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Hepatitis Associated with Aplastic Anemia: Do CD8(+) Kupffer Cells Have a Role in the Pathogenesis?

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Introduction

Hepatitis-associated aplastic anemia (HAAA), first described by Lorenz and Quaiser in 1955 [1], is a rare phenomenon which usually affects adolescents and young men [2] and is more common in the East than in the West [3–5]. It represents a minor proportion of all aplastic anemia cases [6] and may occur in 28% of young adults after liver transplantation for non-A, non-B hepatitis [7]. Although a limited number of aplastic anemia cases have been reported in association with hepatitis A, B, and G, parvovirus B19, Epstein-Barr virus (EBV), transfusion transmitted virus (TTV), and echovirus [8–20], the causative agent in most of the cases still remains unknown [21, 23]. Patients typically develop severe aplastic anemia 2 to 3 months after an episode of acute hepatitis. Aplastic anemia is always fatal if untreated [2, 11] and the hepatitis associated with this may be mild and transient [2, 22], fulminant [7, 23], or chronic [2]. There is no known relation to blood transfusion, drugs, or toxins. Though not clearly defined thus far, immunologic mechanisms seem to be responsible for HAAA, particularly because immunosuppressive therapy has shown some promising results. Nonetheless, most successful outcomes have been achieved by HLA-matched bone marrow transplantation (BMT) [21].

We report an adolescent male admitted with acute hepatitis of unknown etiology whose course was subsequently complicated by severe aplastic anemia. As an original observation, many CD8-expressing Kupffer cells were detected in the liver biopsy specimen. Despite continuing immunosuppressive therapy, the patient died after an intracranial hemorrhage. Etiopathogenesis of and treatment options for this intriguing case are also discussed.

Case report

A 16-year-old male student was admitted to an inner-city state hospital with 4 weeks of generalized weakness, malaise, and jaundice. His initial biochemical tests showed profound transaminitis and hyperbilirubinemia, with ALT of 1186 U/L, AST of 948 U/L, and total bilirubin of 8.94 mg/dl. Initial viral markers including HBs, anti-HBc IgM, anti-HBc IgG, anti-HBs, anti-HAV IgM, and anti-HCV were negative. The patient was referred to a central state hospital with an initial diagnosis of non-A, non-B, non-C hepatitis. There he was tested again for hepatitis A, B, C, and E as well as EBV and cytomegalovirus (CMV) serology and only anti-HAV IgG was found to be positive. The tests for Wilson disease, primary biliary cirrhosis, and α-1 antitrypsin deficiency were unremarkable. Meanwhile, his complete blood count started to drop and he developed pancytopenia, upon which he was

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Table 1 Initial laboratory values

	Results		Results
Chemistry		Immunology	
Alanine		IgG (8.0–17.0 g/dl)	20.14 g/L
aminotransferase	1026	IgM (0.5–3.2 g/dl)	1.93 g/L
(U/dl)		IgA (1.0–4.9 g/dl)	1.31 g/L
Aspartate amino	679	ANA	Negative
transferase(U/dl)		AMA	Negative
Alkaline phosphatase	902	Anti-LKM	Negative
(U/dl)		CD4 (% lymphocytes)	6.5
γ-Glutamyl transferase	119	CD8 (% lymphocytes)	74
(U/dl)	8.63	CD4/CD8	0.08
Total bilirubin (mg/dl)	5.76	HLA-DR(+) CD8 (%)	74.6
Direct bilirubin (mg/dl)	3.8	Serology	
Albumin (mg/dl)	2.4	Anti-HAV IgM	Negative
Globulin (mg/dl)		HBs/anti-HBs	Negative/negative
Hematology		Anti-HBcIgM/anti-	
White blood cells	1,350	HBcIgG	Negative/negative
Absolute neutrophil count		Anti-HCV/HCV RNA	Negative/negative
(cells/mm^3)	681	Anti-HEV	Negative
Absolute lymphocyte		HGV RNA	Negative
count(cells/mm^3)	657	EBV IgM	Negative
Hematocrit (%)	26.9	CMV IgM	Negative
Hemoglobin (mg/dl)	9.4	HSV-1 IgM	Negative
Platelets (cells/mm^3)	26,000	Parvovirus B19 DNA	Negative
Coagulation		TTV DNA	Negative
International			
normalization ratio	1.22		

