Could Parvovirus B19 Induce a Rejection After Bone Marrow Transplantation in a Patient with Diamond-Blackfan Anemia?

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ABSTRACT

A four year-old-girl with Diamond-Blackfan anemia (DBA) that was resistant to corticosteroid treatment and transfusion dependent underwent (bone marrow transplantation) BMT from HLA identical sibling. The patient was conditioned with busulfan and cyclophosphamide and achieved complete marrow engraftment and mixed chimerism in DNA analysis. For the following 13 months she was not transfusion dependent and had a 100% Karnofsky score. But on the 14th month she had anemia following fever, rash and enteritis. Parvovirus B19 IgM seropositivity confirmed Parvovirus infection. Although intravenous immunoglobulin was administered, bone marrow morphology and DNA analysis revealed rejection. Although mixed chimerism detected shortly after the BMT procedure might raise the possibility of an ongoing slow graft rejection during the relatively stable remission period, we think that parvovirus B19 had also contributed rejection.

Key Words: Parvovirus B19, Diamond-Blackfan anemia, Transplantation, Rejection.

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Although corticosteroids seems the most effective pharmacologic agent in Diamond-Blackfan anemia (DBA), many patients are unresponsive and transfusion dependent. Steroid dependency carries adverse effects and chronic red cell transfusion has the risk of hemosiderosis, transmission of infectious agents and isoimmunisation to both cellular and plasma antigens. Desferrioxamine requires long-term administration and compliance of patient in order to be effective in iron chelation. Besides it has high cost, visual and auditory neurotoxicity and may even cause anaphylactoid reactions. Oral iron chelators are either toxic or have insufficient effectivity. IL-3, GM-CSF, CyA, intravenous immunoglobulin, ATG were used with disappointing results[1,2]. High dose steroid therapy is also reported to be effective in some patients unresponsive to standard doses[3-5].

Fortunately, DBA can be successfully treated with bone marrow transplantation (BMT)[6,7]. We believe that BMT from an identical sibling is a rational approach to cure young patients who are dependent to either red blood cell transfusions or high dose steroid therapy. But viral infections may cause engraftment failure during transplantation[8], severe anemia in bone marrow transplant recipients[9] or as in our case may cause rejection after solid organ transplantation[10]. Parvovirus B19 is highly tropic to human bone marrow and replicates only in erythroid progenitor cells. The reason of this erythroid tropism is the tissue distribution of the B19 cellular receptor globoside (blood group P antigen)[11]. In individuals with underlying hemolytic disorders, infection with parvovirus B19 is the primary cause of the aplastic crisis[1]. Similarly, persistent B19 infection may develop in immunocompromised patients (children with acute lymhoblastic leukemia under chemotherapy) who are unable to produce neutralising antibodies and manifests as pure red cell aplasia and chronic anemia[1]. B19 infection in utero can result fetal death or congenital anemia[1].

CASE REPORT

Patient

A four- year-old girl was referred to BMT Department with diagnosis of DBA. She had pallor, malaise, anorexia and poor weight gain since nine-month-old and had nine transfusions ever since. She also had a ten-month course of methyl prednisolon 2 mg/kg/day without any response. There were no associating congenital anomalies. Her physical examination revealed a pale girl with good features, minimal hepatosplenomegaly (2 cm each below costal margins) and 1/6 systolic heart murmur. Her weight was on the 10^{th} and height on the 25^{th} percentile. Laboratory investigations showed anemia, reticulocytopenia, iron overload and increased erythropoetin level. RBC: $2.28x10^{12}$/l, Hb: 6.4 g/dL, PLT: $661x10^{9}$/L, MCV: 83 fL, reticulocyte: 0.1%, total biluribin: 0.4 mg/dL, serum iron: 165 mg/dL, total iron binding capacity: 168 mg/dL, ferritin: 530 ng/mL, BUN: 20 mg/dL, AST: 30 U/mL, ALT: 254 U/mL, direct Coombs test negative, erytropoetin: 26 mu (1.5-2.1) HbF: 4%, serum folic acid: 11 ng/mL, B_{12}: 550 pg/mL. Marrow cellularity was normal with marked hypoplastic erythropoesis. Parvovirus IgM and IgG were negative as other virus serology (Toxoplasma, rubella, cytomegalovirus, herpes, hepatitis A, B, C, EBV). Induced chromosome breakage was also within normal limits.

Conditioning Regimen and GVHD Prophylaxis

She underwent BMT from her HLA identical sibling. ABO blood group of receipient and donor was also identical. Conditioning was Busulfan (Bu) 4 mg/kg/day and cyclophosphamide (CY) 50 mg/kg/day for four days. The number of nucleated marrow cells infused was $5x10^{8}$/kg receipent. GVHD prophilaxis was MTX 15 mg/m^2 on day 1, then 10 mg/m^2 on days +3, +5,+7,+11 and CsA begining on the $+1^{st}$ day.

Supportive Care

The patient was nursed in a single bed isolated room. Oral cotrimaxazole, iv flucanazole, iv acyclovir and iv immunoglobulin (IVIG) support 0.4 g/kg/weekly were administered. G-CSF and GM-CSF were also administered until engraftment.

