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Allograft Liver Biopsy in Patients With Epstein-Barr Virus–Associated Posttransplant Lymphoproliferative Disease

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Allograft liver biopsy specimens (n = 24) obtained in the clinical setting of primarily extrahepatic posttransplant lymphoproliferative disease (PTLD) were studied for histopathology, lymphocyte subsets, and Epstein-Barr virus (EBV)–encoded EBER RNA. Acute rejection was found in 20 (83.3%) of 24 biopsy specimens and graded as indeterminate in 7 (35%) of 20 (35%), mild in 3 (15%) of 20, and moderate in 10 (50%) of 20 cases. EBV hepatitis was the primary diagnosis in two biopsy specimens and a secondary finding in six others. Four biopsy specimens showed nonspecific reactive hepatitis, and five showed recurrence of primary liver disease. Immunoperoxidase staining showed primarily T cells. EBER RNA was detected in 14 (58.3%) of 24 biopsy specimens: 12 (60%) of 20 with and 2 (50%) of 4 without acute rejection. Antirejection therapy resulted in complete or partial response in 4 (36.3%) of 11 and 7 (63.7%) of 11 treated cases, respectively, despite the presence of EBV-infected cells in some tissues. Subsequent follow-up showed early or late chronic rejection in 6 (25%) of 24 patients. Gamma glutamyl transferase, a marker for early or late chronic rejection, was greater than five times the upper limit of normal in 9 (37.5%) of 24 patients. In conclusion, liver biopsy specimens in patients with PTLD show a spectrum of pathologic changes. Rejection may be treated even if EBV is concurrently present. Long-term graft is suboptimal, because low immunosuppression results in a tendency to develop chronic rejection.

Key Words: Allograft—Biopsy—Epstein-Barr virus–Hepatitis—Liver—Posttransplant lymphoproliferative disease.

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Epstein-Barr virus (EBV) infections are ubiquitous in humans, and as many as 90% of adults show serologic evidence of exposure.[15] In immunocompetent individuals, the usual clinical manifestation is a self-limited infectious mononucleosis syndrome, which is frequently associated with mild hepatitis, but may cause jaundice in approximately 5% of patients. Rare cases of cholestatic or fulminant hepatitis are on record in patients with no known immune deficiency.[5,6] Clinically severe liver disease is regularly seen in the setting of X-linked lymphoproliferative syndrome and sporadic fatal infectious mononucleosis.[15] After liver transplantation, primary Epstein-Barr virus infection is reported in 63% to 80% of patients who are seronegative at the time of transplantation, whereas reactivation infections occur in 20% to 22% of patients exposed to the virus before transplantation.[10,24] Infection is asymptomatic in as many as 85% of patients, whereas the remainder show variable abnormalities in liver function tests.[24] Biopsy samples obtained in the latter group of patients are reported to show a wide spectrum of liver disease, including acute rejection, nonspecific reactive hepatitis, EBV hepatitis, fulminant hepatitis, and posttransplant lymphoproliferative disease (PTLD).[11,16,17,21]

Posttransplant lymphoproliferative disease, regardless of the primary site, is managed initially with a reduction in the dosage of tacrolimus or cyclosporine. Even when the allograft is not involved, allograft liver biopsy is used to monitor graft function in these patients. Immunosuppression is usually reintroduced or increased when acute rejection is detected, although interpretation of biopsy findings in this clinical setting can be difficult. This work summarizes our experience with the interpretation of allograft liver biopsy specimens obtained from patients with PTLD. These specimens frequently satisfy Banff criteria for acute rejection, yet show concurrent lobular hepatitic changes, with or without expression of Epstein-Barr virus encoded RNA (EBER RNA). A conceptual framework for explaining these pathologic changes will be outlined. The therapeutic management and clinical outcome of these cases are discussed.

METHODS

The cases selected for this study were liver transplant recipients who fulfilled the following criteria: a docu-

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mented clinical and pathologic diagnosis of primarily extrahepatic PTLD; a temporally related liver biopsy to monitor graft function; tissue remaining in the paraffin block for immunohistochemistry and in situ hybridization; and available clinical follow-up information.

