Bone Marrow Transplantation (2002) 29, 519–521

www.nature.com/bmt

Case report

Allogeneic bone marrow transplantation for active Epstein–Barr virus-related lymphoproliferative disease and hemophagocytic lymphohistiocytosis in an infant with severe combined immunodeficiency syndrome

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Summary:

A 5-month-old male presented with fever, hepatosplenomegaly, leukocytosis with atypical lymphoblasts, anemia and thrombocytopenia. Severe combined imunodeficiency syndrome (T–, B+, NK+), B lymphoproliferative disease and hemophagocytic lymphohistiocytosis triggered by Epstein–Barr virus (EBV) were diagnosed. As his clinical situation deteriorated rapidly, BMT was performed with unmanipulated marrow stem cells from his EBV-positive HLA-identical sister after conditioning with dexamethasone (1.75 mg/kg/day), cyclophosphamide (114 mg/kg) and etoposide (10 mg/kg), with no immunosuppression given post transplant. Engraftment occurred on day 6 with explosive proliferation of donor CD8$^+$ T cells. The patient died 3 days later from acute respiratory distress syndrome. Autopsy revealed full donor engraftment and no signs of hemophagocytic lymphohistiocytosis or B lymphoproliferative disease. Thus, transplanted T cells can expand very rapidly within days after BMT and clear EBV lymphoproliferative disease and hemophagocytic lymphohistiocytosis.
Bone Marrow Transplantation (2002) **29,** 519–521. DOI: 10.1038/sj/bmt/1703396
Keywords: SCID; EBV; lymphoproliferative disease; hemophagocytic lymphohistiocytosis; BMT

Infants with severe combined immunodeficiency syndrome (SCID) are prone to abnormal responses to infection by Epstein–Barr virus (EBV), resulting in lymphoproliferative disease (LPD) due to absent EBV-specific cytotoxic T lymphocyte (CTL) activity. LPD can be treated by decreasing uncontrolled B lymphocyte proliferation with chemotherapy,[1–3] by giving anti-CD 20 monoclonal antibody (rituximab)[4] or by transferring EBV-specific CTLs through allogeneic stem cells, preferably from an EBV-positive donor.[5] Antiviral therapy alone is usually insufficient.[2]

In addition, EBV or other infections can lead to an excessive activation of the monocyte–macrophage system resulting in infection-associated hemophagocytic lymphohistiocytosis (HLH). Cardinal symptoms are fever, hepatosplenomegaly and pancytopenia.[6] Without treatment, mortality from infection-associated HLH is as high as 50%. If virus-associated HLH worsens despite anti-viral therapy, chemotherapy with steroids and etoposide or even BMT is recommended.[6,7]

We report a case of sibling bone marrow transplantation in an infant with SCID (T–, B+, NK+) with fulminant B lymphoproliferative disease and hemophagocytic lymphohistiocytosis due to EBV infection. Conditioning consisted of reduced intensity chemotherapy with dexamethasone, etoposide and cyclophosphamide. LPD and HLH were effectively controlled and rapid engraftment with full donor chimerism was achieved. However, the patient died due to acute respiratory distress syndrome (ARDS) on day 9.

Case report

A 5-month-old infant was admitted because of persistent high fever unresponsive to oral antibiotics for 4 days, tachydyspnea and hepatosplenomegaly. Blood counts revealed leukocytosis (16 000/μl WBCs with 40% neutrophils, 15% lymphocytes, 39% abnormal lymphocytes, 6% monocytes), anemia (8.1 g% Hb) and thrombocytopenia (139 000/μl platelets). Serum levels of liver enzymes (GOT 48 U/l, n: 10–27; GPT 63 U/l, n: 6–23), lactate dehydrogenase (533 U/l, n: 200–400), fibrinogen (502 mg/dl, n: 150–300) and ferritin (625 ng/ml, n: 15–120) were elevated. Active EBV infection was diagnosed by quantitative PCR testing (675 000 genome equivalents/20 000 cells).

