Case report

Cytokine and pathological analyses of hepatic variant Graft-versus-Host disease after allogenic peripheral blood stem cell transplantation in a child

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Typical liver Graft-versus-Host disease (GVHD) is characterized by cholestasis causing bile duct damage. The hepatic variant of liver GVHD shows marked elevations of blood aminotransferase levels without significant elevation of biliary tract enzymes. Hepatic GVHD may account for as many as 36% to 50% of cases of liver GVHD in adults but is rare in children. We describe a 4-year-old girl in whom hepatic GVHD developed after allogenic hematopoietic stem cell transplantation for Philadelphia chromosome-positive acute lymphoblastic leukemia. The pathologic features of liver biopsy specimens were consistent with lobular hepatitis, with marked infiltration of CD8-positive T-cell predominating in portal areas. There was no cholangiolitis or B-cell or plasma cell infiltration, both of which play important roles in autoimmune hepatitis like liver GVHD. The cytokine/chemokine profile showed high expression of monocyte chemotactic protein-1, and macrophage inflammatory protein-1β in liver sample, suggesting that activation of monocytes/macrophages may be related with pathophysiology of hepatic GVHD. (Journal of Hematopoietic Cell Transplantation 5(1): 22–26, 2016.)

Introduction

Typical liver Graft-versus-Host disease (GVHD) is characterized by cholestasis causing bile duct damage.¹ In contrast, the hepatic variant of liver GVHD shows marked elevations of serum aminotransferase levels without significant elevation of biliary tract enzyme.²,³ Such hepatic GVHD may account for as many as 36% to 50% of cases of liver GVHD in adults.⁴ In children, on the other hand, hepatic GVHD is rare, with fewer than 10 reported cases.⁵,⁶ Acute GVHD is generally related to the activation of donor T-lymphocytes. Hepatic GVHD is considered same pathophysiology but resembles autoimmune hepatitis (AIH)-like GVHD, which involves B-cells or plasma cells.⁷ To our knowledge, there have been no reports of the pathologic features or the cytokine/chemokine profile of hepatic GVHD. Therefore, in the present report we describe a case of hepatic GVHD that developed after allogenic peripheral blood stem cell transplantation (PBSCT) for Philadelphia chromosome-positive acute lymphoblastic leukemia (ALL). In addition, we examined the cytokine/chemokine profile to elucidate the etiopathogenesis of hepatic GVHD.

Case report

A 4-year-old Japanese girl was found to have Philadelphia chromosome-positive ALL. The initial blood count showed hyperleukocytosis (881.7 × 10³/μl; blastic cells, 96%), severe anemia (5.9 g/dl), and thrombocytopenia (2.0 × 10⁴/μl). Chromosomal abnormality was found: 46,XX, der (9) del (9) (p13) inv (9) (p11q13), t (9; 22) (q34; q11) [20/20], and chimera screening with the reverse-transcriptase poly-

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merase chain reaction revealed $6.97 \times 10^6$ copies/µg RNA of minor bcr/abl transcripts without mutation. She achieved complete remission after induction chemotherapy. After treatment with intensive chemotherapy including imatinib, followed Hyper-CVAD chemotherapy, the patient underwent allogenic PBSCT from her human leukocyte antigen (HLA)-6/6 matched mother as donor. The conditioning regimen consisted of total-body irradiation (12 Gy in 6 fractions) and cyclophosphamide (60 mg/kg × 2 days) and etoposide (60 mg/kg × 1 day). On day 0, $3.0 \times 10^9$/kg nucleated peripheral blood mononuclear cells (3.0 × 10⁷/kg CD34+ cells) were infused. Intravenous tacrolimus and short-term methotrexate were employed for the prophylaxis of GVHD. Neutrophil engraftment was achieved on day 11. Grade ᶗ cutaneous acute GVHD was observed on day 14. The serum levels of hepatic enzymes began to increase on day 49 (Figure 1). Results of laboratory studies on day 69 were as follows: aspartate aminotransferase (AST), 245 IU/l (normal range, 10~33 IU/l); alanine aminotransferase (ALT), 305 IU/l (normal range, 6~35 IU/l); γ-glutamyltranspeptidase (γ-GTP), 364 mg/dl (normal range, 9~27 mg/dl); and total bilirubin, 1.0 mg/dl (normal range, 0.2~1.3 mg/dl). Serologic tests for hepatitis A, B, and C viruses were negative, as were those for herpes simplex, varicella zoster, cytomegalovirus, and Epstein-Barr virus. All autoimmune markers available for testing were negative. Because we suspected drug-induced liver dysfunction, we began to taper the dose of orally administered tacrolimus. However, laboratory data on day 79 revealed progressive hepatic dysfunction with cholestasis: AST, 233 IU/l; ALT, 274 IU/l; alkaline phosphatase (ALP), 1513 IU/l (normal range, 96~300 IU/l); γ-GTP, 405 mg/dl; and total bilirubin, 2.7 mg/dl (direct, 2.3 mg/dl; indirect, 0.4 mg/dl). Maximum level of total bilirubin was 4.1 mg/dl (direct, 3.0 mg/dl; indirect, 1.1 mg/dl) on day 84, suggesting Grade Ⅲ acute GVHD. Liver biopsy was performed on day 82. Based on the results of pathological and cytokine/chemokine profile described below, treatment with intravenous prednisolone was started at a dose of 1 mg/kg/day, and the dose of tacrolimus was increased. Liver function gradually improved. Prednisolone was tapered and was finally discontinued on day 155.

