

Case Report

Long-term Remission of Hepatitis-associated Aplastic Anemia Possibly due to Immunosuppressive Therapy after Liver Transplantation

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Hepatitis-associated aplastic anemia (HAAA) is an acquired bone marrow failure syndrome that develops after seronegative fulminant hepatitis. Abnormal cytotoxic T-cell activation with cytokine release is a possible pathophysiology. We present the case of a 16-month-old Japanese male who developed HAAA following living-donor liver transplantation for fulminant hepatitis. His aplastic anemia was successfully treated with immunosuppressive therapy. He had been administered tacrolimus for prophylaxis against hepatic allograft rejection. Ten years after the HAAA onset, the patient's bone marrow was found to be slightly hypoplastic. Tacrolimus may be effective in controlling abnormal immune reactions that can cause recurrent impaired hematopoiesis.

Key words: hepatitis-associated aplastic anemia, impaired hematopoiesis, liver transplantation, immunosuppressive therapy, abnormal immune reaction

Hepatitis-associated aplastic anemia (HAAA) is a rare cause of acquired aplastic anemia (AA), which develops within approx. 7 months of the onset of hepatitis [1]. A study conducted in Japan estimated that HAAA accounted for approx. 12% of pediatric cases of acquired AA [2]. Most of those cases were seronegative for hepatitis viruses including hepatitis A, hepatitis B, hepatitis C, Epstein-Barr virus, and cytomegalovirus.

HAAA is a devastating disease without treatment, but it is curable with immunosuppressive therapy (IST) or hematopoietic stem cell transplantation (HSCT). The pathophysiology of HAAA is not fully understood. Abnormal cytokine release and the expansion of cytotoxic T cells is suspected to trigger simultaneous fulminant hepatitis and bone marrow dysfunction [3]. In

most cases of HAAA, hematopoiesis recovers soon after the initiation of IST: we were able to find only two case reports of HAAA recurring > 10 years after a first episode of HAAA treated with IST. In those 2 cases, an abnormal immune reaction against both hematopoietic stem cells and hepatocytes is suspected to have been sustained, even after successful IST [4, 5].

Here we describe the development of HAAA in a male following a living-donor liver transplantation (LDLT) for idiopathic fulminant hepatitis. The patient's hematopoiesis recovered following IST with glucocorticoid and horse anti-thymoglobulin (ATG). The patient had been well, using tacrolimus prophylactic therapy against hepatic allograft rejection, but a bone marrow examination performed 10 years after the LDLT revealed hypocellular marrow with neither dysplastic

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change nor abnormal karyotype. We suspect that an abnormal immune reaction persisted in this patient, and that the tacrolimus treatment may have been effective in preventing recurrent impaired hematopoiesis as well as hepatic allograft rejection.

Case Report

A 16-month-old Japanese male presented with jaundice, clay-colored stool, and hepatomegaly. He underwent surgery to repair an inguinal hernia at 2 months of age and an umbilical hernia at 5 months. He did not have any other remarkable medical history through the prenatal and neonatal period. He did not have any family history of hepatitis or autoimmune diseases. Blood testing revealed abnormal liver function, elevated bilirubin, decreased platelet count, and coagulopathy, suggesting the development of acute liver failure.

The patient was transferred to our hospital as a potential liver transplant candidate. He presented with lethargy, jaundice, and petechiae. His liver was palpable at the right midclavicular line, 5 cm below the costal margin. His spleen was not palpable. Laboratory tests indicated fulminant hepatitis and acute liver failure, with aspartate transaminase (AST) 4,240 IU/L, alanine transaminase (ALT) 3,170 IU/L, albumin 3.77 g/dL, platelet count 35,000/ μ L, ammonia 85 μ g/dL, prothrombin time (PT) 39.8 sec (20%), and activated partial thromboplastin time (aPTT) 139.4 sec. At this time point, pancytopenia was not seen, with a normal white blood cell count (WBC) 7,800/ μ L and slightly decreased hemoglobin at 11.7 g/dL. The anti-nuclear antibody index was 27.3. Other autoimmune antibodies were not examined. The IgG, IgA and IgM levels were 1,318 mg/dL, 159 mg/dL and 180 mg/dL, respectively.

