Fatal Immune-Mediated Pancytopenia and a TRALI-Like Syndrome Associated with High Titers of Recipient-Type Antibodies Against Donor-Derived Peripheral Blood Cells After Allogeneic BMT Following Dose Reduced Conditioning

Pancytopenia occurring after bone marrow transplantation is a rare complication. A 47 year old patient with progression of multiple myeloma after standard therapy received an allogeneic marrow graft from a matched unrelatd donor. The non-myeloablative conditioning regimen consisted of fludarabine, cyclophosphamide, rabbit anti-thymocyte globulin and total body irradiation. GVHD prophylaxis consisted of cyclosporine. Neutrophil engraftment was as expected and the patient was discharged without signs of acute GvHD. On day +34 the patient presented with clinical and laboratory findings consistent with severe pancytopenia. Antibodies against red cells, platelets, lymphocytes and granulocytes were detected in extremely high titers. Immune-mediated pancytopenia was refractory on multiple immunosuppressive treatment strategies. Proliferation of polyclonal plasma cells of recipient-type that was documented post-mortem, was most likely responsible for excessive antibody formation.

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Autoimmune hemolytic anemia (AIHA) is detected in up to 5%[1] of patients following allogeneic stem cell transplantation. A higher incidence was reported after transplantation of T cell-depleted grafts.[2] Immune-mediated cytopenias other than hemolytic anemia including pancytopenia are very rare after bone marrow transplantation. Treatment of immune-mediated pancytopenia post-transplant is often extremely difficult with failure of corticosteroid treatment and even of splenectomy or total lymph node irradiation.V There are reports of successful therapy of AIHA in transplant recipients with the anti-CD 20 monoclonal antibody Rituximab.[2]

Case report

A 47 year old patient presented with progression of stage II multiple myeloma on standard treatment. He was scheduled to undergo high-dose chemotherapy with autologous stem cell support 5. Since stem cell mobiliziation was not successful, the patient underwent a dose reduced conditioning followed by an allogeneic bone marrow transplantation (BMT) from an HLA-matched unrelated donor.[6,7] Donor blood group was 0 D-, the recipient was tested 0 D+ and absence of irregular anti-erythrocyte antibodies was established. The preparative regimen consisted of fludarabine 30 mg/m^2/day for 5 days, cyclophosphamide 20 mg/m^2/day for 2 days, rabbit anti-thymocyte globulin (ATG Fresenius, Bad Homburg, Germany) 20 mg/day for 3 days and 2 Gy total body irradiation. 1.89×10^8 MNC/kg body weight were infused after starting GVHD prophylaxis with cyclosporine (3 mg/kg body weight) on day -1. Neutrophil engraftment (neutrophils above 0.5×10^9/L) was documented on day +10. The patient did not require any platelet transfusions until discharge. He did not develop acute GVHD. Mixed chimerism with an autologous fraction of 20-30% was repeatedly documented on days +12, +56 and +70. Analysis of CD19$^+$ and NK cells was not possible.

The patient required re-admission on day +34 when he presented with fever, jaundice and shortness of breath on exertion. Clinical examination revealed no GVHD nor any infectious focus. Abnormal laboratory values were Hb 3.5 g/dL, WBC 2.64×10^9/L, platelet count less than 5×10^9/L, LDH 391 U/L, bilirubin 5.6 mg/dL (conjugated bilirubin 2.0 mg/dL). Plasma hemoglobin was not elevated, the blood smear examination showed a normal differential blood count and no evidence of fragmentation. Antibody screening test was strongly positive in the indirect antiglobulin test and even more in the enzymatic assay. The antibody did not show specificity in the enzymatic assay. The direct antiglobulin test showed strong positivity with monospecific anti-IgG and less with anti-IgM and anti-C3d and negativity with anti-IgA and anti-C3c. The eluate of autologous red cells did react with all red cells in a test kit without detectable specificity. AIHA as a result of warm agglutinins was diagnosed (see Table 1) and prednisone 2 mg per kg body weight per day was administered. Due to the severe symptomatic anemia transfusion of packed red cells and plasma exchange was performed. Hemoglobin did not exceed 7.2 g/dL during the following days in spite of daily plasma exchange and transfusions. Platelet values remained below 5×10^9/L in spite of platelet substitution. Subsequently WBC fell to 0.11×10^9/L.

Table 1. Assessment of Autoimmunhemolytic Anemia.

Test	*Results Patient R.S.*
Hemoglobin	3.5 g/dL
LDH	391 U/IL
Bilirubin	5.6 mg/dL (direct: 2.0 mg/dlL)
Antibody Screening Test	
Direct Antiglobulin Test	Strongly pos.
Indirect Antiglobulin Test	Strongly pos.
Enzymatic Assay	Strongly pos.
Antibody differentiation	
Direct Antiglobulin Test	Strongly positiv with monospecific Anti-IgG; less with Anti-IgM and Anti-C3d; negativ with Anti-IgA and Anti-C3c.
Indirect Antiglobulin Test	Agglutination with all red cells of a panel; reinforced with E-pos. red cells
Enzymatic Assay	Agglutination with all red cells of a panel; no detectable specificity
Eluate of autologous red cells	Reactivitiy with all red cells of a panel
Blood group	Could not be determined

A bone marrow biopsy showed residual infiltration by plasma cells but no signs of dysplasia and increased erythropoiesis and megakarypoiesis could be demonstrated. Additionally, presence of antibodies against platelets and both lymphocytes and granulocytes could be documented, being of HLA (anti lymphocyte-antibodies) and HNA-2a (anti granulocyte-antibodies) specificity, respectively.