Twenty-four patients who received transplants between 1985 and 1995 at the University of Pittsburgh adult liver transplant program were included in the study. The biopsy specimens selected for study were obtained between 57 days before and 71 days after the diagnosis of PTLD. When multiple biopsy specimens were available, the one closest to the diagnosis of PTLD was selected. The total clinical follow-up ranged from 2 days to 116 months.

Morphologic examination of liver biopsy specimens was performed on 3-μm thick paraffin-embedded sections stained by the routine hematoxylin and eosin technique. Trichrome stains were done in selected cases. These biopsy specimens were evaluated for the presence and grade of acute rejection using the Banff criteria for liver allograft pathology.[2] In essence, a diagnosis of acute rejection was based on the presence of a mixed but predominantly mononuclear portal infiltrate, duct damage, and portal or central venulitis (Figs. 1, 2). The severity of rejection was graded as indeterminate, mild, moderate, or severe. Histopathologic changes previously reported to be associated with EBV-associated hepatitis were also specifically sought in all specimens. These changes include lobular disarray, sinusoidal lymphocytosis, prominent plasma cell infiltration, immunoblasts, and nuclear atypia.[15,21]

Immunoperoxidase staining for lymphocyte subsets was performed by using commercially available antibodies to CD3 (1:800 dilution) and CD20 (L26 clone, 1:50 dilution) antigens (Dako, Carpenteria, CA, USA). These antigens are expressed by T cells and B cells, respectively. The staining procedure was controlled by a commercially available immunostaining program (Ventana Systems, Tucson, AZ, USA, programs 16 and 6).

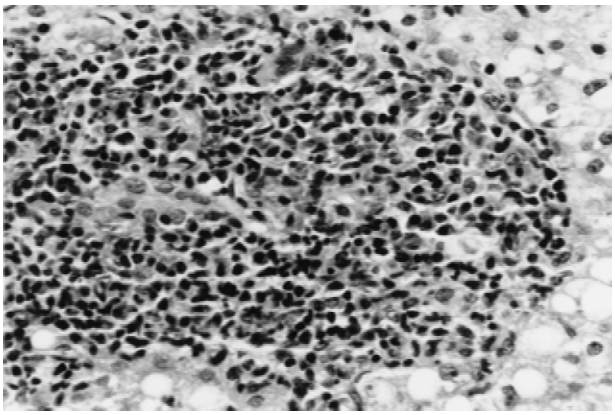

FIG. 1. An allograft liver biopsy specimen from a patient with PTLD, showing a dense mononuclear portal inflammatory infiltrate that includes scattered plasma cells. One entrapped bile duct shows disruption of its normal tubular architecture. These findings, in conjunction with those illustrated in Figure 2, were used to make a diagnosis of moderate acute cellular rejection in this case.

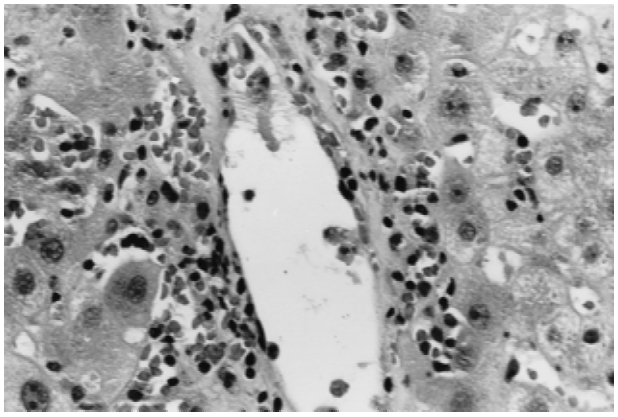

FIG. 2. A photomicrograph prepared from the same case in Figure 1. A central vein is surrounded by a mononuclear infiltrate, which focally extends into the subendothelial zone and causes lifting up of the endothelial cells (so-called central endotheliitis or central venulitis). Focal hepatocyte dropout is seen in the perivenular parenchyma.

