Role of HLA Class II-Specific Alloreactive T Cells in Biliary Epithelium Injury Associated with Liver Transplant Rejection

S. Saidman, B. Markus, A.J. Demetris, J. Fung, A. Zeevi, T. Starzl, and R. Duquesnoy

Abstract: In this study, we report the establishment of lymphocyte cultures from liver allograft biopsies, and correlate their primed lymphocyte testing (PLT) specificity towards HLA class I and/or class II donor antigens with clinicopathological findings. Early posttransplant biopsies generally yielded more class I-specific cells than later biopsies. There appears to be no correlation of hepatocellular enzyme levels (SGOT or SGPT) with PLT specificity. However, the levels of GGTP and AP, which are biliary enzymes, were increased in patients whose biopsies yielded class II-specific rather than class I-specific cells. There was also a trend towards more damage to bile duct epithelium in biopsies yielding class II-specific cells. These results are probably related to the interaction of class II-specific T cells with bile duct epithelium, which has been shown to express class II HLA antigens.

Although liver transplantation has become an accepted treatment for a variety of end-stage liver diseases, the mechanisms of liver transplant rejection are still obscure. A novel approach to learn more about the immunopathology of transplant rejection is the propagation and characterization of lymphocytes directly from the

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testing (PLT) and by cell-mediated lympholysis (CML). Often they demonstrate restricted specificity patterns against a limited number of donor HLA antigens and this alloreactivity can be blocked with HLA-specific monoclonal antibodies against appropriate determinants. 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.

T lymphocytes were propagated from portions of percutaneous liver biopsies taken at times of liver allograft dysfunction, or from removed allografts. The tissue was divided into small segments and cultured in microculture wells in the presence of 200 µl of recombinant IL2 as described previously (1). After approximately 2 weeks, sufficient cells were obtained for PLT assays against cryopreserved donor splenocytes. Class I- and/or class II-specific alloreactivity was determined by testing an informative panel of HLA-typed lymphocytes and/or blocking with anti-class I and class II monoclonal antibodies (3).

Liver tissue from the same biopsy core or from the same region of the liver (in failed allografts) was submitted for histopathologic review with special emphasis on the presence of a mononuclear portal inflammatory infiltrate associated with ductual epithelial and venular endothelial damage, findings considered to be important in hepatic rejection (4). Information about the clinical and biochemical data was obtained from the medical charts and included serum levels of total and direct bilirubin, glutamate oxaloacetic transaminase (SGOT), glutamate pyruvate transaminase (SGPT), alkaline phosphatase (AP), and γ-glutamyl transpeptidase (GGTP). All values were recorded from the day prior to obtaining the biopsy. Statistical analysis of relevant data was performed by the nonparametric statistical tests of Mann-Whitney and Kruskal-Wallis using the SPSSPC software package (5).

For 35 out of 67 lymphocyte cultures established we could assess allospecificity towards HLA class I (n = 8), mixed class I and II (n = 15) or HLA class II antigens

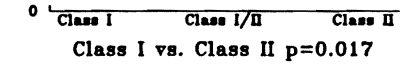

Figure 1. Serum levels of the biliary enzymes alkaline phosphatase (AP) and γ-glutamyl transpeptidase (GGTP) from liver transplant recipients on the day prior to liver biopsy. Patients are grouped according to whether their biopsies grew lymphocytes reactive against only class I antigens, a combination of class I and II antigens, or only class II antigens. Bars indicate the median levels for each group. The p values are for the comparison of median levels in the group yielding only class I-reactive cells versus median levels in the group yielding only class II-reactive cells, using the Mann-Whitney rank sum test.

(n = 12). As shown in Table 1, class I-specific cells were generated from biopsies obtained earlier during the posttransplant time period than those yielding class II-specific cells. This is concordant with previous data from heart transplant biopsies showing that class I-specific cells are more predominant in earlier biopsies whereas class II-specific cells are generally found in later biopsies (6).