Pathological and cytokine/chemokine analyses

Histopathological study of the biopsy liver tissue showed marked infiltration of CD8⁺ lymphocytes in portal areas (Figure 2A and B). Hepatocyte ballooning degeneration was observed (Figure 2A). Immunostaining for cytokeratin 7
Yamaoka et al. suggested the preservation of intralobular bile ducts without inflammatory cell infiltration (Figure 2C). Spotty infiltration of CD20⁺ B-lymphocytes and CD138⁺ plasma cells was observed in Glisson’s capsule and within lobules (Figure 2D and E). Silver staining demonstrated the preservation of limiting plates. These pathological findings indicated lobular hepatitis-type liver GVHD.

We next investigated the cytokine/chemokine profiles of the serum and liver biopsy specimens collected in the same day with the Bio-Plex suspension array system and the 17 Plex Panel (Bio-Rad Laboratories, Tokyo, Japan). The frozen liver sample was homogenized to prepare for the analysis. We evaluated the cytokines/chemokines data of serum and liver samples, compared to previous reported data⁸. As shown in Figure 2F, interleukin (IL)-8 in both serum and liver biopsy samples was increased. Moreover, monocyte chemotactic protein-1 (MCP-1), and macrophage inflammatory protein-1 β (MIP-1β) were increased in the liver sample.

**Discussion**

Our patient had elevated serum levels of aminotransferases without cholestasis after allogenic PBSCT, and hepatic dysfunction with cholestasis gradually developed. The viral and
autoimmune markers associated with hepatitis were negative. The liver biopsy specimen showed a lobular hepatitis pattern with marked infiltration of cytotoxic T-lymphocytes, preservation of limiting plates, and cholestasis without destruction or inflammation in the intraepithelial cells of bile ductules.

Several recent reports suggest that hepatic GVHD after hematopoietic stem cell transplantation (HSCT) resembles Autoimmune Hepatitis (AIH). A summary by Narita et al. of 7 cases of AIH-like GVHD following allogenic HSCT showed that most cases had pathological features, such as interface hepatitis, but autoimmune markers were positive in 5 cases and negative in 2 cases. Typical AIH-like GVHD generally presents with such features as late onset and hypergammaglobulinemia with autoimmune markers. Pathological features include interface hepatitis with infiltration of CD20 B-cells and plasma cells, centrilobular necrosis, and emperipolesis in hepatic cells. In the present patient, however, the pathological findings in liver tissue were more consistent with the lobular hepatitis type of hepatic GVHD than with AIH-like hepatitis or classic liver GVHD. Thus, we propose that hepatic GVHD can be divided into 2 types on the basis of onset time and type of inflammatory cells. The early onset type involves cytotoxic T-cells, whereas the late onset type involves B-cells or plasma cells that can produce autoimmune antibodies.

The cytokine/chemokine profiles are a useful, noninvasive tool. Recent study showed that hepatocyte growth factor and IL-6 increased at least 2 weeks before veno-occlusive disease or GVHD in serum. However, secretion of multiple cytokines in the HSCT recipient is significantly lower than in control group during week 2 to 4 after HSCT, but subsequently rebounded after week 4 that was related with white blood cell count, but still remained below control levels. Thus, the cytokine/chemokine profiles of liver biopsy specimens may provide important clues about the etiopathogenesis of HSCT-related hepatic disorders. In our patient, liver biopsy sample showed high expression of MCP-1, and MIP-1β. These chemokines were secreted primarily by monocytes, macrophages, and dendritic cells, are overexpressed by the liver, spleen, skin, and lungs during acute GVHD. Interestingly, IFN-γ, TNF-α, and IL-2 were low concentration in both serum and liver biopsy specimens. These results suggested that activation of monocytes/macrophages may be related with the pathophysiology of hepatic GVHD. However, the profile may be affected by the timing of measurement. To understand the etiopathogenesis of hepatic GVHD, it must be analyzed in sequence.

The standard therapy for hepatic GVHD is corticosteroids. High doses of corticosteroids, tacrolimus, cyclosporine, and azathioprine have been used to treat cases of refractory GVHD. Recently, pulse-cyclophosphamide and rituximab have been reported to be effective for refractory hepatitis GVHD. In our patient, corticosteroids combined with dose-up of tacrolimus were effective. These treatments might affect monocytes/macrophages, as well as not cytotoxic T-cells, because the cytokine/chemokine profiles showed increases of MCP-1 and MIP-1β, which indicated monocytes/macrophages activation. Further study to clarify the sequential cytokine/chemokine profiles and to identify biomarkers for of HSCT-related hepatic disorders, including hepatic GVHD, may lead to novel therapies.

Conflict of interest
The authors declare no competing financial interests.

Authors’ contributions
MY, concept/design, data analysis/interpretation; MA, data analysis/drafting the article, approval of article; HK, data analysis; WO, data collection; KY, data collection; HI, critical revision of article; MI, data analysis, critical revision of article.

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