Serology for hepatitis A, hepatitis B, hepatitis C, herpes simplex virus, Epstein-Barr virus, and cytomegalovirus was negative. Serology for Parvovirus B19 was not examined.

The patient was diagnosed as having fulminant idiopathic hepatic failure. He received 2 courses of exchange transfusion, but his liver function did not fully improve, and his level of consciousness gradually deteriorated. An ABO-compatible LDLT from his mother was performed. After the LDLT, prophylaxis against liver allograft rejection with prednisolone and tacrolimus was initiated. The patient's liver failure improved following the LDLT, but his platelet count remained below 100,000/ μ L. Moreover, severe anemia and leukocytopenia developed.

A bone marrow examination performed 18 days after the LDLT showed severe hypocellular marrow with a decreased nucleated cell count (NCC) and megakaryocytes (Table 1). A karyotype analysis with the G-banding method revealed 46, XY. The diagnosis of HAAA was made.

Frequent transfusion therapy was required, and no HLA-matched donor for allogenic hematopoietic stem cell transplantation could be found. Therefore, IST was performed with horse ATG at a single dose of 8 mg/kg on the first day and 17 mg/kg for the following 4 days with reference to the drug label. Methyl-prednisolone (mPSL) at a dose of 2 mg/kg/day was administered for 7 days along with ATG. Then mPSL was switched to prednisolone, which was tapered over a year and finally discontinued. Granulocyte-colony stimulating factor (G-CSF) at a dose of 144 μ g/m² was initiated 13 days prior to IST and its dose was increased to 400 μ g/m² on the 9th day of administration. The patient's pancytopenia improved gradually. Because neutrophil count

Table 1 Analysis of bone marrow aspirates and peripheral blood over time since liver transplant

	Time since liver transplant			
	18 days	78 days	6 months	10 years
Bone marrow aspirate				
Nucleated cell count (/ μ L)	12,000	120,000	264,000	87,000
Megakaryocyte count (/ μ L)	7	35	N/A	N/A
Erythroblast count (/ μ L)	360	29,000	33,800	14,500
Peripheral blood				
Absolute neutrophil count (/ μ L)	540	1,700	4,900	2,200
Platelet count (/ μ L)	22,000	124,000	121,000	128,000
Hemoglobin (g/dL)	7.5	11.9	14.0	13.5

increased over 10,000/ μ L, G-CSF was discontinued on the 6th day of IST. Transfusion was no longer necessary at 42 days after LDLT. Bone marrow aspirates taken 72 and 186 days after the LDLT showed hematopoietic recovery (Table 1).

The patient continued receiving oral once-daily tacrolimus for prophylaxis against allograft rejection after the LDLT with a trough level at 4-6 ng/mL. The erythropoiesis and leukopoiesis had been stable for 10 years with hemoglobin levels at 12-16 g/dL and WBC counts 4,000-10,000/ μ L. However, the patient had mild thrombocytopenia, with platelet counts 100,000-200,000/ μ L. He had been well without transfusion therapy during that time. The CD4/CD8 ratio remained within the reference range from 6 years post-LDLT onwards (Table 2). The results of liver function tests had also been within the reference range. However, a bone marrow examination performed 10 years after the LDLT revealed slightly hypoplastic marrow, with NCC 87,000/ μ L (Table 1). Bone marrow cells showed no remarkable dysplastic change, and their karyotype was found to be 46 XY, using the G-banding method.

Discussion

Our patient developed severe AA following an LDLT for fulminant idiopathic hepatic failure. Immunosuppressive therapy with horse ATG and methyl-prednisolone successfully improved his bone marrow failure, and his peripheral blood counts were maintained without transfusion therapy for a decade, although mild thrombocytopenia persisted. Unexpectedly, a bone marrow examination performed 10 years after the onset of HAAA revealed a reduction in the patient's NCC count without dysplastic change, which suggests that his impaired bone marrow function was sustained even after the successful IST and maintenance tacrolimus administration.

Table 2 Peripheral blood lymphocyte subpopulations over time since liver transplant

	Time since liver transplant			
	2 months	6 years	8 years	11 years
CD3 + CD4 + (29-65%)	12%	46%	37%	32%
CD3 + CD8 + (13-40%)	40%	34%	31%	31%
CD4 + /CD8 + (0.6-2.9)	0.3	1.4	1.2	1

Reference ranges are shown in brackets.