RESULT

The engraftment was on the 15^{th} day, (WBC: 0.5 x 10^{9}/L) and last platelet and red blood cell transfusion requirements were on 20 and 14 days, respectively. Bone marrow examination on the $+30^{th}$ day showed normocellularity and maturation of all series with RBC: $4.18x10^{12}$/L, Hb:12 g/dL, WBC: $4.8x10^{9}$/L, PLT: 82 $x10^{9}$/L. No serious infection or GVHD was experienced. She had a 100% Karnofsky performance score. Monitoring chimerism with DNA polymorphism on the 2^{nd} and 8^{th} months showed mixed chimerism. Erythropoetin also declined to normal levels on the following months (+5 months 6 mIU/mL, +9 months 1 mU/mL). CyA was discontinued on the 6^{th} month and IVIG support was continued to maintain an IgG level of 600 mg/dL. She had a normal hemoglobin level. But on the 14^{th} month she had anemia (Hb: 7.2 g/dL, WBC: 7300/mm^3, PLT: 343.000/mm^3) following a short episode of fever, rash and enteritis. Bone marrow aspiration showed almost complete absence of erythroid precursors with a simultaneous elevation of serum erythropoetin level (1200 mIU/mL). Serum parvovirus IgM was positive and IgG negative (enzyme linked immunoabsorbant assay). Although a four-day-course of IVIG therapy (0.5 g/kg per day) was administered, DNA analysis of blood cells revealed loss of chimerism. In the following months her anemia persisted with a hemoglobin value about 6 g/dL. On the third

month of rejection, Parvovirus serology revealed loss of IgM antibodies and IgG positivity. She is transfusion dependent and on iron chelation program. She is alive on the 5th year of transplantation but family refused a second transplant procedure.

DISCUSSION

Since DBA is quite rare and over 50% of patients respond to corticosteroids, this was our first experience of BMT in DBA. Although she had iron overload, BMT procedure was without remarkable complications and engraftment was quite early. In multiply transfused patients, rejection of donor marrow was a major clinical problem due to sensitisation of patients to minor histocompatibility antigens expressed on donor cells via blood transfusion. The patient's transfusion load was moderate so we avoid irradiation in a young patient considering long-term side effects. Conditioning with Bu + CY and number of donor cells transfused seemed adequate because there was a 13 month period with normal erythropoesis and mixed chimerism. She had a late rejection after parvovirus infection. But mixed chimerism detected shortly after the BMT procedure might raise the possibility of an ongoing slow graft rejection during the relatively stable remission period. Tchernia et al[12] reported a 3-year-old girl with DBA who achieved spontaneous transfusion independence for one year, after three-years regular transfusions and corticosteroid resistance. This child also had a relapse due to parvovirus B19 primary infection.

Some authors report successful engraftment (complete chimerism) and good outcome with Bu + CY[6,7,13]. Saunders et al.[14] reported the complication of BMT in transfusion dependent children. He suggested that Bu+CY regimen was inadequate in some beta thalasemia major patients. Three of the 6 beta thalasemia major patients suffered rejection. Two of them had mixed chimerism with a gradually decreasing hemoglobin level and remained in a clinical status of thalasemia intermedia. In this series, patients with DBA did not suffer engraftment failure but they had severe GVHD. In BMT for DBA different conditioning regimens were used. Total lymphoid irradiation, even total body irradiation was administered and outcome was good[15,16]. In recent years, new immunosuppressive agents such as ATG or ALG were also used[17]. ATG/ALG is also combined with TBI or TAI (total abdominal irradiation)[18,19]. Some regimen had procarbazine[17,19]. In our Center also ATG or ALG will be added to conditioning regimen of transfusion dependent children. Despite morbidity and mortality of BMT procedure, it is curative in DBA.

There is limited data about aetiological role of parvovirus in DBA[20]. In a study of 11 patients with DBA, 3 patients had parvovirus DNA in marrow smears and these three patients experienced remission with steroid treatment. It has been hypothesised that parvovirus could induce autoimmune mechanisms leading to autoimmune pure red cell aplasia based on the development of anti-DNA and antilymphocyte antibodies during the acute infection[21].

Fever and skin findings are the most frequent symptoms of parvovirus infections in addition to hematologic abnormalities. Articular disease and long lasting fever is also identified[22].

In a study of 201 allogeneic bone marrow receipients for screening Parvo B19 by PCR and serodiagnostic methods up to 36 months post-transplantation, only three cases were diagnosed in the second year[23]. Although patients receiving allogeneic transplants are more susceptible because of greater immunosupression, few cases with symptomatic B19 infection were reported[23,24]. Antiviral antibodies passively transferred via IVIG infusions might prevent viral replications in the majority of patients. Besides it is suggested that some patients are resistant to parvovirus B19 infection due to lack of virus receptor (erythrocyte P antigen)[25].

Many reports document the transmission of parvovirus by blood components in post-transplant patients but our patient had no transfusion in the last 12 months[26]. Inhalation of respiratory droplets from an asymptomatic people must be the route of infection in this patient.

Ang et al[27] reported that there was no evidence of transfer of donor parvovirus B19 IgG to receipient after allo BMT. Post BMT antibody persistence dependens on prior receipient immunity, probably due to existence of long-lived receipient plasma cells.

Mouse parvovirus potentiates rejection of tumor allografts by immunomodulatory effects on T-cell mediated immune responces in vivo and in vitro. Immunofluorescence staining and in situ hybridization studies of tumors suggested that direct infection of tumor cells

was not responsible for accelerated rejection[28].

Some reports showed successful therapy of Parvovirus infections with IVIG after solid organ transplants. A 2-day course of 1g/kg or 5 day courses were administered[29,30]. Optimal dose is unclear. We could start IVIG therapy on the 15th day of infection after she was referred to BMT center by local physician. This delay might contribute to failure of IVIG therapy.

In immunocompromised patients, parvovirus infection must be considered in the differential diagnosis of fever and skin findings. Prompt treatment with IVIG might control viral replication and prevent rejection.

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