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Treatment of pure red-cell aplasia following major ABO-mismatched T-cell-depleted bone marrow transplantation

Two case reports with successful response to plasmapheresis

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Abstract. Major ABO-mismatched bone marrow transplantation (BMT) may be accompanied by red-cell haemolysis, but pure red-cell aplasia following BMT is a rare complication. Two cases of transient pure red-cell aplasia following T-lymphocyte-depleted BMT for a period of > 20 weeks are described, both of which responded to one cycle of plasmapheresis. The prompt response of the two patients described with red-cell aplasia with no evidence of haemolysis suggests that plasmapheresis may be considered in such clinical situations as a first treatment of choice before attempting more complex modes of therapy.

Key words: Red blood cell aplasia, in bone marrow transplantation – ABO mismatch, in bone marrow transplantation – Bone marrow transplantation, ABO mismatch

Bone marrow transplantation (BMT) from HLA-identical siblings is the treatment of choice for a large number of haematological malignancies, aplastic anaemia, inborn errors of metabolism and immunological deficiencies. Major ABO incompatibility between donor and recipient red blood cells (RBC) exists in approximately 10%–15% of HLA-matched pairs and 15%–20% of HLA-mismatched combinations [1]. A majority of studies have demonstrated that such transplants do not have an increased incidence of graft rejection [1, 3, 8]. Major ABO-incompatible transplants do carry the risk of a haemolytic transfusion reaction at the time of marrow infusion due to the presence of incompatible donor RBC contained in the marrow inoculum. In addition, newly developed RBC may be haemolysed as long as the host's anti-AB antibodies persist in leading to haemolysis and there is a slow rise in the donor RBC for the first few weeks following BMT. In order to avoid the acute haemolytic complication, a number of techniques have been developed that attempt to reduce the titre of the host's anti-AB antibodies or to remove the donor RBC prior to BMT.

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We report two cases of transient pure red-cell aplasia following T-cell-depleted BMT for a time period of more than 20 weeks that resolved immediately after one cycle of plasmapheresis.

Case reports and results

The following represents a summary of the case histories of two patients who developed severe red-cell aplasia following major ABO-mismatched T-lymphocyte-depleted allogeneic BMT.

Patient 1

A 17-year-old male (patient UPN 76), with sudden onset of severe mucosal and cutaneous bleeding, was diagnosed as having severe aplastic anaemia. Relevant laboratory findings included blood group 0+ (N+; Fy^b+), haemoglobin 5.6 g/dl, white blood cells (WBC) 0.4×10^9/l and platelets $10^4 \times 10^9$/l. Bone marrow biopsy showed a marked hypocellularity typical of severe aplastic anaemia. His 14-year-old sister was found to be HLA-A, B, DR-identical, MLR non-responding, with blood group B+ (N–; Fy^b–). The patient underwent BMT in March 1985 following our standard conditioning that includes total lymphoid irradiation (TLI) 150 cGy twice daily for 6 consecutive days (total 1800 cGy) followed by cyclophosphamide (CY) 50 mg/kg IV daily for 4 consecutive days. T-cell depletion was carried out using Campath-1M (rat antihuman lymphocyte antibody) and fresh donor serum as the source of complement, as described in earlier reports [15]. On day –1 the patient received oral cyclosporin A (CyA) 25 mg/kg and subsequently 12.5 mg/kg in two divided daily doses for rejection prevention.

The clinical course after BMT is summarized in Fig. 1. Essentially, the patient had early engraftment, documented by rapid normalization of white blood cells and platelet counts (Fig. 1 a) with excellent clinical condition, no evidence of graft-versus-host disease (GVHD) and documented female karyotype in a bone marrow aspirate. Nevertheless, the patient remained anaemic with no reticulocytosis and no evidence of donor RBC phenotype and was RBC transfusion-dependent. The patient was maintained on CyA for 12 weeks and from the fourth week onwards received a therapeutic trial with high-dose steroids. Bone marrow aspiration and biopsy showed normal differentiation of the myeloid series and a normal number of megakaryocytes, but no evidence of red-cell precursors. From week 8 onwards, the patient received oral oxymetholone 150 mg/day without any response.

