Successful Treatment of a 19-Month-Old Boy with Hepatitis Associated Aplastic Anemia by Infusion of Umbilical Cord-Derived Mesenchymal Stromal Cells: A Case Report

Liu Fang¹, Lim Meikuang², Guo Ye¹, Chen Xiaojuan¹, Yang Wenyu¹, Ruan Min¹, Chang Lixian¹, Wang Weiqiang², Han Zhibo¹,², Han Zhongchao¹,², and Zhu Xiaofan¹

Abstract
Here we presented a case of a 19-month-old boy who developed severe aplastic anemia postacute hepatitis. He was treated successfully with the umbilical cord-derived mesenchymal stromal cells (UC-MSCs) infusion and cyclosporine A (CsA). The boy achieved both hematopoietic recovery and normal lymphocyte proportion. So far, his condition still remains stable. To our knowledge, there is a rare previous report on the utility of MSCs infusion for the treatment of hepatitis-associated aplastic anemia (HAAA). Considering the efficacy, safety, and strong operability, particularly for pediatric patient, the infusion of UC-MSCs combined with CsA could be an effective alternative for the treatment of HAAA.

Keywords
hepatitis, aplastic anemia, umbilical cord, mesenchymal stromal cell

Introduction
Hepatitis-associated aplastic anemia (HAAA) is a variant of acquired aplastic anemia (AA) in which marrow failure follows the development of hepatitis. Hepatitis may be acute and severe, even fulminant; it may also be self-limiting or chronic. The cause of hepatitis appears to be an undetermined virus, and the underlying pathological mechanism may be immune-mediated¹. Several cases of HAAA have been successfully treated by allogeneic hematopoietic stem cell transplantation (HSCT) or immunosuppressive treatment²-⁴. However, HSCT is not feasible for all patients. For the last decades, it has been reported that mesenchymal stromal cells (MSCs) could be isolated from various tissues. Among those, umbilical cord (UC) was an ideal source due to their accessibility, painless procedures to donors, and lower risk of viral contamination. Moreover, UC-derived mesenchymal stromal cells (UC-MSCs) are homologous, genetically stable, and safe⁵. Our previous studies demonstrated that UC-MSCs could ameliorate liver damage caused by grade III acute graft versus host disease (GVHD)⁵. With their immunosuppressive potential, UC-MSCs could effectively reduce the risk of graft failure, decrease the incidence of severe GVHD, and improve patient survival in haplo-HSCT⁶,⁷. Some other studies have also revealed that UC-MSCs could disrupt the development of the inflammatory cascade and greatly improve liver survival for acute liver failure. In this article, we present a pediatric patient with HAAA who recovered from severe pancytopenia after the infusion of UC-MSCs twice in combination with cyclosporine A (CsA). We supposed hematopoietic recovery...
occurred as a result of UC-MSCs infusion by improving the bone marrow (BM) microenvironment.

Case Presentation
A 19-month-old boy presented with deep jaundice and dark urine in January 2018, not accompanying fever, vomit, diarrhea, rash, weight loss, or joint pain. There was no history of contact with infectious patients or any exposure to drugs or toxins before. He had severely damaged liver function with alanine aminotransferase level of 2610 U/L, aspartate aminotransferase of 2128 U/L, and total bilirubin of 137.32 μmol/L. The serologic markers of hepatitis A, B, C, D, and E were all negative, and the blood cell counts were normal at that time. He was diagnosed as acute icterohepatitis and then treated with reduced glutathione, polyene phosphatidylcholine, and methylprednisolone (2 mg/kg body weight for 7 days). One month later, his liver function gradually returned to normal range and the boy became better. But 2 weeks later, he suffered from fever, petechial, and nose bleeding. The blood cell counts revealed pancytopenia, with a white blood cell (WBC) level 1.89 × 10^9/L, absolute neutrophils count 0.11 × 10^9/L, lymphocyte proportion 85.7%, red blood cells 2.22 × 10^12/L, hemoglobin 69 g/L, platelet 17 × 10^9/L, and reticulocyte percentage 1.52%. The chest computed tomography scan showed pneumonia with influenza virus infection. The boy soon became blood-infusion dependent and was admitted to our hospital.