His clinical condition deteriorated over the following 6 days with persistent high fever, increasing hepatosplenomegaly, anemia (Hb 5.9 g%), thrombocytopenia (58 000/μl)

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Received 8 August 2001; accepted 20 December 2001

and leukocytosis (32 000/μl WBCs with 31% lymphocytes, 12% monocytes, 6% plasma cells, 22% atypical lymphocytes). In addition, the bilirubin was increased (3.6 mg/dl, n: 0.4–1.3) as were the other liver function tests (GOT 148 U/l, n: 10–27; GPT 34 U/l n: 6–23), ferritin (5866 ng/ml, n: 15–120) and triglycerides (241 mg/dl, n: 30–140). The fibrinogen was now normal. Cerebrospinal fluid analysis and cranial computer tomography scaning were normal. Chest radiographs and computer tomography scaning showed bilateral bronchopneumonia and signs of thymic hypoplasia.

Immunophenotyping of the peripheral blood showed no T cells but activated mature B cells ($CD19^+$, $CD38^+$, HLA-DR^+, $CD20^+$, $CD22^+$, IgM^+, IgD^+) indicating that the morphologically atypical lymphocytes were EBV-infected B cells. Lymphocyte stimulation with mitogens was absent. Severe combined immunodeficiency syndrome (T–, B+, NK+) was diagnosed. Serum IgA and IgG levels were low; however, IgM levels were extremely high (10.88 g/l, n: 0.1–1). The number and function of neutrophils were normal. X-linked lymphoproliferative disease and IL2Rγc-gene defect were excluded by genetic analysis. Thus, the proliferation of mature B cells in the peripheral blood was interpreted as EBV-induced LPD due to a missing T cell defense to EBV infection in SCID.

Peripheral blood smears on day 6 after admission showed proliferation of mature and immature plasma cells (34%), histiocytes (15%) and 7% atypical lymphoid cells. Bone marrow smears revealed extensive proliferation of mature and immature plasma cells, histiocytes with erythrophagocytosis, but normal granulopoiesis and erythropoiesis. Because of the fever, hepatosplenomegaly, bicytopenia, hypertriglyceridemia and lymphohistiocytic bone marrow infiltration with erythrophagocytosis, HLH was diagnosed, most probably triggered by EBV.

Empiric therapy with ganciclovir and later on with acyclovir, immunoglobulins, broad-spectrum antibiotics and liposomal amphotericin was started. Because of increasing tachydyspnea with inadequate oxygenation due to diffuse interstitial pneumonia, the child had to be intubated and ventilated 2 days later, 8 days after admission. C-reactive protein was 21.1 mg/dl (n: $<$1.5). *Pneumocystis carinii* was not isolated from bronchoalveolar lavage and endotracheal secretions. Trimethoprim–sulfamethoxazole was given in therapeutic doses for 5 days until day −2, and then at prophylactic doses twice a week. Despite anti-viral therapy, EBV persisted in blood, nasopharyngeal and endotracheal secretions and cerebrospinal fluid. Thus, generalized EBV infection leading to uncontrolled proliferation of B cells, HLH and ARDS presented as a life-threatening disease. Since the patient had an HLA-identical EBV-positive sibling, a BMT was planned in this desperate situation.

