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# The Lymphocytotoxic Crossmatch in Liver Transplantation: A Clinicopathologic Analysis

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**P**REFORMED donor-reactive antibodies in kidney transplantation predispose to accelerated or precipitous graft failure, sometimes within hours after transplantation (hyperacute rejection).<sup>1</sup> However, liver allografts have been shown to be resistant to this form of injury.<sup>2</sup> Improvements in surgical techniques, preservation methods, patient selection and management,<sup>3</sup> as well as the introduction of the new immunosuppressant, FK 506,<sup>4</sup> have improved early liver allograft survival, reducing many of the nonimmunologic causes of early graft dysfunction.

This study was undertaken with consent on a subset of patients previously reported showing a deleterious effect of lymphocytotoxic antibodies.<sup>5</sup> We wanted to determine the effect, if any, of preformed lymphocytotoxic antibodies on early liver allograft function and histology, and determine whether immunologic injury to the graft could be detected. The following is an overview of our results, to be presented in detail elsewhere.<sup>6</sup>

## PATIENTS AND METHODS

Between November 31, 1989 and September 9, 1990, 243 adult patients received primary liver allografts under FK 506 and low-dose steroid therapy. There were 26 (11%) patients who received crossmatch-positive primary hepatic grafts during this time. Fifty-two crossmatch-negative control patients were selected on the basis of a sequential OLT number assigned to each patient during the same period of time, as previously described.<sup>7</sup> No statistically significant difference in either sex, age, UNOS status, original disease, donor demographic data or cold ischemic time between crossmatch-positive and control cases was detected. All patients received ABO-identical hepatic grafts.

The recipient's sera obtained immediately before liver transplantation were tested for cytotoxic antibody activity against T lymphocytes isolated from donor lymph node at room temperature (37°C) for 30 minutes followed by 60 minutes of incubation with rabbit complement. Target cell lysis was determined by trypan blue exclusion with dithiothreitol (DTT) treatment,<sup>8</sup> interpreted as positive when more than 50% of lymphocytes were killed.

Clinical events of the patients were reviewed, and graft and patient survival were calculated by the life-table method of Kaplan-Meier. Differences in survival curves were measured using the generalized Wilcoxon test. Statistical comparisons were made by Student's *t* test and by chi-square analysis.

Liver allograft biopsies were performed immediately before implantation, after complete revascularization, and thereafter, when clinically indicated. All routine H&E slides and selected needle biopsy and all failed allograft tissue specimens (stained for the presence of IgG, IgM, IgA, C1q, C3, C4,  $\alpha$ -2-macroglobulin,

transferrin, and fibrinogen) were reviewed by two of the authors (K.N., A.J.D.).

## RESULTS

Early postoperative graft function was assessed by daily median values of platelet counts and standard liver function tests for both groups. The crossmatch-positive patients showed lower peripheral platelet counts and higher total bilirubin levels during the first 30 days than control patients. The canalicular enzymes were similar in the two groups.

The incidence of clinically indicated needle biopsies performed during the first 10 days after surgery was significantly higher in the crossmatch-positive cases (77% vs 42%;  $P < .01$ ). Employing previously published histologic criteria,<sup>9</sup> the diagnosis of acute cellular rejection was more common in the crossmatch-positive cases in the first 10 days ( $P < .05$ ) and of "preservation" injury during the first 20 days. Also, the mean time for the first onset of cellular rejection was  $9 \pm 6$  days in crossmatch-positive patients compared to  $14 \pm 6$  days in the controls ( $P < .05$ ). Other histologic findings with a statistically significant difference between the two groups included vascular platelet aggregation in postreperfusion biopsies ( $P < .05$ ), neutrophilic portal venulitis in the first 10 days ( $P < .05$ ), and cholangiolar proliferation between 20 and 30 days ( $P < .05$ ).

Among the control group, 3 (6%) grafts failed within 180 days, compared with 7 (27%) in the crossmatch-positive group. A statistically significant difference was seen for both primary graft ( $P < .01$ ) and patient ( $P < .05$ ) survival. In the crossmatch-positive patients, portal inflammation with neutrophilia and cholangiolar proliferation, hepatocellular swelling, focal large hilar bile duct necrosis with biliary sludge, organized intrahepatic portal vein, and arterial thrombi were common findings. Although necrotizing or neutrophilic arteritis was not seen, arterial findings included a thickened media with medial myocyte vacuolization (indirect evidence of spasm) and marked endothelial cell hypertrophy, at times with platelet margin-

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ation coating the luminal surface. At the time of this writing, nine of the crossmatch-positive patients and seven controls had died, but no differences in the causes of death were noted.

Direct immunofluorescent analysis for immunoglobulin and complement deposition were detected only in samples taken immediately or shortly after transplantation and consisted of relatively faint granular IgG, C1q, and C3 deposits, predominantly in the sinusoids. Focal weak deposits were detected in hepatic arteries, while portal and central veins were generally negative. No immune deposits were detected in any of the control cases examined.

#### DISCUSSION

The results of this study demonstrate that IgG lymphocytotoxic antibodies can adversely influence human liver allograft function. Compared to patients without such antibodies, humorally sensitized patients in this series more frequently experienced early allograft dysfunction, for which they were subjected to needle biopsies, earlier and more often. These sensitized patients also suffered from an earlier onset and more frequent and relapsing episodes of acute cellular rejection, and risked earlier graft failure from apparent immunologic injury, which pathologically resembled ischemic or "preservation" injury.<sup>5</sup>

Recognition of the pattern of injury associated with lymphocytotoxic antibodies on needle biopsy was extremely difficult to separate from "preservation" injury and sepsis, even in retrospect with the use of immunofluorescent staining. This difficulty was highlighted by the

fact that a diagnosis of "preservation" injury was significantly more common in the crossmatch-positive patients, even though no difference in the cold ischemic time was appreciated.

Although a deleterious effect of preformed antibodies on patient and graft survival was seen in this series, it was relatively small compared to the experience in renal transplantation. It is likely that the "protective" mechanisms of the liver account for this difference.<sup>10</sup> Furthermore, we have since adopted a more aggressive immunosuppressive regimen in presensitized liver allograft recipients, which is initiated immediately after transplantation.

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