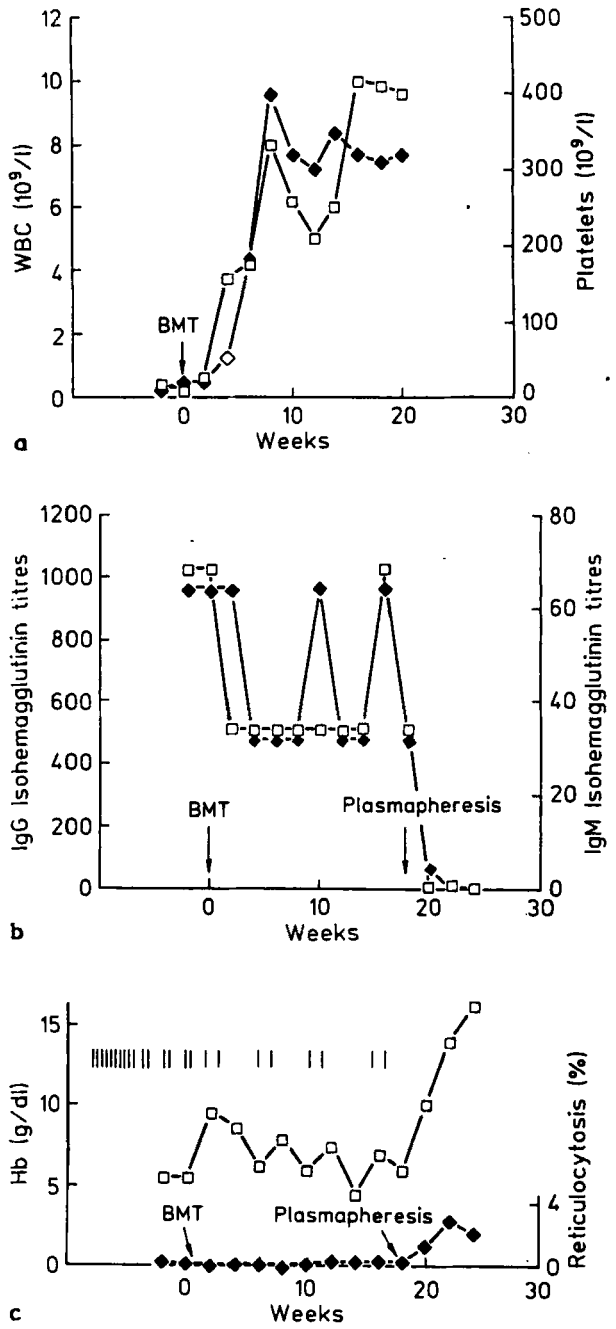

Fig. 1 a–c. Clinical course of patient 1 (UPN 76). **a** Engraftment of WBC (□) and platelets (■). **b** Anti-B + isohaemagglutinins before and after plasmapheresis. □ IgG; ■ IgM. **c** Response of RBC to plasmapheresis. □ Hb; ■ reticulocytosis; II RBC Tx

The patient never showed any laboratory evidence of haemolysis. The isohaemagglutinin titres of both IgG and IgM remained high until 18 weeks post-BMT (Igb 1:1024 and IgM 1:64) (Fig. 1b). At that time, continous flow plasmapheresis was performed and 2500 ml of plasma was exchanged with plasma of blood-group B donors. The response to the plasmapheresis was dramatic with immediate reticulocytosis (Fig. 1c) and subsequently documentation of donor type RBC. The isohaemagglutinin titres dropped and eventually disappeared from the blood (Fig. 1b). Direct and indirect Coomb's tests remained negative. The haemoglobin level normalized within 16 days and the patient became transfusion independent. Donor RBC phenotype was documented from day 168 onwards (B + ; N − ; Fy^b −). The patient is currently more than 5 years post-BMT on no medication, without chronic GVHD or any other complication.

