Case Report

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Donor Lymphocyte Infusion Induced Acute Hepatitis

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Hepatic graft-versus-host disease (GVHD) post allogeneic hematopoietic stem cell transplantation generally presents as cholestatic jaundice and increased serum alkaline phosphatase (ALK-P). Currently accepted standards for evaluating the clinical severity of hepatic GVHD are not based on serum aminotransferase levels but on the serum bilirubin levels. We describe a 25-year-old female who initially had no liver damage at all after an allogeneic peripheral blood stem cell transplantation (allo-PBSCT) from her HLA-indentical sister. Markedly elevated aminotransferases, without hyperbilirubinemia, however, developed 7 and 9 weeks after the first and second donor lymphocyte infusion (DLI), respectively. Liver biopsies performed in both events revealed lymphocytic infiltration of the portal tracts and pericentral necrosis of the lobuli. There was also a picture of periductal lymphocytic infiltration and vacuolization of the biliary epithelial cells, which was compatible with the diagnosis of GVHD of cholangiohepatitic type. These findings indicate that hepatic GVHD may present as acute hepatitis and should be included in the differential diagnosis for patients with increased aminotransferases after DLI.

Key Words
acute hepatitis;
allogeneic peripheral blood stem cell transplantation (allo-PBSCT);
donor lymphocyte infusion (DLI);
graft-versus-host disease (GVHD)

Liver dysfunction is a common complication after hematopoietic stem cell transplantation (HSCT), which may be complicated by the concurrent adverse effects of pretransplant chemo/radiotherapy, graft-versus-host disease (GVHD), GVHD prophylactic medication, veno-occlusive liver disease (VOD) and various viral hepatitis. The hepatic manifestation of a typical GVHD is characterized by cholestatic jaundice which starts with an an increased serum alkaline phosphatase (ALK-P), followed by hyperbilirubinemia and mild hepatomegaly.[3] On the other hand, elevation of serum aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) is usually mild. We describe here a patient of acute lymphoblastic leukemia, who received donor lymphocyte infusions twice after an uneventful allogeneic peripheral blood stem cell transplantation (allo-PBSCT). Both, however, were complicated by marked elevation of the AST/ALT 7 and 9 weeks after each DLI, respectively. The clinical manifestations were highly suspicious of acute hepatitis but histologically comfirmed as hepatic GVHD.

CASE REPORT

A 24-year-old female with acute lymphoblastic leukemia was diagnosed in Febuary, 2002. She received remission induction chemotherapy consisting of oncovin

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(2 mg/day i.v. on day 1), daunorubicin (50 mg/m^2 i.v. for 3 days), leunase (10000 IU/day i.v. for 5 days) and oral prednisolone (40 mg PO QD for 14 days). There was no treatment-related liver injury, yet more than 50% of nucleated cells remained to be lymphoblasts on day 14 bone marrow study. Re-induction chemotherapy with idarubicin (12 mg/m^2/day i.v. for 3 days) and cytosine arabinoside (Ara-C) (100 mg/m^2/day i.v. for 7 days) was immediately administered and serum levels of AST and ALT transiently increased up to 129 IU/L (normal <40 IU/L) and 314 IU/L (normal <45 IU/L), respectively. After the successful re-induction, she received 1 course of early intensification chemotherapy with Ara-C (3000 mg/m^2/day i.v. twice daily for 4 days) and mitoxantrone (6 mg/m^2/day i.v. for 5 days) and decadron (20 mg/m^2/day i.v. for 5 days) without any liver enzyme elevations.

She received allo-PBSCT from her HLA-identical sister during first complete remission (CR) on May 18, 2002. Before the transplantation, her liver enzymes results including aminotransferases and bilirubin were all within the normal limits. The pretransplant conditioning regimen consisted of total body irradiation (150cGy for 8 fractions) and intravenous cyclophosphamide (60 mg/kg/day i.v. for 2 days). The previous cryopreserved allogeneic peripheral blood stem cells (PBSCs) were thawed and transfused to the patient via Hickman catheter smoothly, which contained 9.6 × 10^6/kg CD34$^+$ cells and 2.5 × 10^8/kg CD3$^+$ cells. GVHD prophylaxis composed of standard dose cyclosporin-A (CsA) and short course intravenous methotrexate. Rapid engraftment of neutrophils and platelets was noted with sustained granulocytes count exceeding 0.5 × 10^9/L on day 10, and platelet count exceeding 50 × 10^9/L on day 15.