Lab results showed a pancytopenia pattern (Fig. 3), while the liver function, renal function, and thyroid function were normal. The levels of B12 and folic acid were within the normal limit. Serological tests for hepatitis A, B, C, D, E, G, Parvovirus B19, cytomegalovirus, Epstein-Barr virus, and human immunodeficiency virus were negative. The BM aspirate showed that the marrow was hypocellular with no signs of fibrosis, dyshematopoiesis, or malignancy (Fig. 1), consistent with the criteria for AA. Bone marrow clot sections revealed hypocellular marrow with decreased granulocytes and erythrocyte counts. No megakaryocytes but lymphocytes were observed (Fig. 2A). Immunohistochemistry showed only a few lymphocytes expressed CD3 (Fig. 2B), a few granulocytes, and mononuclear cells positive for lysozyme and myeloperoxidase (Fig. 2C, 2D). Bone marrow cytogenetic study revealed a normal male karyotype. There was no evidence of paroxysmal nocturnal hemoglobinuria (PNH). Flow cytometry showed no PNH clones were detected in erythrocytes and mature granulocytes. A mitomycin-C-induced chromosomal breakage study was also performed and showed normal results. Therefore, myelodysplastic syndrome, PNH, and Fanconi’s anemia were excluded.

Based on the above-described medical history, laboratory, and other examination results, the patient was diagnosed with HAAA. From the second day of hospitalization, the immunosuppressive treatment with CsA was given orally, once every 8 h, 20 mg each time. To stimulate WBC proliferation, the recombinant human granulocyte-colony stimulating factor (G-CSF) was administered subcutaneously (10 μg/kg weight), once per 2 days, totally 4 times. With written informed consent from his legal guardians and approval by the ethics committee of the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences, the patient received twice infusion of allogeneic UC-MSCs which were prepared by the National Engineering Research Center of Cell Products, Tianjin AmCellGene Engineering Co., Ltd, China at a dose of 1 × 10^6 cells/kg body weight each time, at the second day and the 12th day postadmission, respectively. CsA continued to be taken orally, during the interval of administering UC-MSCs. The UC-MSCs preparation was performed according to our previous publication.

After UC-MSCs treatment, his blood profile showed a continuous up-trend, and no blood transfusion was required. Sixty-five days after the first UC-MSCs treatment, his blood counts were completely normal (Fig. 3). No adverse events and toxicity during and after UC-MSCs infusion were noted. During the 1-year follow-up after UC-MSCs infusion, his condition remained stable with normal blood counts (Fig. 3).

Discussion
HAAA is a rare but well recognized and distinct variant of AA, in which acute hepatitis leads to pancytopenia and marrow failure. The onset of pancytopenia syndrome usually takes 2 weeks to 2 to 3 months after the attack of acute
hepatitis. It is not considered relative to age, sex, and severity of hepatitis, and predominantly it has been found in children, adolescent boys, and young age men³. Hepatitis shows variability in clinical features but generally follows a benign course, showing a partial or complete resolution before the onset of AA. A BM failure can be rapid and severe and is usually fatal if untreated. The mean survival time after developing severe BM aplasia is 2 months, and the fatality rate ranges from 78% to 88%⁸. The pathogenesis of HAAA remains unclear. Many possibilities have been reported, which may include pathogenic viruses, autoimmune responses, liver transplantation⁹, and radiation¹⁰–¹³. Several hepatitis viruses such as hepatitis A, B, C, E, G, and other viruses like parvovirus B19, cytomegalovirus, Epstein-Barr virus, Echovirus 3, GB virus C, transfusion-transmitted virus, SEN virus, and non-A-E hepatitis virus have been implicated as a causative agent of AA⁵. However, most cases of HAAA are seronegative for known viruses. Clinical features and experimental results strongly indicate that the liver function and BM abnormalities in HAAA are immune mediated. Various immunological abnormalities have been responsible for the development of AA following hepatitis, including increased activation of circulating cytotoxic T cells which tend to accumulate in the liver, broad skewing pattern of T cell repertoire in peripheral blood, a large number of T cell infiltration in the liver parenchyma, defective monocyte to macrophage differentiation, decreased circulating level of interleukin-1, and liver infiltration by activated CD8⁺ cells⁵,¹⁴. Recent studies have demonstrated that the expansion of a liver-infiltrating cytotoxic T lymphocyte clone takes part in the development of HAAA, especially CD8⁺ cells might be important mediators of HAAA. Immunosuppressive therapy such as antithymocyte globulin (ATG) and cyclosporine has been reported to be effective, without eliciting any acute side effects⁸,¹⁵.

The first-line treatment of HAAA is allogeneic stem cell transplantation from HLA-matched siblings. However, if there is no sibling donor, immunosuppression ATG combined with CsA is recommended for patients¹¹. Our patient did not have a compatible sibling donor for stem cell transplantation. Since his parents refused ATG treatment due to the high cost and its possible side effects, CsA was given alone, combined with the recombinant human G-CSF.