Table 1. In vitro colony formation of bone marrow cells obtained during red-cell aplasia

	Erythroid colonies BFU-E/2 × 10^5 cells	Non-erythroid colonies GM-CFU/2 × 10^5 cells
Normal controls	10–50	50–100
Patient 1 cells + erythropoietin 2 U/ml	26 (B type)	20
Patient 2 cells + erythropoietin 2 U/ml	36 (B type)	24
Patient 1 cells + conditioned media	0	55
Patient 2 cells + conditioned media	0	55

Patient 2

A 33-year-old male (patient UPN 89), developed acute nonlymphoblastic leukaemia (ANLL) diagnosed in April 1985. The patient entered complete remission after two cycles of daunorubicin and ARA-c (7 + 3) and then received two consolidations with the same drugs (5 + 3). His 33-year-old twinsister was found to be HLA-A, B, DR-identical and MLR nonresponding with a blood group B + (E + ; C + ; N + ; S +). The recipient's blood group was 0 + (E − ; C − ; N − ; S −). The conditioning for BMT included low-dose TLI 150 cGy twice daily for 2 days (for prevention of T-cell depleted graft rejection), total-body irradiation 1200 cGy (200 cGy twice daily for 3 days) and CY 60 mg/kg × 2 IV for 2 days.

In July 1985, BMT was performed following depletion of bone marrow T cells with Campath-1M [15]. No post-transplant anti-GVHD prophylaxis was administered. The clinical course after BMT is summarized in Fig. 2. The patient showed rapid engraftment, with normal reconstitution of WBC and platelet counts (Fig. 1 a) and documented normal female karyotype in bone marrow aspirates without any evidence of GVHD. No reticulocytes were observed in the peripheral blood and the patient remained RBC transfusion-dependent. There was no evidence of donor RBC phenotype in the peripheral blood and the bone marrow showed a complete absence of RBC precursors, a classical morphology of pure RBC aplasia. The isohaemagglutinin titres of both remained elevated (IgG 1:128 and IgM 1:8; Fig. 2b).

Continuous flow plasmapheresis was performed at 22 weeks post-BMT. After one 3 l exchange cycle, the patient showed reticulocytosis and gradual normalization of haemoglobin (Fig. 2c). Donor RBC were documented from day 140 onwards in peripheral blood (B + ; C + ; N + ; S +) and the isohaemagglutinin titres disappeared from the serum. The patient did not show any evidence of haemolysis, and direct and indirect Coomb's tests remained negative. The patient is transfusion-independent, with no chronic GVHD, and in excellent health more than $4^1/_2$ years following BMT.

A study of the erythroid and granuloid-committed colonies from the bone marrow of both patients carried out at the time of red-cell aplasia showed normal BFU-E colonies (with erythropoietin) with RBC phenotype compatible with donor blood type (group B) and normal levels of GM-CFU (Table 1).

Discussion

We report here two patients who underwent allogeneic BMT across major ABO blood group incompatibility following depletion of donor RBC and lymphocytes for two