In situ hybridization for EBV-encoded EBER RNA was performed on routinely fixed biopsy tissue by adapting published techniques.[20] Briefly, tissue sections on Superfrost Plus slides were paired with double-thickness capillary gap slides (Fisher Scientific, Pittsburgh, PA, USA), deparaffinized using an autodewaxer (Research Genetics, Huntsville AL, USA) at 105°C, and moved through an alcohol series to water. Endogenous peroxidase was blocked with 10% hydrogen peroxide in methanol for 5 minutes. The sections were digested with pepsin for 1 minute at 110°C, washed, and exposed to a biotinylated EBER probe (Research Genetics, 1 ng/mL) at 110°C for 5 minutes, and then at 47°C for 20 minutes. Washing at room temperature was as follows: twice in phosphate buffer saline, once each in 2×, 1×, and 0.5× sodium salt citrate buffer (SSC), followed again with phosphate buffer saline. Finally, the slides were exposed to peroxidase conjugated streptavidin and amino-ethyl carbazole chromogen, each for 30 minutes at 47°C. A positive reaction consisted of a reddish brown intranuclear signal in the portal or lobular mononuclear cells.

Liver function tests, sequential changes in immunosuppression, and other clinical parameters were obtained by direct chart review and from a clinical database maintained by The Thomas E. Starzl Transplantation Institute. Response to antirejection therapy was classified as complete or partial depending on whether it resulted in liver function test results returning to baseline.

RESULTS

The age (19–64 years), sex (12 males and 12 females), primary cause of end-stage liver disease, and other clinicopathologic parameters pertaining to these cases are

presented in Table 1. These patients had a primarily extrahepatic presentation of PTLD. While describing the distribution of lesions (column 5 in the Table 1), the term systemic PTLD refers to cases in which more than two organs were affected. The liver biopsy specimens chosen for study had been obtained between 57 days before and 71 days after the initial diagnosis of PTLD. Clinical management after the diagnosis of PTLD consisted of reduction in the dose of tacrolimus or cyclosporine, a course of acyclovir or ganciclovir, and surgical resection if clinically indicated. This resulted in regression of the disease in most patients. Chemotherapy was necessary to induce regression in patients 3, 22, and 24. Progressive PTLD led to death of patients 5, 14, and 17, despite chemotherapy in patients 5 and 17.

Evaluation of biopsy material showed changes of acute cellular rejection in 20 (83.3%) of 24 (83.3%). Onset of rejection occurred 1 to 57 days before (median, 9 days) or 0 to 71 days after (median, 15 days) the diagnosis of PTLD. The intensity of rejection was graded as indeterminate in 7 (35%) of 20, mild in 3 (15%) of 20, and moderate in 10 (50%) of 20 cases. In addition to changes of acute rejection, wherein inflammation and tissue injury were centered on portal triads and central veins, all biopsy specimens showed mild lobular abnormalities (Fig. 3). These changes consisted of focal hepatocellular swelling, mild lobular disarray, occasional acidophilic bodies, small clusters of Kupffer cells, and sinusoidal lymphocytes arranged in aggregates or linear arrays. These lobular alterations were interpreted as