She received the first course of donor lymphocyte infusion with CD3+ cell dosage 11.4 × 10^6/kg on June 1. She stood the process well without major complication until July 17 when serum levels of AST and ALT increased up to 1152 IU/L and 1256 IU/L, respectively. While the bilirubin level remained within normal range, the ALK-P and γ-GT levels were markedly elevated to 579 IU/L (normal < 100 IU/L) and 386 IU/L (normal < 40 IU/L), respectively. She was afebrile. There was no right upper quadrant abdominal pain, no significant weight gain, no hepatomegaly, no ascites. Cutaneous and gastrointestinal manifestations of acute GVHD were totally absent. Serological tests for hepatitis A, B and C viruses, varicella-zoster virus (VZV), herpes simplex virus (HSV) and cytomegalovirus (CMV) were all negative. To rule out the possibility of drug-induced liver injury, all drugs were withdrawn except CsA and trimethoprium-sulfurmethoxazole (TMP-SMX). However, the liver dysfunction did not get improved.

She underwent an ultrasound-guided liver biopsy on July 25. Pathology revealed a picture of abnormal interlobar bile duct with destruction of basement membrane, clouding of nuclei, and suspicious inflammatory cells infiltration. Mild portal fibrosis, portal infiltration, periportal fibrosis and necrosis are also present with positive acidophilic bodies. The liver parenchyma showed degenerative change and obliteration of sinusoids. No CMV was demostrated by special stain. These histological findings were compatible with the cholangiohepatitic changes of hepatic GVHD. She was then treated with prednisolone (1 mg/kg/day). AST and ALT levels promptly decreased to 585 IU/L and 529 IU/L, respectively, and turned normal gradually on tapering. In contrast, the ALK-P and γ-GT levels went down much more slowly than ALT and AST. Finally, all ALT, AST, ALK-P, and γ-GT levels returned to normal ranges in mid-August.

In early September, a bone marrow examination showed 5% lymphoblasts, which implied an early relapse of acute leukemia. CsA was discontinued and re-induction chemotherapy with the same regimen as early intensification given before transplantation was given. Lumbar puncture was checked, which showed no evidence of central nervous system involvement. Intrathecal chemotherapy was then administered. Neutropenic fever was noted later, and chest X-ray revealed bilateral interstitial infiltration. CMV pneumonitis was impressed. In addition to the use of empiric broad spectrum of imipenem/teicoplanin, amphotericin B, gancyclovir and large dose intravenous immunoglobulin (IVIG) were administered. Followed-up chest X-ray got complete resolution. The following BM examination revealed no lymphocyblast and DNA short tandem repeat (STR) study confirmed a complete donor's chimerism.

The second course of DLI was given on October 1. The patient received CD3$^+$ cells at a dose 12.5 × 10^6/kg. Liver dysfunction was not observed until December 13

when serum levels of AST and ALT stroke again up to 1016 IU/L and 905 IU/L, respectively. Although the bilirubin level remained normal (0.80 mg/dL), the ALK-P and γ-GT levels were again elevated to 319 IU/L and 270 IU/L. She underwent a second ultrasound-guided liver biopsy on December 31. Acute hepatitis with bridging necrosis, collapsed hepatocytes with confluent necrosis, and ballooning degeneration were observed. There were also eosinophilic degeneration and abdundant acidophilic bodies. Bile ducts with degenerative changes with cluster of CD3+ cells were also noted. These histological findings were compatible with the cholangiohepatitic changes of hepatic GVHD. Cyclosporin and prednisolone were prescribed, and the AST and ALT levels decreased promptly to 352 IU/L and 231 IU/L, respectively. With the gradually tapering dose of prednisolone, her ALT, AST, ALK-P and γ-GT levels all declined steadily. On Feb 10, 2003, all the hepatic enzymes returned to normal (Figs. 1 and 2). Up to the present, she has been doing well for more than 11 months after the second course of DLI.

DISCUSSION

We describe a patient with acute lymphoblastic leukemia who had received allo-PBSCT and repeatedly developed hepatic GVHD presenting as acute hepatitis after 2 separate courses of DLI. The differential diagnosis included hepatic VOD, viral infection, hepatic GVHD and drug related toxicity.[1] Previous chemo/radiotherapies including pre-transplant conditioning regimen and re-induction for early relapse of acute lymphoblastic leukemia may both induce acute hepatic dysfunction and even severe VOD, but clinical manifestations of VOD were absent in our patient. Acute viral hepatitis due to herpes viruses (HSV, VZV or CMV) and hepatitis A, B and C viruses were all excluded serologically or pathologically.