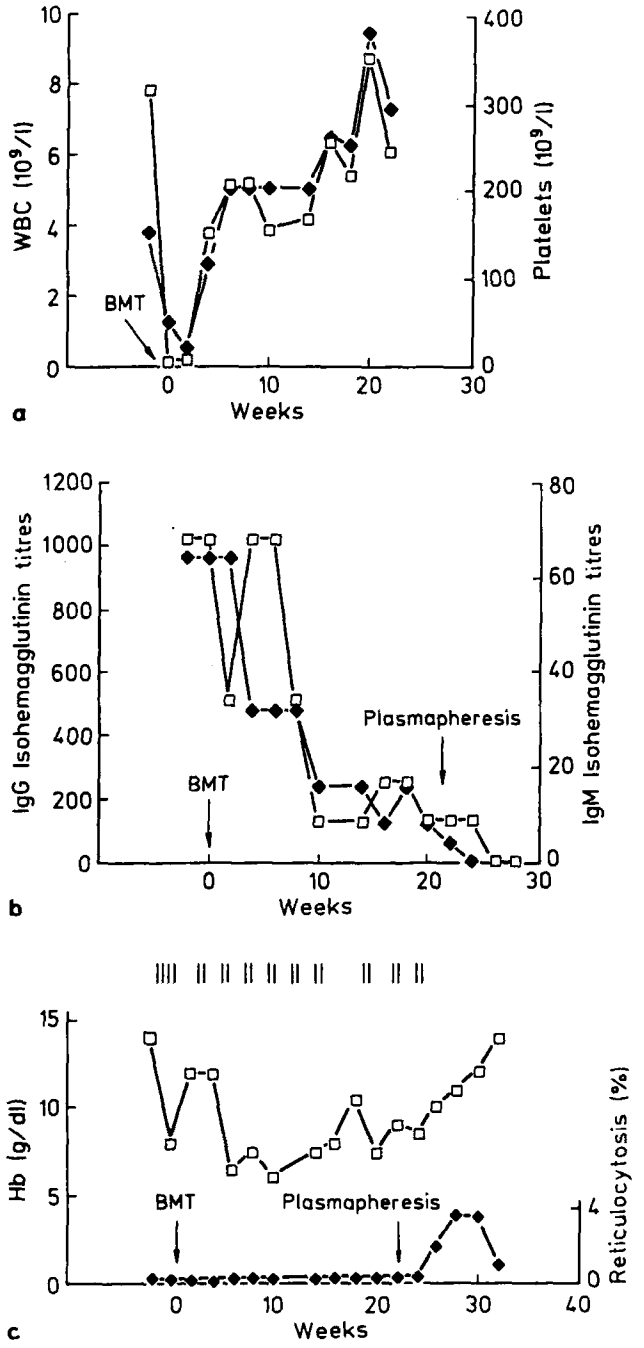

Fig. 2a–c. Clinical course of patient 2 (UPN 89). **a** Engraftment of WBC (□) and platelets (■). **b** Anti-B + isohaemagglutinins before and after plasmapheresis. □ IgG; ■ IgM. **c** Response of RBC to plasmapheresis. □ Hb; ■ reticulocytosis; II RBC Tx

different diseases that developed pure red-cell aplasia with persistent anti-AB titres, both of which responded to one-cycle plasmapheresis. The infusion of the processed marrow was well tolerated because 98% of donor RBC were removed prior to infusion as part of our routine T-cell depletion procedure using hetastarch (Volex) at 1 g sedimentation for 1 h, as previously described [15]. There was no evidence of immediate or late haemolytic reaction in either patient. The bone marrow morphology of both patients post-BMT was typical, showing pure red cell aplasia with normal appearance of the myeloid series and megakaryocytes.

The persistence of anti-B isohaemagglutinins beyond 20 weeks post-BMT without onset of effective erythropoiesis and the BFU-E findings suggest that factors present in the plasma may have been the cause of the delayed onset of erythropoiesis. Indeed, both patients showed an excellent and immediate response.

BMT recipients of major ABO-mismatched transplants do not usually experience a greater incidence of graft rejection, suggesting that early red cell precursors, and probably stem cells as well, do not seem to express appreciable amounts of cell surface ABO antigens [9]. Similarly, GVHD has not been shown to occur with greater frequency, presumably because most nonhaematopoietic tissues do not express ABO antigens. The immediate haemolysis is preventable by removal of donor-incompatible RBC or host anti-RBC antibodies. We use the simplest method for removal of RBC from the marrow by 1 g sedimentation with hydroxyethyl starch. Other equivalent polysaccharide solutions or a ficoll hypaque gradient are equally effective for RBC removal [7, 12].