MSCs represent a type of adult stem cells found in multiple tissues and organs, with potential for self-renewal and multilineage differentiation. MSCs also have the ability to regulate immune function. Our previous studies showed that co-transplantation of HSCs and UC-MSCs could induce

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Fig. 2. Histological features of the patient’s bone marrow clot sections prior to umbilical cord-derived mesenchymal stromal cell injection. (A) Hematoxylin and eosin staining showing hypocellular marrow with reduced counts of granulocytes and erythrocytes. No megakaryocytes but lymphocytes were detected. Immunohistochemical staining demonstrating (B) a very low percentage of lymphocytes positive for CD3; (C) a very low percentage of granulocytes and mononuclear cells positive for lysozyme, and (D) myeloperoxidase (MPO). A, B, C, and D × 600.
hematopoietic recovery in severe AA (SAA) patients\(^6,7\), and injection of UC-MSCs significantly improved the liver damage and pancytopenia caused by grade III acute GVHD\(^5\). Thus, allogeneic transplants of MSCs may be a potential supplementary alternative to HAAA. Moreover, UC-MSCs seemed to be safe in children with SAA who received allogeneic HSCs transplantation\(^16\). Hence, we decided to infuse UC-MSCs into this patient after obtaining the informed consent of his parents. Surprisingly, after the UC-MSCs infusion twice with an interval of 10 days, his blood count profile was ameliorated gradually and became completely normal on day 65. The patient was completely recovered and remained stable during the 1-year follow-up.

In this case, immune-mediated pathogenesis could not be excluded. It has been shown previously that acquired AA is caused by the destruction of HSC and progenitor cells associated with MSC abnormalities. These events lead to an imbalance among CD8\(^+\) and CD4\(^+\) T cells which correlates with apoptosis of HSC and progenitor cells and consequently BM aplasia and pancytopenia. BM failure also promotes MSC aberrant alteration and leads to impairment in maintaining the immune homeostasis. The reduction of CD146\(^+\) MSC and its secretion, which impaired MSC to support hematopoiesis, collaborates to accelerate the progress of the disease\(^17\). In this patient, we suppose BM failure improvements occur as a result of UC-MSCs infusion through restoring the imbalance between CD8\(^+\) and CD4\(^+\) T cells in peripheral blood, which increase CD4\(^+\) T cells and

**Table 1. Flow cytometry Examination Results of Lymphocyte Subsets Before and After UC-MSCs Infusion.**

| Peripheral blood | Result |
|-----------------|--------|
| **Before UC-MSCs infusion (%)** | **11 days after the first UC-MSCs infusion (%)** | **Reference value (%)** |
| Lymphocytes constitute nuclear cells | 91.6 | 61.1 | 20 to 40 |
| CD3\(^+\) CD4\(^+\) T cells constitute lymphocytes | 18.7 | 33.4 | 33 to 58 |
| CD3\(^+\) CD8\(^+\) T cells constitute lymphocytes | 30.7 | 23.2 | 13 to 39 |
| CD3\(^+\) T cells constitute lymphocytes | 53.9 | 61.5 | 56 to 86 |
| CD19\(^+\) B cells constitute lymphocytes | 14.3 | 27.1 | 5 to 22 |
| CD3\(^+\) CD16/CD56\(^+\) NK cells constitute lymphocytes | 22.1 | 4.3 | 5 to 26 |

NK: natural killer; UC-MSCs: umbilical cord-derived mesenchymal stromal cells.
decrease CD8\(^+\) T cell proportion, and other immune modulation mechanisms such as suppression of both natural killer (NK) cells and increase CD19\(^+\) B cells (Table 1). In AA, B lymphocytes were decreased\(^{18}\), but NK cells were increased\(^{19}\). Recent studies demonstrated that MSCs suppress NK cells and support B cell proliferation, and because B cell responses are T cell-dependent, the effects of MSCs might also be influenced by MSC-mediated T cell inhibition\(^{20}\). In summary, according to our knowledge, this is the first case which illustrates that UC-MSCs infusion was effective in the treatment of post-hepatitis AA without notable toxicity and adverse effects. Therefore, UC-MSCs infusion, instead of BM transplantation, could be proposed as an alternative means for the treatment of HAAA, particularly for pediatric patients. However, this is a single case report and further large-scale multicenter clinical studies are warranted.

**Ethical Approval**

Ethical approval to report this case was obtained from ethics committee of the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences (NI2019005-EC-1).

**Statement of Human and Animal Rights**

All procedures in this study were conducted in accordance with the ethics committee of the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences (NI2019005-EC-1) approved protocols.

**Statement of Informed Consent**

Written informed consent was obtained from a legally authorized representative for anonymized patient information to be published in this article.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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