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Allospecificity of Liver Allograft-Derived Lymphocytes and Correlation With Clinicopathologic Findings

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PREVIOUS studies have shown that activated T cells can be propagated in vitro from liver allograft biopsies in presence of exogenous recombinant IL 2. Further analysis of these biopsy-grown lymphocytes has revealed the presence of alloreactive T cells specific for donor HLA antigens. Often enough, these cell cultures exhibit restricted specificity patterns against one or few of the donor HLA antigens. This alloreactivity could also be blocked with monoclonal antibodies (MoAb) against appropriate HLA determinants.^{1,2} Similar observations have also been made on lymphocyte cultures grown from heart transplant biopsies. In certain patients, there appeared a sequential infiltration of the allograft by class I-specific lymphocytes in earlier biopsies followed by class II- or mixed class I/II-specific lymphocytes in later biopsies.³

The aim of this study was to evaluate the association of the HLA class I and class II allospecificity of lymphocytes grown from liver allograft biopsies with clinical, biochemical, and histopathologic findings.

MATERIALS AND METHODS

All liver transplant recipients received cyclosporine and steroids as immunosuppressive drugs. As of December 1984, OKT3 MoAb therapy had been added to treat acute rejection episodes. Samples of hepatic allografts were obtained from percutaneous liver biopsies, intraoperative liver biopsies, or removed allografts. Indications for sampling were derangements in liver function tests and bile composition. All samples were taken in a sterile manner for propagation of infiltrating cells and histologic evaluation. Liver biopsies were divided into smaller segments and cultured with recombinant IL 2. The cultured lymphocytes were tested against cryopreserved donor splenocytes, exogenous IL 2, and an informative panel of unrelated lymphocytes with known HLA type. In blocking studies, different anti-class I and anti-class II MoAbs were tested for their inhibitory effect on the PLT response of these cultured T cells.¹

Information about the clinical and biochemical data

prior to taking a biopsy was obtained from the medical charts with special attention to the time interval after the liver transplant procedure, bilirubin total and direct (TOT BIL, DIR BIL), serum glutamate oxalocetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (AP), and gamma glutamyl transpeptidase (GGTP). All values were recorded from the day prior to obtaining the biopsies.

All liver tissue submitted for histopathologic review was taken from the same biopsy core or from the same region of the liver (in failed allografts) as samples used for lymphocyte cultures. The tissues were fixed in neutral buffered formalin, sectioned at 6 μ m and routinely stained with hematoxylin, eosin, and trichrome stains. Histologic sections were examined in a blind manner by one of the authors (A.J. Demetris). Each biopsy and/or resection specimen was reviewed according to a defined protocol with special emphasis on the presence of a mononuclear portal inflammatory infiltrate associated with ductal epithelial and venular endothelial damage.⁴⁻⁶

Statistical analysis of relevant data was performed by the nonparametric statistical tests of Mann-Whitney and Kruskal-Wallis using the SPSSPC software package.⁷

RESULTS

For 25 of 49 lymphocyte cultures established from liver allograft biopsies, we could determine the specificity towards HLA class I (n = 6), mixed HLA class I and II (n = 9), or HLA class II antigens (n = 10). The median of the interval from transplant to biopsy date was eight days (range from four to 18) for HLA class I, 13 days (range from two to 75)

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for HLA class I/II, and 31 days (range from five to 753) for HLA class II-specific T cell cultures (statistically significant between class I and class II, $P = .039$). There was no statistically significant difference observed in the bilirubin, SGOT, and SGPT values for the different groups, although a trend towards higher values for biopsies exhibiting class II-specific T cells was noted. In contrast we observed a statistically significant elevation of GGTP and AP values associated with the appearance of HLA class II-specific cells in the biopsy-derived lymphocyte cultures (class I v class II, for GGTP $P = .017$, for AP $P = .030$; class I v combined class I/II + II, for GGTP $P = .022$, for AP $P = .011$) (Fig 1 left and right).

Histopathologic examination of tissues from which HLA class I only specific cells were expanded showed mild to severe portal tract inflammation consisting of an admixture of cells typical of that described for acute cellular rejection.^{5,6} The percentage of biliary ducts damaged was equal to or less than 60% in all cases.

In general, biopsies from specimens with an HLA class II specificity only showed a mild to moderate portal tract infiltrate. In no instance was the portal inflammation scored as "severe." Two biopsies of liver allografts from

which lymphocyte cultures expressed only HLA class II specificity were obtained during the first week posttransplant. Both were diagnosed as having harvesting injury. Rejection was not thought to be the primary cause of graft dysfunction in these instances, even though the lymphocytes expanded in culture showed donor-specific reactivity. Additionally, the markedly elevated cytosolic hepatocellular enzymes from both of these patients at the time of biopsy were supportive of this contention.⁶ The other allograft biopsy specimens (greater than 1 week posttransplant) from which lymphocyte cultures showed class II only reactivity, showed a mild ($n = 4$) or moderate ($n = 4$) portal infiltrate. Bile duct damage was higher than in both other groups. Subsequent biopsy samples from three of these eight patients continued to show an indolent, ongoing chronic rejection process with prominent duct damage. In fact, two of these grafts were later lost because of a paucity of bile ducts and obliterative vasculopathy.

