocytes expressed lytic molecules when freshly isolated and did not require further Ag stimulation.

Blocking studies using the perforin inhibitor, CMA (29, 30), partially blocked killing of Hepa 1.6 cells equivalently by both OT-1 mice and OT-1\lpr liver T cells (Fig. 6C). Fas-Fc also partially blocked target killing to similar degrees in both mice (Fig. 6C). This is consistent with the view that both FasL and perforin were partly responsible for the lysis of the hepatocytes. Positive control for Fas-Fc blocking of FasL killing was defined using FasL-expressing 293 T cells (Fig. 6D). To confirm that the perforin pathway was activated in T cells from mice given OVAp, further cytolyis assays were performed using the thymoma target EL4 that is not sensitive to FasL-mediated apoptosis (N. J. Kennedy, unpublished observations). Liver lymphocytes isolated from OT-1 or OT-1\lpr mice given OVAp killed EL4 targets, and this was completely blocked by CMA, but unaffected by Fas-Fc (Fig. 6E). By contrast, anti-TNF-\alpha did not block cytolytic activity from liver lymphocytes of either OT-1 or OT-1\lpr mice (data not shown). These observations show that liver lymphocytes from mice administered OVAp kill targets in vitro using both the perforin- and FasL-mediated pathways, and support the view that these same pathways are involved with liver damage in vivo in OVAp-treated OT-1 mice.

To further assess the cytolytic capacity of liver lymphocytes from OT-1 vs OT-1\lpr mice, semiquantitative PCR was performed to assess the levels of expression of perforin, TNF-\alpha, and FasL, using the levels of expression of the hprt gene as a comparison. An example of the PCR results of cDNA titrations from liver lymphocytes is illustrated in Fig. 7A and summarized in Fig. 7B for all three experiments analyzed. A striking finding was the significantly higher levels of message for perforin, TNF-\alpha, and FasL, and in liver lymphocytes compared with lymph node or spleen cells from the same animals. As T cells in lymph nodes, spleens, and livers in OT-1 mice are nearly all CD8\(+\), these differences did not reflect different composition of CD4\(\)- vs CD8\(+\) cells. This underscores the notion that liver CD8\(+\) lymphocytes are enriched for activated T cells following Ag stimulation. A less striking feature was the slightly increased expression in lymph nodes of perforin, TNF-\alpha, and FasL by day 2 after OVAp. A somewhat opposite trend was apparent in the spleen. These trends paralleled the initial increase of Va2\(+\) CD8\(-\) T cells in lymph nodes after OVAp, but their rapid decline in number in the spleen. Conceivably, the activated splenic T cells may have rapidly migrated to the liver. There were only minimal nonstatistical differences between both strains of mice in message levels of these molecules within liver lymphocytes. This further supports the view that the difference in hepatocyte cell death in vivo was not the result of diminished cytolytic capacity of OT-1\lpr CD8\(-\) cells. Finally, in lymph node and spleen cells, there was a moderate increase in message for...
FasL from OT-1pr mice compared with OT-1 mice. This is in agreement with earlier findings (31) and might reflect a compensation by lymphocytes for the lack of Fas (32, 33).

Consistent with the mRNA studies for FasL, surface FasL was considerably induced on liver lymphocytes by day 2 after OVAp (Fig. 7C). These levels were also higher than from lymph nodes from the same day 2 animals, especially OT-1 mice. Surface FasL was also much higher on OT-1pr lymph node T cells than from OT-1 mice.

Discussion
The current findings extend our earlier results that Ag-activated CD4+ and CD8+ T cells migrate to the liver, but that liver damage is preferentially caused by CD8+ cells (5). OVAp-activated OT-1 CD8+ liver lymphocytes used the perforin and Fas mechanisms of cell death in vitro to kill target hepatocytes. This was comparable between OT-1 and OT-1pr liver lymphocytes as defined by both functional assays and PCR analysis of effector molecules. There was a moderately increased expression of FasL by lpr T cells, which may explain the slightly more efficient killing of the Hepa 1.6 target cells by OT-1pr liver lymphocytes in vitro. Nonetheless, considerably less hepatocyte damage was observed in vivo in OT-1pr mice compared with OT-1 mice following OVAp. These findings underscore the importance of the Fas death pathway in this model of liver injury.