Hepatic GVHD is widely known to manifest as cholestatic jaundice in conjunction with cutaneous and/or intestinal GVHD. The liver is rarely involved as a sole initial site of GVHD. In typical hepatic GVHD, liver function tests reveal a cholestatic picture with increased serum ALK-P and bilirubin. Liver aminotransferases are generally only mildly elevated. Clinical manifestations of liver dysfunction after DLI in our patient were totally different. Cutaneous lesions and gastrointestinal tract symptoms of acute GVHD were absent and liver aminotransferases were markedly elevated up to > 45 folds of upper normal, suggestive of an acute hepatitis. We performed a ultrasound-guided liver biopsy on 2 separate episodes when serum aminotransferases rose remarkably after both DLIs to make a definitive diagnosis. Such histological findings of the liver are reported to be characteristic of the cholangiohepatitic lesions of chronic GVHD.[4-6]

Gholson *et al*[7] reported a case of chronic hepatic GVHD without extrahepatic involvement. In their case, progressive cholestasis developed 145 days after bone marrow transplantation while on tapering of immunosuppressives and responded well to corticosteroid therapy. In our case, the administration of steroid during the 2 episodes of liver GVHD after DLI also showed good

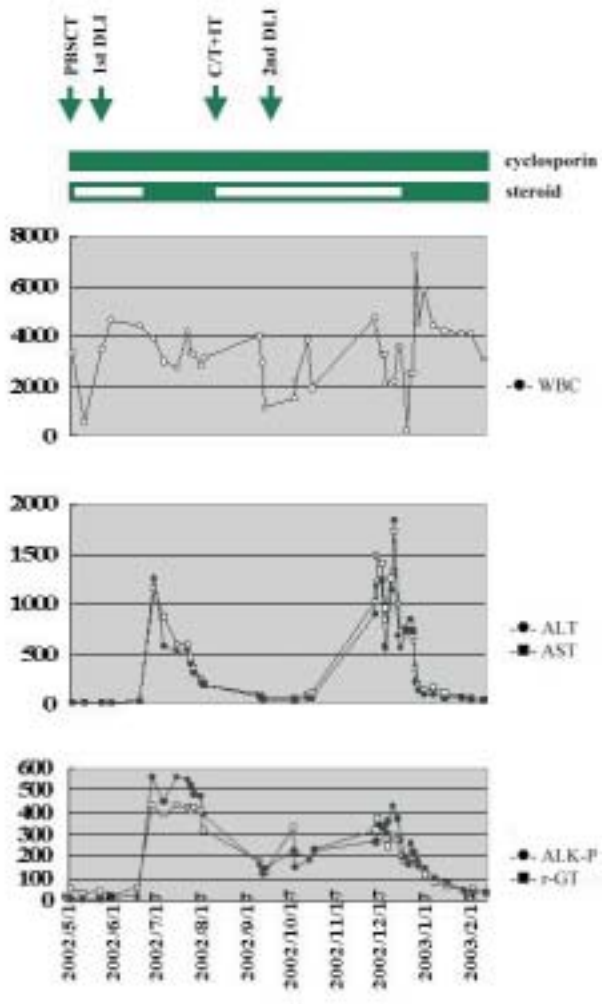

Fig. 1. Clinical course of the patient.

response. Strasser *et al* [8] reported similar cases who developed chronic GVHD of the liver, presenting as acute hepatitis. They described a syndrome of abrupt hepatitic onset of chronic hepatic GVHD in patients receiving mild or no immunosuppression, but their patients developed hepatitis 70 days after transplant. In our case, hepatic GVHD developed in 7 and 9 weeks after the first and second DLI, respectively. DLI represents the efficacy of immunotherapy as a means of curing cancer.[9,10] Immunotherapy with alloreactive donor lymphocytes gives the chance to eliminate the residual tumor cell even in patients with hematological malignancies resistant to maximally tolerated doses of chemoradiotherapy. Alloreactive lymphocytes, which can mediate anti-tumor effects following induction of host-versus-graft tolerance induced by transplantation of donor stem cells, can induce graft-versus-malignancy (GVM) effects which are usually accompanied by graft-versus-host disease (GVHD).[11]

Although DLI can provide curative therapy through a graft-versus-tumor effect, complications still result in significant morbidity and mortality. Prominent among these complications is acute and chronic GVHD, as are the major factors affecting the outcome in these patients. In general, more than half of the patients have acute or chronic GVHD, and half of these patients die of GVHD-related complications.[12] Beside its high incidence and severity, DLI-related GVHD appears to have distinct clinical features. It seems to have more complicated and prolonged clinical course, as compared with PBSCT-related GVHD.[13]

Recently, akpek[14] *et al* demostrated 11 patients with an unusual presentation of hepatitic-variant GVHD in DLI recipients. They suggested that GVHD occuring after DLI may have distinct clinical features. The diagnosis of a hepatitic-variant of GVHD is based on pathological evidence of lobular hepatitis (n = 5), elevation of maximum serum ALT or AST level more than 10 times