Biopsies in which there were both HLA class I and II reactivity displayed histopathologic findings intermittent between the two groups. The diagnoses varied from preservation injury to severe rejection. Notably, one of the patients with both class I and II donor-

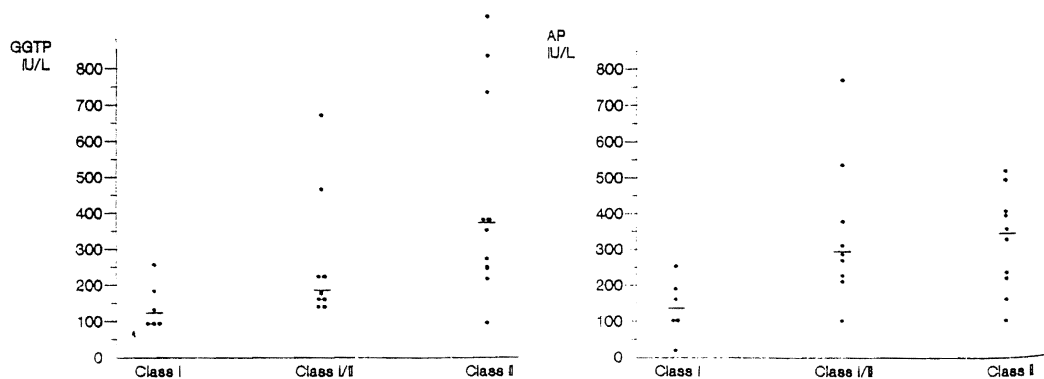


Fig 1. GGTP (left) and AP (right) values and median for each group of HLA specificities. A statistically significant elevation of GGTP and AP values associated with the appearance of HLA class II-specific cells in the biopsy-derived lymphocyte cultures was noted. (Class I v class II, for GGTP $P = .017$, for AP $P = .030$; class I v combined class I/II + II, for GGTP $P = .022$, for AP, $P = 0.11$.)

specific reactivity experienced progressive graft dysfunction resulting in graft loss from chronic rejection.

DISCUSSION

Previous studies have shown that biopsy-grown lymphocytes from certain heart allografts exhibited a trend towards HLA class I-specific cells being more predominant in earlier biopsies, followed by mixed HLA class I/II- or class II-specific cells in later biopsies.³ The same trend has been noted here in lymphocytes grown from liver allografts. The infiltration with class II-specific cells was associated with a rise in GGTP and AP values as indicated by the statistically significant elevation of mean GGTP and AP in these groups. GGTP is specifically released at times of biliary epithelial cell (BEC) damage. In addition, BECs are the main structural element capable of expressing HLA class II antigens in the liver allograft and are a preferential target for invading lymphocytes.⁸ Therefore it seems possible that the main effector cell in the rejection cascade attacking the BECs are class II-specific lymphocytes.

Class I-specific lymphocytes might play a role in inducing the primary steps of the rejection cascade. On vascular endothelial cells class I antigens rather than class II are present early after transplantation. These class I antigens can serve as specific recognition structures for class I-specific lymphocytes, thereby facilitating the preferential adherence of class I-specific lymphocytes to vascular endothelium and their subsequent migration into the surrounding tissues.^{9,10}

Through the release of lymphokines, specifically γ -IFN, these invading class I specific lymphocytes can promote additional HLA class II antigen expression on the vascular endothelial cells¹¹ with subsequent class II-specific lymphocyte adherence and invasion.¹⁰ In addition, various other causes such as ischemia and viral infections might serve as class II antigen promoters on VEC and BEC, thereby facilitating class II-specific infiltrates

and promoting class II-specific intragraft effector mechanisms. Interestingly, harvesting injury was determined to be the major cause of liver dysfunction in both cases in which biopsies were taken during the first week after transplantation and the subsequent lymphocyte culture displayed HLA class II specificity.

Immunocytochemical analysis has previously shown a selective destruction of bile ducts in liver allografts undergoing rejection as demonstrated by striking elevations of GGTP activity.⁸ It was postulated that the induced expression of DR/Ia antigens on structures targeted for immune destruction may be an important event in the pathogenesis of liver allograft rejection. The current analysis suggests that the increased destruction of bile duct epithelium might be associated with HLA class II-specific lymphocytes invading the allograft tissue, and that this damage might be selectively restricted to HLA class II-bearing target structures, namely the biliary epithelium. Class I-specific T cells could thereby function as a promotor for class II-specific lymphocyte invasion. This could finally lead to recognition of class II antigens on BEC and subsequent damage to BEC as shown by elevated serum levels of GGTP and AP in times of a class II-specific T cell infiltrate.

The histopathologic findings described in this report, although limited in number, offer some interesting avenues for further investigation. It has been postulated that the ideal situation for the development of chronic rejection or loss of bile ducts is partial or complete matching at the class II locus with a complete disparity at the class I region.¹² The findings reported here, however, suggest the possibility that some of the duct damage observed in this syndrome may be the result of class II antigen disparity between the donor and recipient. However, further evaluation is needed in terms of defining the type of donor specificity (ie, proliferative v cytotoxic response), the addition of greater numbers to confirm these

observations, and the sequential culturing of samples from the same patient.

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