Although hepatocytes are clearly sensitive to death following in vivo injection of anti-Fas Ab (10), it has been less certain to what
extent Fas contributes to hepatocyte damage by liver-infiltrating lymphocytes. In three studies using in vivo Con A, liver damage induced by T cells was mediated by perforin or IFN-γ, but not by FasL (11, 12, 34). Other studies using viral-specific T cells from mice bearing transgenically expressed hepatitis B virus yielded conflicting results, either supporting a role for Fas in liver damage (15), or arguing against any involvement of Fas (14, 35). These differences may have resulted in part from the heterogeneity of infiltrating T cells. However, in none of these studies were liver lymphocytes isolated and studied directly for their cytolytic activity or expression of cytolytic effector molecules. The current studies represent the first analysis of the cytolytic activity and mechanisms used by the actual infiltrating lymphocytes in the liver. Hepatocyte damage in OT-1 mice given OVAp results from both perforin- and FasL-mediated killing pathways, as both the perforin inhibitor CMA as well as Fas-Fe block in vitro killing of hepatocyte targets by OT-1 liver lymphocytes. The partial involvement of perforin would be consistent with our findings in vivo that OT-1/lpr mice were not completely devoid of histological evidence of damage of hepatocytes or elevation of serum AST, although it was dramatically less than in Fas-bearing OT-1 mice.

Liver lymphocytes were directly cytolytic when freshly isolated and did not require in vitro restimulation with OVAp, unlike spleen or lymph node T cells from the same mice. This is consistent with the view that CD8+ cells may become activated in peripheral lymphoid tissues, but then migrate to the liver, where cytolytic activity would become concentrated. This model would suggest that OVAp-induced liver injury in mice represents an “innocent bystander” phenomenon. In this system, Ag presentation by the target organ may not be necessary for either the trafficking of lymphocytes to the organ or the subsequent tissue injury induced by infiltrating lymphocytes. We (J. Q. Russell, unpublished observations) and others (2) have observed that Ag-activated T cells also traffic to the lung and kidneys, and we are examining the degree of tissue injury that results at these sites. This model might also serve to explain the liver dysfunction that is often observed after situations in which the immune system has been strongly activated largely outside the liver, such as by a superantigen in toxic shock syndrome (36, 37). It also suggests that the normal number of resident liver lymphocytes in wild-type mice might induce tonic low levels of hepatocyte apoptosis in a Fas-dependent manner. This would be consistent with the observation that Fas-deficient mice have larger livers than wild-type mice (38).

The current findings also raise questions regarding the origin of the CD4−CD8−B220− T cells that accumulate in lpr mice. Prevaling evidence suggests that these unusual lpr T cells derive from a CD8+ precursor. This is supported by persistent demethylation of the CD8α gene in CD4−CD8− T cells (39), and the absence of these cells in lpr mice deficient in β2-microglobulin (18, 40). Although we have previously observed that administration of OVAp to OT-1 mice results in the transient appearance of Vα2+CD4−CD8− T cells (25), the current studies did not observe an accumulation of these cells in OT-1/lpr mice. Conceivably more persistent administration of OVAp to OT-1/lpr mice might result in accumulation of Vα2+CD4−CD8− T cells. However, even with the limited administration of OVAp in this protocol, Vα2+ cells, either CD8+ or CD4−CD8−, were largely eliminated from lymphoid tissues of OT-1/lpr mice by day 7 with kinetics that was nearly identical to OT-1 mice. These findings suggest that the derivation of CD4−CD8− T cells from CD8+ precursors in lpr mice may not result from chronic antigenic activation, but by a different process.