No statistically significant differences were found in the bilirubin, SGOT, and SGPT values between the groups yielding class I- and/or class II-specific cells, although a trend towards higher levels in the group of biopsies yielding class II-specific cells was noted. In contrast, significant differences were found for levels of AP and GGTP, both of which are biliary enzymes. Figure 1 shows that the median levels of serum AP were almost twice as high in the class II group as in the class I group (232 versus 170 IU/ml, normal range < 100 IU/ml). Intermediate levels of AP were found in the group of biopsies yielding a mixture of class I- and II-specific cells. Similarly, the median GGTP levels were approximately 2.5 times higher in the class II group than in the class I group (365 versus 142 UI/ml, normal range males <44; females <32 IU/ml).

Table 1. HLA-Associated Alloreactivity of Liver Biopsy-Grown Lymphocyte Cultures

Specificity	No. of Cultures	Posttransplant Day of Biopsy	
		Median	Range
Class I	8	8.5*	4–48
Class I + II	15	17.0	2–237
Class II	12	29.0	5–753
	35†		

*Class I versus class II differences for all biopsies (n = 20), p = 0.076; for biopsies with rejection (n = 16), p = 0.003.
†Thirty-five of 67 biopsy-grown lymphocyte cultures showed class I and/or class II HLA-specific reactivity.

allograft. Previous studies have shown that activated T-cell cultures can be generated from liver allograft biopsies by incubation with recombinant IL2 (1,2). Such cultured lymphocytes frequently exhibit alloreactivity towards donor cells as measured by primed lymphocyte testing (PLT) and by cell-mediated lympholysis (CML). Often they demonstrate restricted specificity patterns against a limited number of donor HLA antigens and this alloreactivity can be blocked with HLA-specific monoclonal antibodies against appropriate determinants. 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.

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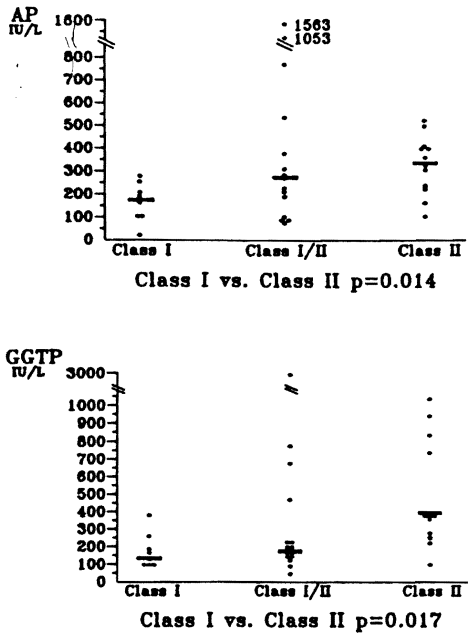

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Other studies have shown that increased GGTP levels reflect injury to the biliary epithelium (7). Thus greater elevations of GGTP (and probably also AP) levels in the class II group may suggest that a cellular infiltrate of class II-specific cells in the liver allograft is associated with more injury to the biliary epithelium. Normal biliary epithelium expresses class I HLA antigens, but no or very low levels of class II antigens (8,9). In contrast, increased expression of class II HLA antigens is frequently observed in liver allografts, especially during rejection (10). The expression of class II antigens on biliary epithelial cells can be induced by lymphokines released by activated T cells, such as γ interferon (11). During rejection, the biliary epithelium is often surrounded by infiltrating lymphocytes (12,13). Our studies have shown a marked trend towards increased bile duct damage in biopsies yielding class II-specific lymphocyte cultures as compared to biopsies growing class I-specific cells (3).

These data suggest that a cellular infiltrate of class II-specific alloreactive T cells may lead to injury of the bile duct epithelium. At the same time, an increased expression of class II HLA antigens may render the biliary epithelium a preferred target in the cellular rejection of the liver allograft.

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