Others have reported plasma exchange or plasma immunoadsorption [2, 14], as described by Bensinger et al. [4]. Results from Seattle show that RBC depletion can be successfully achieved with semiautomated cell washers or apheresis equipment. Immunoadsorption was found to be less effective than plasma exchange for removal of antibodies, since patients treated with plasma immunoadsorption or whole-blood immunoadsorption had delays in RBC transfusion independence compared with patients conditioned with plasma exchange. The nature of the synthetic antigen and the physical presentation of the immunoadsorbent material may play an important role in determining the efficacy of immunoadsorption, whereas plasmapheresis is technically simpler.

Sniecinski et al. [13] recently reported the immunohaematological consequences of major ABO-mismatched BMT. After receiving major ABO-incompatible marrow, 21% of evaluable patients developed significant haematological complications in the post-BMT period. Red-cell production, as indicated by a reticulocyte count > 0.5%, was delayed to 40 days or more, and in five out of 66 patients reticulocytosis was markedly delayed to 170 days or more. One patient showed no red-cell production after day 605 despite 13 plasma exchanges. Half of the patients showed overt haemolysis manifested by a sudden drop in haemoglobin. Haemolysis and a delay in the onset of erythropoiesis beyond 170 days were more frequent in 30 patients treated with CyA and prednisone as compared with 28 patients treated with methotrexate and prednisone for GVHD prophylaxis.

Reviron et al. [11] have studied the obstacle to RBC engraftment due to major ABO incompatibility, and showed a clear relationship between delayed RBC engraftment and high residual antibody titre at day 20 post-BMT. Removal of plasma antibody seemed essential for overcoming the barrier to ABO-incompatible RBC engraftment. Tichelli et al. [14] evaluated the role of extensive plasma exchange and extracorporeal adsorption for removal of anti-A or anti-B blood group antibodies. Since

1985 these authors have returned to simple in vivo adsorption with incompatible RBC infusion.

Sniecinski et al. [13] suggest the fact that recipients of red-cell-depleted marrow do not undergo procedures to reduce their isohaemagglutinin titres prior to BMT may cause more frequent post-BMT immunohaematological complications. Lasky et al. also stated that red-cell transfusions are required in significantly larger numbers and for longer periods of time in recipients of RBC-depleted marrow than in patients treated by plasma exchange [10]. However, in a larger series of patients, Braine et al. [6] did not find an increased requirement for RBC transfusions in recipients of RBC-depleted marrow. In our experience with 16% major ABO-incompatible patients out of more than 300 who underwent T-cell depleted BMT, only the two cases reported here showed RBC aplasia; no significant complication related to erythropoiesis was observed in ABO-matched recipients.

It appears from the present and previous studies that anti-self isohaemagglutinins may prevent maturation of RBC in the bone marrow. The absence of normoblasts in vivo and early RBC precursors in vitro (BFU-E and CFU-E) suggests that ABO antigens may be expressed on early RBC precursors. Blacklock et al. [5] have shown that a small proportion (approximately 5%) of pluripotent progenitor cells (CFU-GEMM) express A or B antigens. This raises the question of whether exceptionally high ABO incompatibility may represent an occasional cause of engraftment failure. Although, theoretically, plasma cell survival may be prolonged in recipients of T-cell-depleted BMT, no evidence exists for 'graft versus plasma cell' interaction and, in addition, only two out of the many patients undergoing T-lymphocyte-depleted BMT developed a significant complication. In the present cases, RBC aplasia may have resulted from the combination of high titres of anti-RBC antibodies and lack of immunocompetent T cells during engraftment. Reduced titre of anti-RBC antibodies as a result of the plasmapheresis procedure may have tilted the balance towards take-over by donor hematopoiesis, further absorption of residual isohaemagglutinins and eventual total replacement by the donor immunohaematopoietic system.

In conclusion, we would like to suggest that, unless pretransplant removal of anti-ABO antibodies is considered in cases with high titres, early plasmapheresis should certainly be considered post-BMT whenever delayed RBC production is observed in the presence of anti-RBC antibodies.

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