Note. ANA, antinuclear antibody; AMA, antimitochondrial antibody; anti-LKM, anti-liver/kidney microsomal antibody; EBV, Epstein-Barr virus; CMV, cytomegalovirus; HSV-1, herpes simplex virus-1; TTV, transfusion transmitted virus.

referred to our hospital's gastroenterology clinic for further evaluation.

On admission to our unit, the patient did not give any history of blood transfusion, drug intake, or toxic substance exposure. On physical examination, vital signs were normal, however, jaundice and conjunctival pallor were noted. There was no sign of hepatic decompensation. His initial laboratory values (Table 1) revealed the following: ALT, 1026 U/L (normal: 0–41 U/L); AST, 679 U/L (normal: 0–38 U/L); ALP, 902 U/L (normal: 0–270 U/L); GGT, 119 U/L (normal: 0–61 U/L); total bilirubin, 8.63 mg/dl (normal: 0.1–1.2 mg/dl); direct, 5.76 mg/dl (normal: 0.0–0.8 mg/dl); Hb, 9.4 mg/dl (normal: 13–17 g/dl); Hct, 26.9% (normal: 40–52%); total WBC count, 1350/mm^3 (normal: 3800–10,600/mm^3); neutrophil count, 648/mm^3; and platelet count, 26,000/mm^3 (normal: 150,000–440,000).

Serologic tests for hepatitis B and C as well as HBV DNA PCR and HCV RNA PCR were negative. EBV, CMV, and HSV-1 serology ruled out acute infection. Serum copper and seruloplasmin values were in normal limits. Autoimmune markers including antinuclear antibody, antimitochondrial antibody, anti-smooth muscle antibody, and Coombs tests, serum haptoglobin, and acid-sucrose lysis test were unremarkable.

Serum IgG level was increased, but serum IgM and IgA were within normal limits. Bone marrow biopsy showed a severely hypoplastic marrow (Fig. 1). Liver biopsy revealed chronic active hepatitis (Fig. 2). HBs immunohistochemistry was negative. Based on these findings, the diagnosis of HAAA was made and other rarely seen viruses that could be responsible for this disease entity were investigated. However, HGV RNA PCR, parvovirus B19 DNA PCR, and TTV DNA PCR were all negative.

On day 22 of the patient's admission, immunosuppressive therapy with antithymocyte globulin (ATG; Lymphoglobulin; Aventis Pasteur) at 20 mg/kg per day and cyclosporine A (Cy A) at 5 mg/kg per day was initiated for aplastic anemia since blood counts continued to decline. He was also pretreated with 40 mg of methylprednisolone before ATG infusions. He was further supported with G-CSF, 30 MU per day, and platelet and washed red blood cell infusions as needed. His liver function recovered promptly, with ALT and AST levels normalizing on day 35, 13 days after the initiation of therapy with ATG and Cy A (Fig. 3). However, the patient's complete blood count still continued to decline, and by day

