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CASE REPORT

Differences in Liver Pathology and Clinical Outcome Between Two Patients with Hepatitis B Virus and Graft Versus Host Disease

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Our two patients undergoing allogeneic bone marrow transplantation (AlloBMT) had both Hepatitis B virus (HBV) and graft-versus-host disease (GVHD). In the first patient, liver enzymes elevated three months after AlloBMT, and GVHD was diagnosed. Two weeks after the diagnosis of GVHD, HBsAg appeared in his serum. At that time, liver biopsy was not able to discriminate two disorders, but his sequential liver biopsies disclosed GVHD. Despite the patient was treated with cyclosporin A (CsA), he died for chronic GVHD. In contrast to the first patient, the second patient had HBsAg prior to GVHD. His liver enzymes deterioration was detected in the first month after AlloBMT, and reached the highest level in the third month while withdrawing CsA. In the fifth month he developed sclerodermatous skin changes, and skin biopsy revealed chronic GVHD, whereas concurrent liver biopsy revealed chronic active hepatitis. This observation showed that immunosuppressive conditions such as GVHD or its prophylaxis may affect the appearance of liver pathology caused by HBV, which depends on the time of GVHD development, and the duration and depth of GVHD prophylaxis. (Pathology Oncology Research Vol 5, No 3, 229–232, 1999)

Keywords: alloBMT, hepatitis B virus, graft versus-host disease

Introduction

Hepatitis B virus (HBV) and graft-versus-host disease (GVHD) are two frequent disorders that may cause deterioration of liver enzymes after allogenic bone marrow transplantation (AlloBMT). Pretransplant prevalence of HBV was reported in European Group for Blood and Transplantation (EBMT) study up to 15% (0–15%) in European recipients.[8,11] After transplantation, it is often difficult to determine which of these disorders is responsible for hepatic deterioration. Furthermore both of them may affect each other through some aspects. The reactivation of hepatitis B virus after immunosuppresive conditions, including chemotherapy and transplantation has been reported.[2,3,5,10,13] This reactivation, not always,[9,12] but sometimes may cause fulminant B type hepatitis and deaths following the reconstitution of immune system.[10,13] It is well-known that GVHD or GVHD prophylaxis induce immunosuppression.[1,7] Therefore, GVHD and GVHD prophylaxis may have impact on the clinical and histological outcome of HBV.[2] On the other hand, Lau et al have shown that the presence of HBV may increase GVHD frequency after transplantation.[6]

We present here, our two patients who had both GVHD and HBV infection. The appearance time of HBV and GVHD, and the prophylaxis and treatment of GVHD were not similar between these patients. Therefore, clinical and histopathological outcome of these patients regarding liver damage were very different.

Methods

All five specimens were fixed in 10% buffered formalin and paraffin embedded. Sections were routinely stained with hematoxylin-eosin. The PAS (periodic-acid

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Schiff) stain was used to evaluate the bile duct damage. Masson's trichrome was performed to show fibroblastic activity. Hepatitis B virus infection was searched by staining surface and core antigens immunohistochemically (IHC) (Biogenex).

Case Report

Case 1

In July 1993, a 25-year-old man with chronic myelogenous leukemia in chronic phase (CMLcp) was donated his HLA- identical sibling's bone marrow. The patient was conditioned with busulphan 4 mg/kg/d p.o.for four days and cyclophosphamide 50 mg/kg/d for two days. Methotrexate 15 mg/m^2 on day+1, and 10 mg/m^2 on days +3, +6, +ll, and cyclosporin A (CsA) 3 mg/kg/d i.v. for the first month, then 6 mg/kg/d p.o. were administered for GVHD prophylaxis. The engraftments of neutrophil and platelet occurred on day+18 and day+20, respectively. In the 3rd month skin rash, diarrhea and deterioration of liver functions were detected. Acute GVHD (grade IV) was diagnosed clinically and was also confirmed by his skin biopsy. Two weeks after this, HBsAg, HBeAg and anti-HBc IgM were positive. Differential diagnosis between acute type B hepatitis and acute GVHD could not be made by liver biopsy. Methylprednisolone 80 mg/d p.o. was added to CsA treatment. In the seventh month he developed scleroderma, dry mouth, dry eyes and jaundice. The second percutaneous liver biopsy showed that there was no visible bile duct in the portal tract. Ursodeoxycolic acid (10 mg/kg/d) was added to therapy. In the ninth month, clinical and laboratory findings did not improve despite the therapy. GVHD findings became prominent in the third one. He died from chronic GVHD with HBsAg, HBeAg, anti-HBc IgM and anti-HBc IgG positivity.

