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Apoptosis of Graft-Infiltrating Cytotoxic T Cells: A Mechanism Underlying "Split Tolerance" in Mouse Liver Transplantation

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C⁵⁷BL/10 (B10; H2^b) mouse liver grafts are accepted in C3H (H2^k) recipients without immunosuppressive therapy whereas skin and heart grafts are rejected acutely.¹ However, *in vitro* mixed leukocyte reactions (MLR) and the generation of cytotoxic T lymphocytes (CTL) show the persistence of anti-donor responsiveness.² The mechanism underlying this "split tolerance" phenomenon is unclear. The present study was undertaken to test the hypothesis that split tolerance might be due to the elimination of effector CTL through apoptosis, while CTL precursors, unsusceptible to apoptosis persist, as shown by the MLR and CTL responses *in vitro*.

MATERIALS AND METHODS

C3H recipients were sacrificed 4, 7, 14, and 60 days after B10 liver transplantation.³ Liver graft non-parenchymal cells (NPC) and splenocytes from the recipients were isolated for assay of CTL activity by ⁵¹Cr release assay. Apoptotic activity was determined by DNA fragmentation,⁴ and 2-color (propidium iodide [PI] and lineage-specific monoclonal antibody [FITC]) flow cytometric analysis.⁵ Apoptotic cells in liver grafts were detected by *in situ* nick-end labeling (TUNEL)⁶ of cryosections. Rejection of B10 livers by C3H recipients was induced by the intraperitoneal injection of mouse IL-2 (4 × 10⁵ U/d, Cetus, Emeryville, Calif) for 5 days, starting on the day of transplant.

RESULTS

Freshly isolated liver NPC exhibited high anti-donor CTL activity that peaked at postoperative day (POD) 4 (45% specific target cell lysis), declined to 37% at POD 7, to 12% at POD 14, then gradually decreased to control level (5%), indicating that donor-specific CTL were induced initially and then eliminated shortly after liver transplantation. Compared with NPC, freshly isolated recipient spleen cells showed much lower levels of cytotoxicity against donor targets (12% on POD 4). However, when the spleen cells were cultured with donor spleen cells for 4 days, elevated levels of donor cell killing (43% to 70%) were generated at all time points, indicating persistence of CTL precursors.

The fate of the CTL within liver graft NPC was investigated by the detection of apoptotic activity using both TUNEL and DNA fragmentation assays. Compared with isografts, numerous TUNEL-positive infiltrating lymphocytes were observed in the portal triads of liver allografts. Apoptotic activity peaked at POD 7 and declined thereafter.

In rejecting liver grafts (median survival time 5 d), there was a significantly lower incidence of apoptotic NPC, but extensive apoptosis of hepatocytes. Apoptosis was quantitated in freshly-isolated NPC by DNA fragmentation assay. Cells from accepted allografts showed much higher DNA fragmentation on POD 4 (34.8%), 7 (40.1%), and 14 (25.7%) compared with cells from isografts (12.6%, 14.5%, and 17.5%, respectively). NPC from rejecting allografts (IL-2 treatment) showed significantly lower DNA fragmentation (7.2% on POD 4), indicating that IL-2-induced rejection was associated with inhibition of infiltrating cell apoptosis. Two-color flow cytometric analysis of the NPC confirmed that augmented apoptosis of CD8⁺ T cells occurred within the allografted liver.

DISCUSSION

Allogeneic mouse liver transplantation without immunosuppression results in graft acceptance and donor-specific tolerance.¹ However, recipient T cells persistently exhibit MLR and CTL responses against donor alloantigens.² The transplanted grafts initially undergo a brief episode of rejection evidenced by graft infiltration that subsides within a few weeks.² Our data suggest that this is due to the programmed cell death of CTL, probably triggered by interaction with donor antigen-presenting cells. Apoptosis of donor-reactive T cells may also occur in recipient lymphoid organs, because donor-derived antigen-presenting cells have been shown to migrate to and persist in these sites after liver transplantation.^{1,7} Detection of apoptotic CTL in recipient lymphoid organs may not be as easy as within the graft due to the relatively low percentage of donor-reactive cells. Use of T cell receptor (TCR) transgenic mice, in which a large proportion of T cells express donor specific TCR, may shed additional light on mechanisms of liver transplant tolerance.

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