TABLE 1. *Clinicopathologic features of cases studied*

Case no.	Age (yrs)	Sex	Primary liver disease	PTLD site	Liver biopsy day*	Grade of rejection	EBER RNA	Response to rejection therapy	Clinical follow up (mos)	Last liver function tests†
1	56	F	Cryptogenic & hepatoma	Lymph node	7	Moderate	Yes	Yes	Recurrent cryptogenic hepatitis and cancer (41)	1.7/194/926/756/500
2	20	M	Sclerosing cholangitis	Colon Lymph node	6	Indeterminate	No	Not treated	Chronic rejection, graft loss, sepsis, died (1.5)	5.4/60/22/401/167
3	54	M	Cryptogenic	Systemic	27	None	Yes	Not treated	Chemotherapy for recurrent PTLD (51)	0.6/15/18/11/–
4	64	F	Primary biliary & cirrhosis	Systemic	4	Moderate	Yes	Yes	Died of chronic rejection (2)	23.6/476/562/1909/1275
5	57	M	Hepatitis C alcoholic	Lymph node	0	None	No	Not treated	Died of lymphoproliferative disease (8)	1.1/131/46/–/142
6	49	F	Alcoholic	Lung	15	None	No	Not treated	Allograft hepatitis B; died of heart failure (10)	0.8/125/145/12/107
7	22	M	Sclerosing cholangitis	Lymph node	12	Moderate	Yes	Partial	Mild liver dysfunction (91)	0.8/31/78/245/748
8	58	M	Hepatitis C	Lung	71	Indeterminate	Yes	Partial	Graft loss, hepatic artery thrombosis, mild liver dysfunction	3.7/1946/1274/504/117
9	52	F	Primary biliary cirrhosis	Systemic	46	Moderate	Yes	Partial	Mild liver dysfunction (116)	0.5/26/51/279/260
10	44	F	Cyrptogenic	Liver	13	Mild	No	Partial	Died of cranial bleed (3)	0.5/53/56/147/470
11	37	M	Hepatitis C	Lymph node	–1	Indeterminate	Yes	Not treated	Recurrent hepatitis C (94)	0.4/35/37/–/306
12	24	M	Sclerosing cholangitis	Systemic	25	Indetertiminate	Yes	Not treated	Died of sepsis (2 days)	5.3/89/90/244
13	48	F	Hepatitis C	Lymph node	10	Indeterminate	No	Not treated	Graft loss to chronic rejection and cholangitis (84)	9.1/91/145/–/3033
14	58	M	Alcoholic	Brain	7	Moderate	Yes	Not treated	Died of PTLD (28)	0.7/18/18/126/139
15	25	F	Caroli disease	Gastrointestinal Lymph node	39	Indeterminate	No	Not treated	Early chronic rejection (49)	0.8/91/83/311/377
16	28	F	Anti-trypsin deficiency	Lymph node	4	None	Yes	Not treated	Died of pneumonia (3)	0.4/29/31/203/65
17	48	F	Primary biliary cirrhosis	Systemic	–57	Indeterminate	Yes	Not treated	Died; chronic rejection, liver/systemic PTLD (26)	1.4/166/267/–/775
18	63	M	Hepatitis B	Lymph node	–2	Moderate	No	Not treated	Died with severe acute rejection (2 days)	11.7/4024/1440/186/103
19	40	M	Primary biliary cirrhosis	Gastrointestinal	18	Moderate	Yes	Yes	Normal liver function (76)	0.6/27/17/78/23
20	42	F	Hepatitis C	Gastrointestinal	22	Moderate	No	Partial	Mild liver dysfunction (3)	1.3/95/113/202/313
21	59	M	Hepatitis C	Gastrointestinal	–16	Mild	No	Yes	Recurrent hepatitis C, early chronic rejection (62)	1.8/23/58/–/612
22	26	F	Autoimmune	Systemic	18	Mild	No	Partial	Systemic and hepatic PTLD, chemotherapy (83)	1.3/235/272/362/1322
23	19	F	Autoimmune	Tonsil	14	Moderate	Yes	Partial	Recurrent autoimmune hepatitis (102)	1.0/95/183/195/280
24	44	M	Sclerosing cholangitis	Spleen, colon	39	Moderate	Yes	Not treated	PTLD spread to liver, chemotherapy (67)	0.4/19/–/62/50

* The interval between the time of liver biopsy and the diagnosis of PTLD is specified in days.
† The liver function tests are listed in the following order: serum bilirubin (mg/dl), AST, ALT, AP, GGT (U/L).

hepatitis and further subcategorized as indicated in Table 2. In six patients (patients 1, 2, 9, 14, 16, and 19), the lobular inflammatory infiltrates had a readily recognized component of plasma cells and lymphoblasts. Atypical mononuclear cells with nuclear membrane irregularities were present in patients 22 and 24.