Pathological findings – The first biopsy did not show any change consistent with GVHD, venoocclusive disease (VOD) or infectious hepatitis. Cytoplasmic HBsAg and nuclear HBcAg were not detected by IHC. The following biopsies shared similar features regarding bile ducts. However, the last one had more extensive bile duct damage. Portal tracts of the second biopsy displayed varying degrees of inflammatory infiltrate, being mild to moderate, and the infiltrate consisted of mononuclear cells. The interlobular bile duct epithelia showed degeneration, cytoplasmic swelling and segmental destruction *(Figure 1)*. Diffuse balloon degeneration of the hepatocytes, spare focal necrosis and mild Kupffer cell hyperplasia were the only parenchymal changes. Comparing with the previous biopsy, the bile duct changes were found to be consistent with GVHD. The phase of the disease, whether acute or chronic, was debatable since the patient was under therapy, and the histopathology was expected to be affected by the situation. In the third biopsy the portal tracts were widened and the bile duct damage was more prominent. In addition, there was a bile duct loss in some small portal tracts *(Figure 2)* and perivenular canalicular and hepatocellular cholestasis was striking. The hepatocytes showed nuclear regenerative features and there was a mild parenchymal inflammation. Although slight increase in collagenous tissue was observed with trichrome stain, the portal tracts were not fibrotic at all. The hepatocytes with ground glass cytoplasm were found to be stained with surface antigen, but the core antigen was negative *(Figure 3)*. In conclusion, since the last material was evaluated as showing progression regarding the bile duct damage, the case was accepted as both GVHD and true HBV carrier.

Case 2

In June 1990, a 32-year-old man with CMLcp received bone marrow from a HLA-identical sibling. The patient had also been known to be a HBV carrier four months

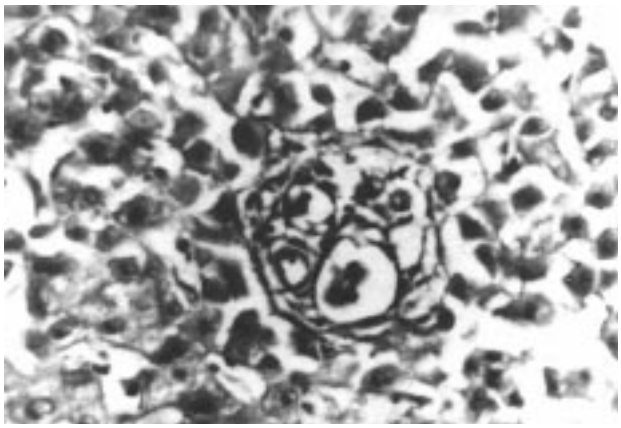

Figure 1. *The bile duct epithelia showed degeneration, aptoplasmic swelling and segmental destruction in HE stain (x400).*