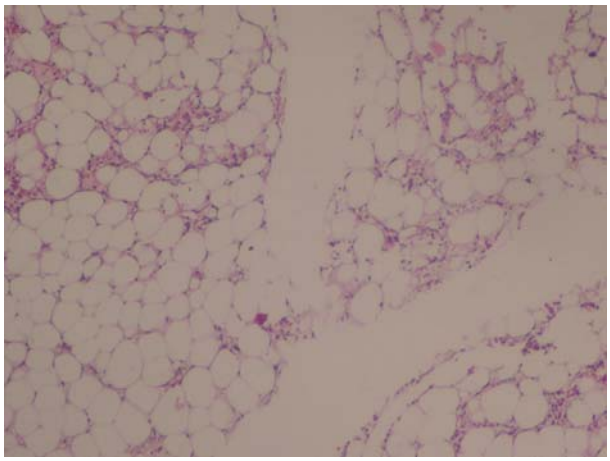

Fig. 1 Bone marrow biopsy showing severe marrow hypoplasia

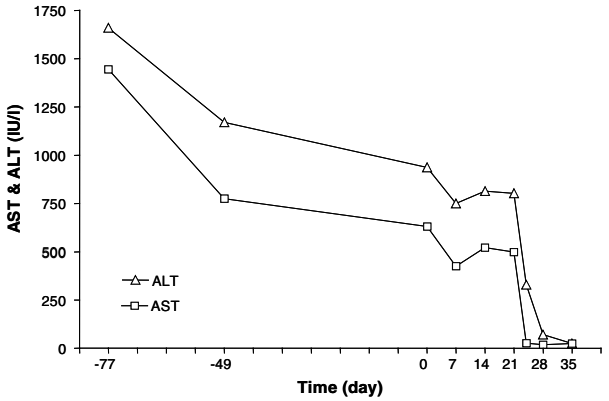

Fig. 3 The course of liver enzymes before and after admission to our hospital (Day 0). Slowly declining liver enzymes were promptly reduced to normal after starting ATG + Cy A (Day 22)

32 his WBC count was down to 400/mm^3; Hb, 4.9 mg/dl; and platelet count, 3000/mm^3. At this point, he developed febrile neutropenia, bilateral retinal hemorrhage, and bleeding gums. He was treated with intravenous broad-spectrum antibiotics, blood products, and G-CSF as well. The patient clinically recovered within 18 days, although the complete blood count was still at very low levels (Fig. 4). Cy A was continued without any hematologic response and therefore a search for a BMT donor was carried out and one of the patient's siblings was found to have 100% HLA-matched bone marrow. He was listed for BMT with priority. Unfortunatelly, on day 104, 82 days after starting therapy with ATG and Cy A, he died suddenly of acute massive intracerebral hemorrhage as evidenced by physical examination and cranial CT.

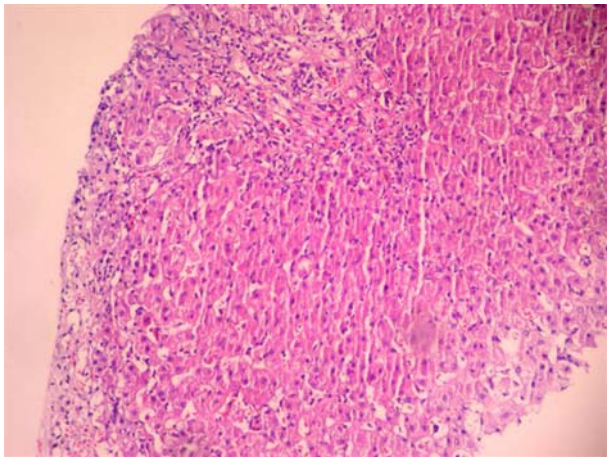

Fig. 2 Liver biopsy demonstrating features of chronic active hepatitis, with lymphocyte infiltration and mild fibrosis in portal tracts with mild interface hepatitis. There is mild parencymal cholestasis and a few spotty necroses, in addition to sinusoidal dilatation and focal hyperplasia of Kupffer cells. (H&E.)

Discussion

HAAA is typically a severe aplastic anemia which usually ensues within 2 to 3 months after the onset of acute hepatitis. In our case this interval was almost 6 weeks. Except for the rare cases that might be associated with hepatitis A, B, and G, parvovirus B19, and EBV, hepatitis in the majority is thought to be caused by a still unknown virus. Hashimato *et al*. [12] showed some structures resembling the nuclear capsids of paramyxovirus and some particles by electron microscope in liver specimens at autopsy. Brown *et al*. suggested that seronegative acute viral hepatitis and HAAA might share a single infectious cause that could be a member of Flaviviridae [24]. We performed various serologic tests to investigate the possible etiologic agent responsible for hepatitis, however, we could not detect any known virus or any other disease entity that might be relevant to such a clinical condition.