Histopathologic review of biopsy material performed in the course of this study showed only minor clinically insignificant discrepancies compared with the original pathologic diagnoses rendered on these specimens. In three biopsy specimens, a component of rejection was thought to be present, but not clearly graded. For the purposes of this study, these cases were graded as moderate rejection in view of the presence of prominent central venulitis. Two of these patients received steroid therapy, and one was not treated because of progressive PTLD. In four biopsy specimens, the presence of lobular inflammatory and regenerative changes was not mentioned in the official surgical pathology report. Three

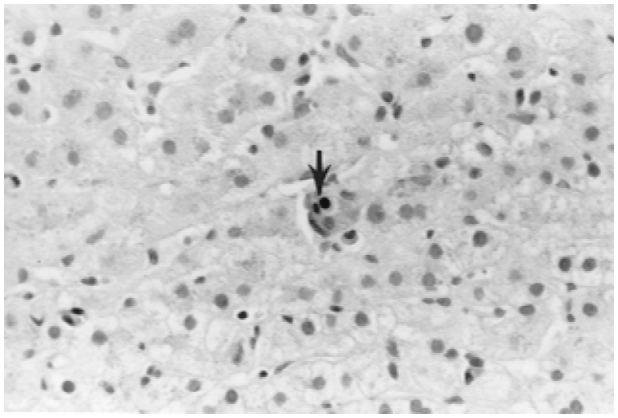

FIG. 3. An allograft liver biopsy specimen showing lobular unrest characterized by loss of the normal cell plate architecture, pleomorphism of hepatocyte nuclei, and a sinusoidal mononuclear infiltration. These changes were interpreted as a low-grade lobular hepatitis. The arrow points to a sinusoidal mononuclear cell showing nuclear staining for EBER RNA.

TABLE 2. *Summary of Biopsy Interpretation*

24 Patients with PTLD treated by reduced immunosuppression

20 biopsies showed hepatitic changes with acute rejection

4 biopsies showed hepatitic changes without acute rejection

2 Epstein-Barr virus hepatitis (EBER +)
1 Hepatitis B (EBER −)
1 Non-specific reactive hepatitis (EBER −)

12 EBER RNA +

8 EBER RNA −

1 biopsy with moderate rejection and nonspecific reactive hepatitis progressed to severe rejection & died (not treated)

2 biopsies with mild or moderate rejection and non-specific reactive hepatitis showed partial therapeutic response

1 biopsy with mild rejection, hepatitis C and early chronic rejection showed partial therapeutic response

1biopsy with mild rejection and transitional hepatitis preceding chronic rejection responded to steroids

3 biopsies with indeterminate rejection and transitional hepatitis preceding chronic rejection not classified with regards to therapeutic steroid response (not treated)

3 biopsies with moderate and 1 biopsy with indeterminate rejection showed partial therapeutic response, and interpreted as rejection superimposed on EBV hepatitis. One of these biopsies was from patient with autoimmune hepatitis.

4 biopsies with moderate rejection showed complete response to increased immunosuppression. Positive EBER RNA may reflect increased number of EBV infected cells in circulation. One of these biopsies was from patient with non A, non B hepatitis.

1 biopsy with moderate rejection not classified (not treated)

3 biopsies indeterminate for rejection interpreted as EBV hepatitis (not treated for rejection). One of these biopsies was from patient with hepatitis C.

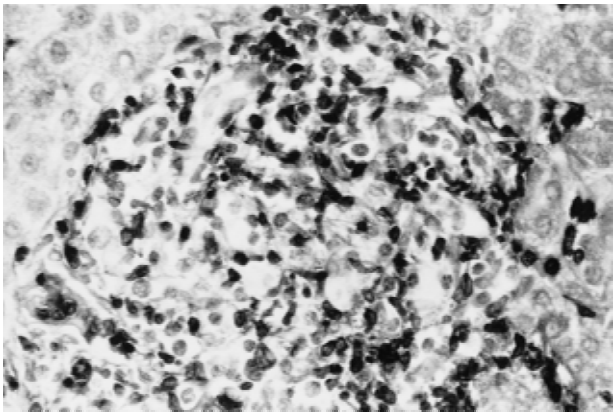

FIG. 4. Immunoperoxidase staining for CD3 antigen, showing that the portal infiltration in all cases consisted primarily of T cells.

cases were listed as showing no rejection, but were classified as indeterminate for rejection on slide review.