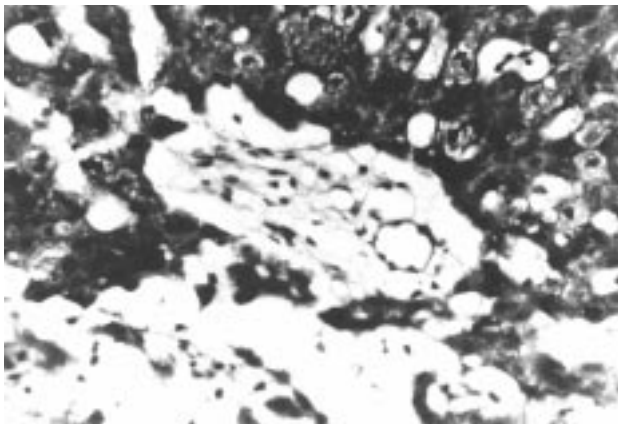

Figure 2. *There is no visible bile duct in PAS stained section (x400).*

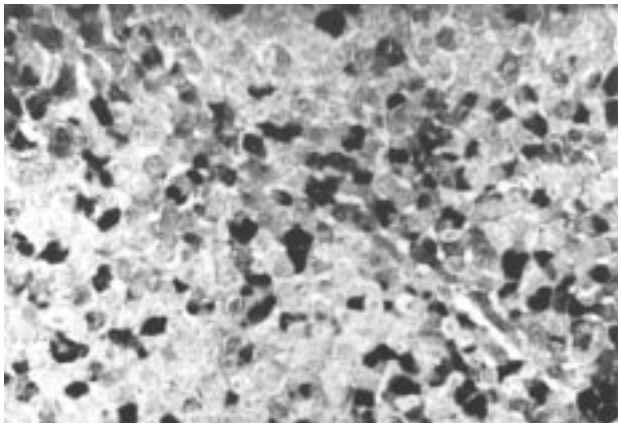

Figure 3. *Cytoplasmic HBs positive staining (x200).*

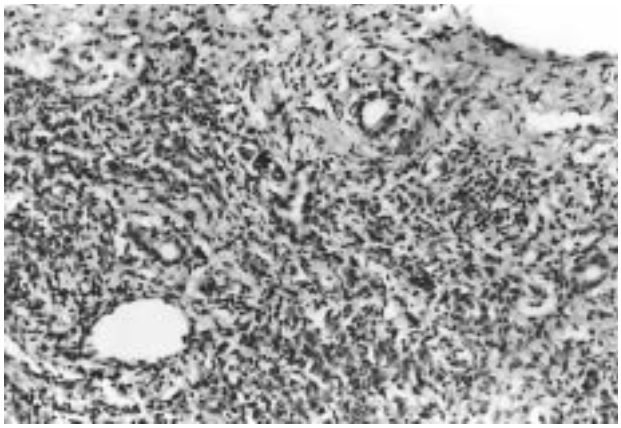

Figure 4. *Portal areas are widened with prominent lymphocytic infiltration and connective tissue increase. Lymphocytes infiltrate hepatic parenchyma in many areas in HE stain (x40)*

before AlloBMT. The conditioning regimen consisted of cyclophosphamide, busulphan and total body irradiation. MTX and CsA were given to prevent GVHD. CsA was initiated as a dose of 1.5 mg/kg/d i.v., then reduced to a dose of 1 mg/kg i.v. ten days after BMT. Neutrophil and platelet engraftments occurred on the +23th and on the +25th day, respectively. On the +24th day the deterioration of liver function tests and hyperglycemia were detected for the first time. AST and ALT remained under two times the upper limit of normal range until the third month. When CsA was stopped, these enzymes began to rise rapidly. He received only supportive treatment and low dose insulin in this period. In the +5th month liver function tests relatively improved, but did not return to normal level. Sclerodermatous changes developed, and his skin biopsy revealed the findings of chronic GVHD. Liver biopsy was consistent with viral chronic active hepatitis rather than chronic GVHD. The low dose of CsA was initiated again for the therapy of chronic GVHD. Liver function tests began to decrease steadily. During his follow-up period serologic HBV markers did not change (HBsAg and anti-HBc IgG were positive, HBeAg and anti-HB IgM negative) In the sixth month the patient died due to diabetic ketoacidosis.

Pathological findings – In the liver biopsy sections, there was severe lymphocytic infiltration and moderate fibrosis in the portal tracts and portal bile ducts could be observed *(Figure 4)*. The limiting membrane was disrupted in many areas and piece-meal type hepatocyte destruction caused by infiltrating lymphocytes was prominent. No cholestasis or pseudoxanthomatous changes were seen. With these findings, the liver pathology was thought to represent viral chronic active hepatitis rather than chronic GVHD. IHC examination could not be available.

Discussion

GVHD enhances severity and duration of immunosuppression after transplantation in patients allografting with bone marrow.[1,7] Chen et al. reported two cases who had HBsAb, HBeAb, HBcAb prior to transplantation, and these antibodies disappeared 2–4 months after the onset of chronic GVHD following immunosuppressive treatment.[2] HBsAg reappeared in patients' sera 6-10 months later and the authors suggested that chronic GVHD and immunosuppressive drugs may reactivate HBV in HBsAb positive patients. In our first patient having HBc IgG antibody before AlloBMT, we do not know whether the appearance of HBsAg in his serum following GVHD was reactivation or reinfection of HBV. However, the positive staining of HBsAg in hepatocyte cytoplasm supports reactivation of the virus[3,9] This staining was negative in his first liver biopsy. Despite serologic markers consistent with active viral replication, HBV did not present its characteristic histological findings due to immunosuppression caused by GVHD and its prophylaxis in the patient.

In the second patient HBsAg positivity preceded GVHD, and his liver biopsy revealed chronic active B hepatitis. Comparing with the first patient, immunoreconstitution was faster because the dose and duration of CsA was inadequate and because the development of GVHD was later in the second patient. This observation is consistent with that HBV causes liver damage through immune mediated rather than direct cytopathic effect.[4]

On the other hand, Lau et al. have shown that HBsAg positive recipients had an increased incidence of post-BMT hepatitis, hepatic failure and acute GVHD.[6] Chronic HBV infection can increase the production of cytokines by which can augment the development of GVHD. In our second patient, his inadequate GVHD pro-

phylaxis is seem to be a major reason responsible for GVHD development, but that HBV has an additional role is not clear.

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