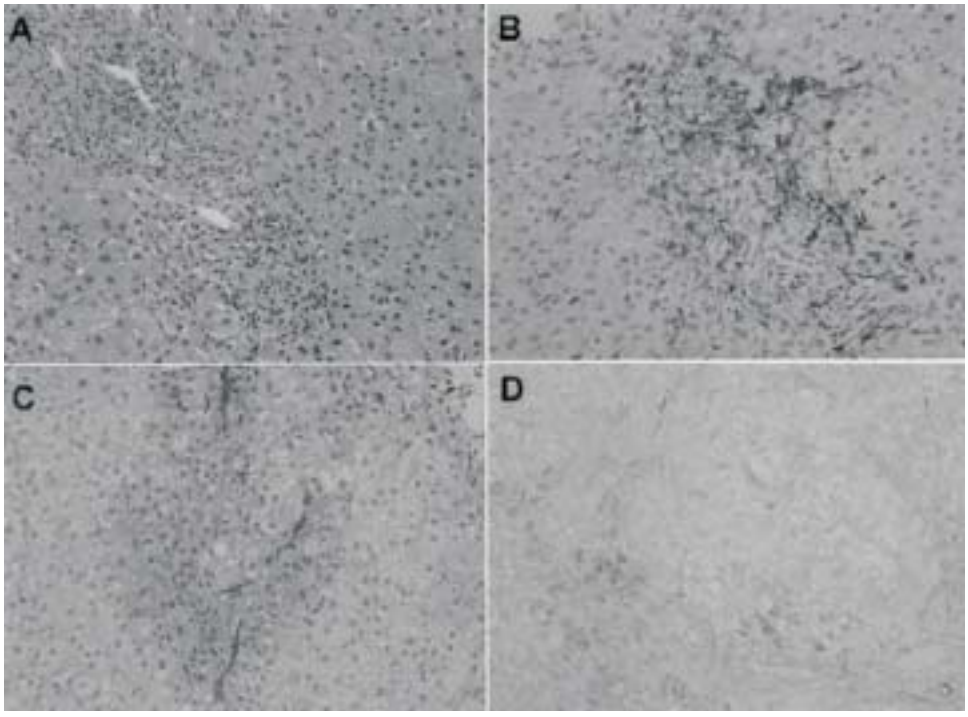

Fig. 2. Liver biopsy 7 weeks after first donor lymphocyte infusion for fig (A) and (B); and biopsy 9 weeks after second donor lymphocyte infusion for (C) and (D). (**A**) The biopsied specimen of the liver revealed lymphocytic infiltrates, intermingled with a few eosinophils and plasma cells in the portal tracts. Limiting plates were not clearly defined with cellular infiltrates (H & E stain). (**B**) There are lymphocyte infiltration of the small bile ducts with epithelial cell collapse, lymphocytic cholangitis and portal lymphoid infiltration. Immunohistochemical stain revealed CD3+ cell. (**C**) There were periductal lymphocytic infiltration and vacuolization of the biliary epithelial cells (H & E stain). (**D**) There was pericentral necrosis accompanied by lymphocytic infiltration. (Immunohistochemical stain with CD3).

the upper normal limit (n = 9), or both. The sequential episodes of acute hepatitis following 2 DLI in our case were compatible with the description of hepatitic-variant GVHD. Given the similar presentation reported after marrow or blood stem cell transplantation, it dose not appear unique to DLI. However, hepatitic-variant GVHD seems more prevalent among DLI recipients. Liver biopsy specimen were obtained from the patient twice to rule out viral hepatitis and to confirm the diagnosis of liver GVHD. Liver biopsy is crucial for the diagnosis, as shown in this case, which reflected the correlation between pathological finding and serum ALT/AST levels.[4,5]

One may argue that the present study lacks a more sensitive molecular study to rule out the possible viral etiology. Although the evaluation of the liver biopsy specimens for HBV or HCV by PCR would be complementary, it is not routine in our institution for the diagnosis of viral hepatitis. Culture of liver tissue is not a sensitive way to identify other viral infections, either. They are best excluded by pathological examination (e.g., CMV, HSV), immunostaining of liver tissue (e.g., adenovirus) and serologic testing. Nonetheless, all the clinical and laboratory features of this patient were in consistence with hepatic GVHD, rather than viral or drug-induced hepatitis.

In conclusion, we report our experience in a case with atypical hepatic GVHD after DLI, which presented as acute hepatitis picture. The possibility of GVHD should be considered when liver dysfunction suggesting acute hepatitis occurs after DLI.

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