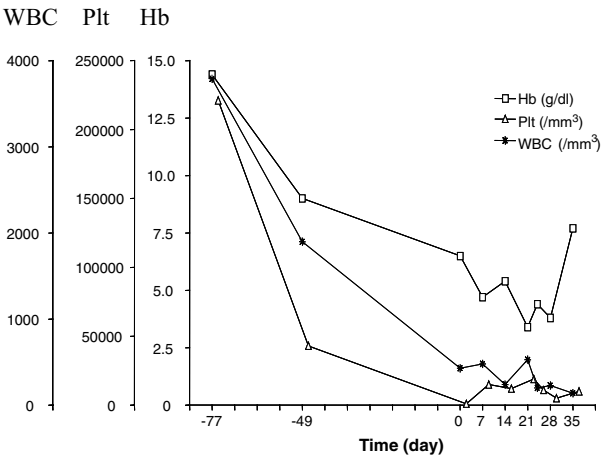

Fig. 4 Blood count showing no response to immunosuppressive therapy

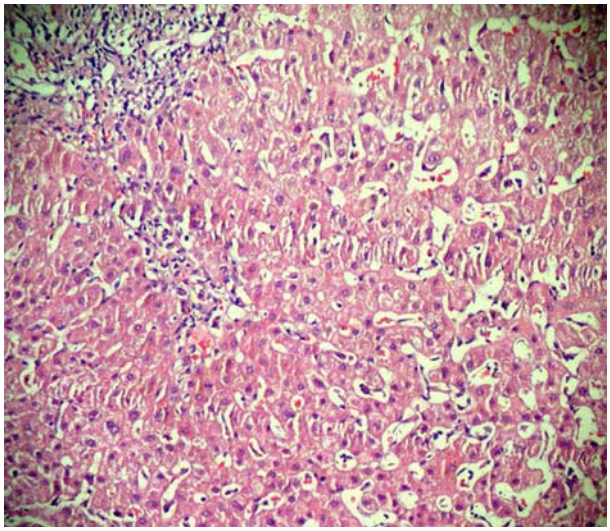

Fig. 5 Increased Kupffer cells, more frequent in periportal areas. (H&E.)

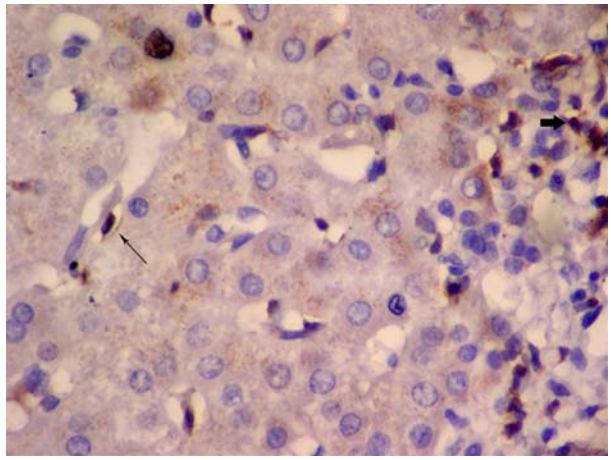

Fig. 6 A section showing a lymphocyte (thick arrow) and a Kupffer cell (thin arrow), both of which are positively immunostained for CD8