Immunosuppressive therapy with ATG is known to be effective in improving the hematopoiesis of HAAA patients without HLA-matched donors [1]. A prospective study conducted by the Japan Childhood Aplastic Anemia Study Group enrolled 318 children newly diagnosed with AA. Of the 318 patients, AA was associated with hepatitis in 44 patients (14%) [6]. The response ratio 12 months after IST reached 75%, which was comparable with the response ratio of IST for idiopathic AA (60-80%). Moreover, the recurrence of HAAA has not been reported among patients who responded to IST.

Immunosuppressive therapy is also known to be beneficial for HAAA patients receiving LDLT [7,8]. Following an LDLT, a long-term administration of immunosuppressive agents such as cyclosporine or tacrolimus is often necessary to prevent graft rejection. Maintenance immunosuppressive therapy after LDLT has been reported to recover hematopoiesis in patients with HAAA, suggesting that it is effective in suppressing abnormal immune activation [9].

Two cases of recurrent HAAA that was identified > 10 years after the first episode have been reported [4,5]. One case was a Caucasian male who first developed HAAA at 7 years of age. The etiology of hepatitis was not identified. Immunosuppressive therapy with ATG, cyclosporine A (CsA) and prednisolone was performed, and his hematopoiesis recovered. The patient remained well until 20 years of age, when the second episode of HAAA developed. An investigation of the etiology of hepatitis failed again. A lymphocyte subset panel revealed that his CD4/CD8 ratio had fallen to 0.05. Immunosuppressive therapy was performed with ATG and CsA. His hematopoiesis recovered 4.5 months after the initiation of the IST [4].

The other case was a Japanese male who first developed HAAA at 13 years of age. Pancytopenia developed following idiopathic liver failure, and he was diagnosed as having HAAA. Immunosuppressive therapy with dexamethasone and CsA was performed. His hematopoiesis recovered within 5 months, and the CsA was tapered off over 1 year. Ten years later, fulminant hepatitis developed, but once more, its etiology was not identified. His CD4/CD8 ratio decreased to 0.156. Pancytopenia developed, and he was diagnosed as having recurrent HAAA. Immunosuppressive therapy with ATG, methyl-prednisolone, and CsA was performed. The patient's hematopoiesis recovered in 6 months, and

his CD4/CD8 ratio increased to 0.42 [5].

These two recurrent HAAA cases raise the speculation that latent, abnormal immunologic responses might have persisted more than a decade after the first HAAA episode was successfully treated, and were re-activated by an unknown source and caused the patients' second episodes.

Immune-mediated mechanisms are hypothesized to play a pivotal role in the pathophysiology of HAAA. Flow cytometry investigations of peripheral blood lymphocytes of HAAA patients before they underwent IST revealed elevated percentages of CD8 T cells and HLA-DR-positive CD8 cells, suggesting the activation of cytotoxic T cells [10]. In our patient's case, the lymphocyte subpopulation analysis also showed an elevated percentage of CD8 cells during the active phase of HAAA. However, it declined to within the reference range after the administration of IST (Table 2).

Analyses of the T-cell repertoire in the liver and peripheral blood lymphocytes in HAAA patients revealed highly skewed V β patterns, as seen in viral or autoimmune hepatitis [3]. Such a skewed spectratype pattern of HAAA patients' T cells can be reversed back to a normal pattern by intensive IST. These results suggest that an antigen, possibly from an unknown virus, induces an abnormal autoimmune reaction toward both hepatocytes and hematopoietic stem cells, leading to severe acquired AA following hepatitis.

Our patient presented with hypoplastic bone marrow, even after 10 years of successful immunosuppressive therapy for his HAAA. This indicates that cytotoxic T cells which attacked bone marrow cells during the HAAA were not completely removed by the IST, and the abnormal immune reaction continued against some antigen in the bone marrow cells. The long-term administration of tacrolimus for the prophylaxis of liver allograft rejection in our patient's case may have been effective in preventing the relapse of HAAA.

In conclusion, we have reported a case of HAAA with persistent impaired hematopoiesis 10 years after successful treatment with immunosuppressive therapy.

A long-term administration of tacrolimus for prophylaxis against liver allograft rejection might be beneficial for suppressing the re-activation of cytotoxic T cells, which could cause a second episode of HAAA.

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