Immunoperoxidase staining showed a portal and lobular primarily T-cell infiltrate associated with duct injury, venulitis, and sinusoidal lymphocyte beading (Figs. 4, 5). The proportion of B cells generally varied from less than 1% to 25% in different portal triads. In patient 23, who had a clinical history of autoimmune hepatitis, a few portal triads contained up to 40% B cells.

In situ hybridization for EBER RNA was positive in 14 (58.3%) of 24 biopsy specimens. Expression of EBER RNA in biopsy specimens with and without acute rejection was demonstrable in 12 (60%) of 20 and 2 (50%) of 4 biopsy specimens. If different grades of acute rejection were considered separately, EBV infection was found in 4 (57.1%) of 7 biopsy specimens indeterminate for rejection, 0 (0%) of 3 biopsy specimens with mild acute rejection, and 8 (80%) of 10 biopsy specimens with moderate acute rejection. Among biopsy specimens with no evidence of acute rejection, 2 (50%) of 4 showed EBER

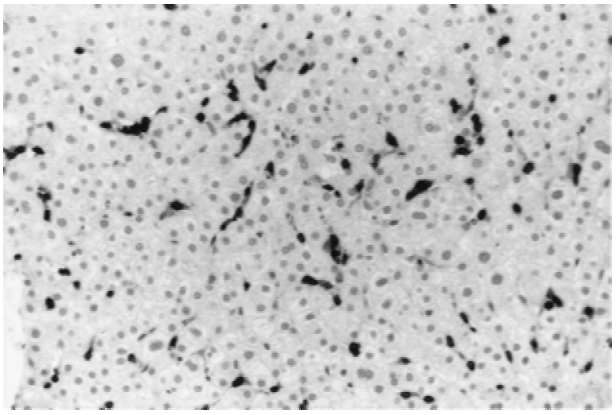

FIG. 5. Lobular inflammatory infiltrates also were comprised predominantly of T cells. Note that some of the sinusoidal cells are arranged in linear arrays, a feature previously reported in EBV hepatitis. Immunoperoxidase stain for CD3.

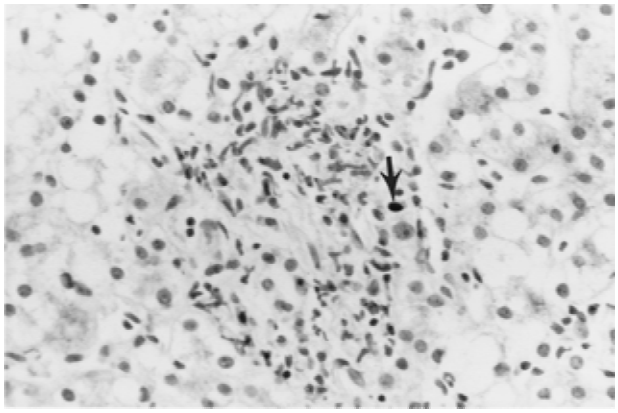

FIG. 6. This allograft liver biopsy specimen contained a single-portal EBV-infected cell (arrow). Portal and lobular inflammatory infiltrates in the remaining biopsy tissue did not hybridize with the EBER RNA probe.

RNA–positive cells (cases 3 and 16). Staining for EBER RNA was observed in small, intermediate and large mononuclear cells, including occasional plasmacytoid cells. In most samples, the total number of EBER RNA–positive cells was extremely small, varying from 1 to 10 in the entire core of liver tissue examined (Figs. 3, 6). Cases 16, 19, 23, and 24 showed scattered positive cells accounting for up to 10% of the total mononuclear cell population (Fig. 7), and one of these cases (case 24) went on to develop PTLD in the allograft liver.