Chronic active hepatitis was observed on liver biopsy, though it was performed at the time of marked transaminitis. Interestingly, many Kupffer cells were observed, crowding particularly in periportal areas (Fig. 5). We also immunostained the sections to see if there is any role of CD8(+) cytotoxic lymphocytes in the pathogenesis of hepatitis in HAAA as shown previously for aplastic anemia. Scant intralobular CD8(+) lymphocytes were noted (Fig. 6). Surprisingly, however, a lot of Kupffer cells were demonstrated expressing CD8. We hypothesize that this may be related to inefficient clearing and therefore, possibly, persistence of a virus and consequent chronic hepatitis in this patient as shown very recently by Tang *et al*. in chronic hepatitis B [25]. They revealed that the number of intralobular CD8(+) T cells was significantly decreased and that of Fas-L(+) Kupffer cells was upregulated in biopsies of patients with high ALT, and conversely low viral replication was associated with increased intralobular CD8(+) T cells. Interestingly, CD8(+) macrophages, a population of monocytes/macrophages with a cytotoxic phenotype, were also reported recently in rat experimental models of human autoimmune diseases such as multiple sclerosis infiltrating at inflammatory sites [26, 27]. The authors suggested a specific role of CD8(+) macrophages in the pathogenesis of inflammatory tissue damage. Likewise, they may have a specific, yet unidentified role in the immunopathogenesis of hepatitis in HAAA.

Several immunologic abnormalities and a good response to immunosuppressive therapy have been described in some previous studies [24, 28, 29], leading to a consideration that the underlying pathologic mechanism in HAAA is immune-mediated. Brown *et al*. [24] reported that these patients had a decreased CD4/CD8 ratio in the peripheral blood, in association with increased activated cytotoxic T cells. Our findings support those of Brown *et al*. in that the patient had a very low CD4/CD8 ratio (0.04; normal range, 0.8–2.8) and a high percentage of HLA-DR-positive CD8 cells (74.6%). Kagan *et al*. [30] demonstrated in vitro that in the case of aplastic anemia these activated CD8-positive lymphocytes could be cytotoxic to myelopoietic cells in the bone marrow itself. In addition, several other studies showed that activated cytotoxic lymphocytes settle and produce increased amounts of interferon-γ in the bone marrow in patients with aplastic anemia [31, 32]. The same events may also hold true for the liver inflammation associated with HAAA as in the case of hepatitis B and C [33, 34]. In a recent report, Bowen and colleagues concluded that intrahepatic accumulation of activated CD8-positive T cells with hepatitis could result from primary activation of these cells by the liver resident bone marrow-derived cells, inducing bystander damage to non-antigen-bearing hepatocytes in the transgenic mouse model [35]. They also showed that this bystander hepatitis was dependent on tumor necrosis factor α and interferon-γ, suggesting that this mechanism may play a role in some forms of biologically significant hepatitis. Thus, whatever the offending agent, it may lead to chronic changes in the liver despite normalization of liver enzymes by immunosuppressive therapy as was the case in our patient.

The poor prognosis of HAAA justifies a search for an HLA-identical sibling donor to be able to proceed with a prompt BMT. Long-term survival after transplantation from an HLA-matched sibling donor is 60% to 90% [36–38] and the success rate is increased with an immunosuppressive conditioning regimen. Moreover, the survival rate after BMT with stem cells from an HLA-matched sibling is similar to that for patients with non-hepatitis-associated aplastic anemia [21]. Our experience supports the previous reports favoring early BMT [38–40], although the analysis of a 10-patient

series by Brown *et al*. [24] and some other case reports [28, 29) showed good response to ATG and Cy A combination therapy in HAAA. Brown *et al*. continued Cy A therapy for at least 6 months, with a complete response in the majority of cases. Early treatment with this combination seems to be more effective.

Overall, aplastic anemia may follow cryptogenic hepatitis, and although we could not determine the etiologic agent, our observations clearly indicate an immune-mediated process leading to both aplastic anemia and hepatitis. Aplastic anemia may have a grave prognosis. Hepatitis may vary from mild to acute or chronic forms and may recover with immunosuppressive therapy. CD8-expressing Kupffer cells may be important mediators of hepatitis associated with aplastic anemia. We suggest an early attempt at BMT if aplastic anemia does not respond to short-term (i.e., 1-month) immunosuppressive therapy.

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