Immunosuppressive treatment was further intensified. But unfortunately neither switching from plasmapheresis to immunoadsorption nor irradiation of the spleen significantly improved the severity of immune-mediated pancytopenia. The patient developed dyspnea with the chest computed tomography showing massive bilateral pulmonary infiltration. Mechanical ventilation had to be instituted.

Since pancytopenia persisted and antibody levels against all blood cell lineages remained high, rituximab, a monoclonal chimeric antibody with its anti-CD20 specificity directed against proliferating B-cells, was infused. Rituximab was given at a dose of 375 mg/m^2. To further reduce recipient-type antibody production 8.5×10^8/kg donor lymphocytes were infused (DLI).

The patient died 2 days after DLI of worsening pulmonary infiltration and hemorrhage due to persistent

pancytopenia. Autopsy that was performed with consent from the patients's family revealed diffuse alveolar damage, sclerosing alveolitis with interstitial fibrosis and intracerebral hemorrhage as causes of death. Molecular biological investigation of lymph nodes revealed the presence of polyclonal i.e. non-malignant plasma cells by PCR using primers for the hypervariable region of the immunoglobulin heavy chain gene indicating their origin from the recipient. In contrast to this finding, bone marrow examintion showed a monoclonal lane reflecting infiltration by malignant plasma cells.

Discussion

Here, we report on a case of fatal immune-mediated hemolytic anemia, neutropenia, lymphocytopenia and thrombocytopenia shortly after a BMT from an HLA-matched unrelated donor following reduced intensity conditioning. Our findings demonstrate the severe pancytopenia in our patient to be due to immune destruction of blood cells of all lineages. The patient's bone marrow cellularity was normal with even increased erythropoiesis and megakaryopoiesis early after transplantation. In addition, antibody formation against red cells, platelets and leukocytes with titers especially for anti-neutrophilic and anti-lymphocyte antibodies being extremely elevated (1:1024) was demonstrated. The rapidly progressing respiratory insufficiency with pulmonary infiltration was most likely caused by granulocyte antibody-mediated lung injury (transfusion related acute lung injury, TRALI). This syndrome is caused by recipient-type antibodies that lead to aggregation and sequestration of donor granulocytes with subsequent bilateral pulmonary edema.[8] Antibodies against all cell lineages could be found in this patient subsequent to transplantation whilst their formation prior to transplantation had not been detected retrospectively. The anti-lymphocyte antibodies were directed against HLA molecules and anti-neutrophilic antibodies against HNA-2a (NB1, CD177).[9] Both were of recipient type. Onset of AIHA of warm type is typically 2 - 25 months after BMT 10 and is difficult to treat. Treatment modalities reported in literature comprise corticosteroids, intravenous immunoglobulins, plasma exchange, splenectomy and total lymphoid irradiation [1-3,10] In our patient immune-mediated pancytopenia was found to be refractory to all these therapeutic strategies. Persistence of host cells (or host antibodies) early after transplantation were shown to induce immune thrombocytopenia and mild hemolysis following BMT.[11] Imbalanced donor B cell and T cell function after transplantation might contribute to the formation of anti-blood cell antibodies.[2] The higher incidence of AIHA alone in T cell depleted grafts and in patients treated with cyclosporine[3, 10, 11] may reflect the importance of re-establishing T cell function after transplantation. Whether incidence of AIHA is increased after non-myeloablative BMT remains unclear.[12] In correspondence to the very high titers of antibodies reactive with donor-derived peripheral blood cells, a massive proliferation of non-malignant plasma cells of recipient type could be demonstrated in lymph nodes on autopsy. Taken together, we describe the first case of immune-mediated pancytopenia following non-myeloablative BMT caused by a documented proliferation of non-malignant plasma cells of recipient type associated with extensive antibody production. Recipient origin of antibodies seems to be most likely on the background of proliferation of recipient-type plasma cells. In addition, excessive titers of anti-neutrophilic and anti-lymphocyte antibodies may neither reflect presence of an autoantibody nor an antibody induced by transfusion. Thus, in patients with immune-mediated cytopenia following allogeneic stem cell transplantation, chimerism of B-cells and plasma cells should be screened for and intensive treatment strategies introduced to induce full B cell chimerism. In addition, the use of anti-proliferative agents like methotrexate or mycophenolate mofetil for GVHD prophylaxis might help to reduce recipient type B-cell proliferation and thus prevent immune-mediated pancytopenia especially in patients undergoing an allograft following a dose reduced conditioning regimen.

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