When episodes of mild or moderate acute rejection developed, they were managed by administration of steroids or an increase in the dose of tacrolimus or cyclosporine. No antirejection therapy was given in patients14, 18, and 24 because of clinical concern about progressive PTLD (Table 1). Biopsy specimens showing changes indeterminate for acute rejection were not treated, with the exception of patient 8, who received steroids and showed partial therapeutic response. A total of 11 patients received therapy for their rejection epi-

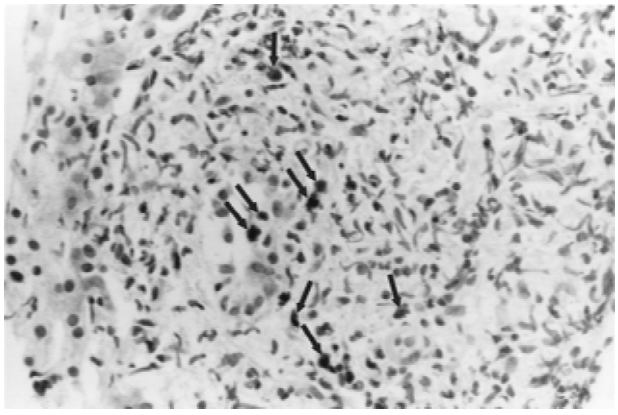

FIG. 7. This liver biopsy specimen contained several portal triads with scattered EBV-infected cells (arrows). A follow-up allograft liver biopsy showed PTLD. In situ hybridization for EBER RNA.

sodes. Response to antirejection therapy was judged to be complete in 4 (36.3%) of 11 cases and partial in 7 (63.7%) of 11 cases. Occasional EBER RNA–positive cells were present in three biopsy specimens, each with complete and partial therapeutic response respectively. On longer follow-up for a median of 46.5 months (range, 2 days–116 months), histopathologic examination of needle biopsy specimens showed PTLD in the liver in three cases (cases 17, 22, and 24), recurrent episodes of mild to moderate rejection in five cases (cases 4, 9, 10, 15, and 23), and early or late chronic rejection in six cases (cases 2, 4, 13, 15, 17, and 21). Five patients developed recurrent hepatitis classified as hepatitis C (patients 11, 21), hepatitis B (patient 6), non A-non B (patient 1), or autoimmune (patient 23) in cause. Two patients died of chronic rejection (patients 4 and 17), and in one of these disseminated PTLD was also a contributing cause of death. One death each was attributed to congestive heart failure, intracranial hemorrhage, and sepsis. The last available liver function tests showed elevations of serum alkaline phosphatase or gamma glutamyl transferase that were disproportionately high compared with aspartate aminotransferase and alanine transferase in 14 (58.3%) of 24 patients. Gamma glutamyl transferase greater than five times the upper limit of normal (i.e., >325 U/L) was observed in 9 (37.5%) of 24 patients. Five of the latter nine cases had histologic evidence of chronic rejection. One patient (patient 22) had an extremely high gamma glutamyl transferase level, but no documented chronic rejection.

DISCUSSION

It is generally agreed that the correct therapeutic strategy for managing patients with EBV infection after liver transplantation is reduction of immunosuppression.[12,25] This allows the host immune response to recoup and mount a successful antiviral response. Unfortunately, this maneuver leads to acute rejection, which was observed in 83.3% of patients at a median of 14 days after the diagnosis of PTLD. This high incidence of acute rejection is comparable with a 74% incidence reported previously in pediatric liver transplant recipients.[4] The data presented show that rejection episodes can be managed with steroids and a judicious increase in the dose of tacrolimus or cyclosporine. Complete therapeutic response, as assessed by restoration of liver function test results to baseline, was observed in only 36.3% of patients. Patients showing only partial response were not subjected to more aggressive immunosuppression for fear of causing flare-up of PTLD. Regression of PTLD was generally observed, except in the three patients who died of disseminated disease. Unfortunately, the relatively favorable prognosis for PTLD was achieved at the cost of allowing continuing low-grade duct damage, which led to progressive graft dysfunction in several patients. Early or late chronic rejection was documented in 6 (25%) of 24 patients, and many other patients who did not undergo another biopsy showed high alkaline phosphatase or gamma glutamyl transferase levels on long-term follow-up. In previous studies, the incidence of chronic rejection in patients with posttransplant lymphoproliferative disease has been quoted as 6%,[4] 38%,[19] and 45%.[7] The overall incidence of chronic rejection in the adult liver transplant program at the University of Pittsburgh is 3.3%.

We observed a 58.3% (14 of 24 samples) prevalence of intrahepatic EBER RNA–positive cells in these adult patients with PTLD. This compares with a prevalence of 71% reported in pediatric allograft liver biopsies performed before a diagnosis of PTLD.[20] The highest prevalence, 8 (80%) of 10 cases, was seen in cases of moderate acute rejection, although typically only rare EBER RNA–positive cells were seen even in these specimens. The significance of this observation is not clear, and it may simply reflect the participation of EBV-infected cells in an allogeneic inflammatory response. However, it is pertinent to recall that a related virus of the herpes group, namely cytomegalovirus, is thought to play an active role in the pathogenesis of acute rejection after solid organ transplantation.[23] Cellular localization of EBV in this study was exclusively to mononuclear cells. Rarely, EBV may infect hepatocytes.[9,20]

The histopathologic diagnoses we assigned to liver biopsies performed during the course of this study are outlined in Table 2. In patients who responded completely to antirejection therapy, rejection was considered to be the primary diagnosis. The presence of occasional EBV-infected cells in these samples was thought to reflect an increased circulating viral burden in these patients. In our hands, a positive in situ hybridization for EBER RNA in liver biopsies is only seen in the context of a high viral load in patients with PTLD or those at high risk for developing this complication.[20] This experience is similar to that reported by Lones et al.,[13] Rizkalla et al.,[22] and Alshak et al.[1] Other investigators, however, presumably using more sensitive methodology, found evidence of EBV infection in a significant proportion of individuals with no clinical evidence of a lymphoproliferative syndrome.[3,8]

In patients with no rejection, changes indeterminate for rejection, or with rejection showing only partial response to antirejection therapy, the presence of EBV-infected cells was thought to be consistent with an underlying component of EBV hepatitis. The primarily T cell lobular infiltrate present in these biopsy specimens does not negate this diagnosis. Even small numbers of EBV-infected B cells in the lesion could conceivably interact with specifically sensitized T-cytotoxic cells and cause sufficient release of cytokines to elicit the inflammatory reaction responsible for the hepatitic change in these biopsy specimens. It is pertinent to recall that in

some cases of PTLD, EBV-infected B cells elicit an intense inflammatory infiltrate, in which T cells can outnumber B cells.[18] Likewise, patients with acute infectious mononucleosis and atypical lymphocytosis can have peripheral blood lymphocytes marking primarily as T cells.[6]

In patients with or without rejection, presence of lobular hepatitic change and negative staining for EBER RNA, the lobular inflammatory reaction was often interpreted as nonspecific reactive hepatitis. This is a pattern of liver injury described in response to numerous extrahepatic disease processes and can presumably also occur in PTLD.[14] In three cases, presence of duct loss in follow-up biopsies suggested a diagnosis of so-called transitional hepatitis, which is described in the setting of early chronic rejection. Recurrent hepatitis B, hepatitis C, and autoimmune hepatitis could explain the lobular inflammation in some of these cases. A final possibility to keep in mind in this group of patients with negative EBER RNA staining is that small numbers of virus-infected cells were present, but not shown because of a sampling problem or insufficient sensitivity of the in situ hybridization procedure.

In summary, the most important diagnostic question that must be addressed in liver allograft biopsies performed in the setting of PTLD is a determination of whether acute rejection is present. This determination should be made on the presence of a mixed, predominantly mononuclear infiltrate that lacks nuclear atypia and is accompanied by duct injury or portal or central venulitis. Presence of frequent eosinophils favors a diagnosis of rejection. If rejection is present, cautious increase in immunosuppression is safe and may improve graft function, even with the concurrent presence of EBV-infected cells. A biopsy diagnosis of EBV hepatitis can be suggested if there is a prominent component of plasma cells and lymphoblasts within the portal and lobular inflammatory infiltrates, and there is lack of proportionate bile duct injury and venulitis. Frankly atypical lymphocytes may also be present. Confirmation requires the demonstration of scattered EBV-infected cells. Milder forms of EBV hepatitis cannot be diagnosed without in situ hybridization studies and exclusion of other clinical entities such as hepatitis B, hepatitis C, or autoimmune hepatitis. The long-term liver allograft function in patients with PTLD is suboptimal, because maintenance of a lower than usual level of immunosuppression results in progressive duct injury and a tendency to develop chronic rejection. □

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