Three days later, chemotherapy was started with dexamethasone (1.75 mg/kg/day until day 4, then reduced to 0.75 mg/kg/day) and cyclophosphamide (7 mg/kg/day on days −6 and −5 to avoid tumor lysis syndrome in B-LPD, then increased to 50 mg/kg/day on days −4 and −2). Low-dose etoposide (10 mg/kg) was given on day −3. On day 0, 17 days after admission, the child received unmanipulated bone marrow from his HLA-identical 4-year-old healthy EBV-positive sister (8×10^8 PNC/kg recipient body weight, 21×10^6 $CD34^+$ cells/kg, 75.25×10^6 $CD3^+$ cells/kg). No graft-versus-host disease prophylaxis was given, but G-CSF was given from day 1 until day 6. WBC counts were: 200/μl on day 0, 300/μl on the morning of day 6 and 3100/μl in the evening, 18 100/μl on day 8, increasing to 45 400/μl on day 9. On day 8, fluorescence *in situ* hybridization (FISH) analysis for X and Y chromosomes revealed 100% donor lymphocytes and granulocytes in the peripheral blood. On the same day, WBC differential analysis showed 3.5% granulocytes (absolute: 630/μl), 1.5% monocytes and 95% lymphocytes (absolute 17 200/μl). Immunophenotyping of the lymphocytes revealed absent B cells, 4% $CD4^+$ cells and 70% activated HLA-DR^+ $CD8^+$ cells (absolute 12 670/μl). To delay the rapid increase in T lymphocytes, additional dexamethasone (0.9 mg/kg) was given. On day 3 after BMT, aspergillus antigen was detected in the endotracheal secretions. The dose of liposomal amphotericin was increased from 3 mg/kg/day to 5 mg/kg/day and since there was no improvement on day 7 this was changed to voriconazole. On the evening of day 8, the ARDS rapidly worsened and no adequate oxygenation was possible. The patient died on the morning of day 9 due to cardiopulmonary failure.

Autopsy confirmed that all three hematopoietic cell lines were of donor origin. Immunohistochemistry showed EBV-LMP1-antigen in some B lymphocytes but no B-LPD in the lymphatic system. There was a marked proliferation of oligoclonal $CD8^+$ T lymphocytes (as detected by T cell receptor rearrangement) of donor origin in the whole lymphatic system, liver and lung interstitium. The cause of death was cardiopulmonary failure secondary to ARDS with aspergillosis present in the right lower lobe, fibrinopurulent bronchopneumonia in the left lower lobe and a diffuse interstitial infiltration with $CD8^+$ donor lymphocytes.

Discussion

In this infant, suffering from an as yet undiagnosed severe combined immunodeficiency disorder, B lymphoproliferative disease and hemophagocytic lymphohistiocytosis were triggered by fulminant EBV infection. The SCID was subclassified as T–, B+, NK+. The underlying molecular defect, however, was not identified. X-linked lymphoproliferative disease which worsens following exposure to EBV and IL2Rγc-gene defect was excluded.

For SCID patients, allogeneic BMT is the treatment of choice. In order to transfer sufficient T cell function in the T– immunodeficiencies, allogeneic stem cells from an HLA-identical sibling donor can be given without conditioning.[8] However, in this case, progressive LPD and HLH with a rapidly deteriorating clinical situation forced us to use more intensive therapy. For LPD alone, we could have used rituximab, a monoclonal antibody recognizing the B cell surface molecule CD20.[4,9] However, with the rapid B cell proliferation, we considered antibody treatment to be insufficiently cytotoxic and immunosuppressive and not to be curative for the SCID.

In EBV-related HLH, immunochemotherapy with steroids and etoposide induces remission in most children, but

this tends to be of short duration.[6,7] Thus, allogeneic BMT has been suggested as the treatment of choice for EBV-HLH, using a preparative regimen including myeloablative, antihistiocytic and immunosuppressive agents, such as etoposide, busulfan and cyclophosphamide.[10] Therefore, by combining low-dose etoposide (10 mg/kg), dexamethasone (1.75 mg/kg/day) and cyclophosphamide (114 mg/kg) as conditioning agents, and T cells from unmanipulated marrow stem cells from the EBV-positive HLA-identical sister, we achieved bone marrow aplasia of short duration (6 days) and rapid donor engraftment. Both LPD and HLH were effectively controlled by this regimen.

Unfortunately, the infant died on day 9 due to a deterioration in the pre-existing ARDS together with a massive increase in oligoclonal $CD8^+$ donor lymphocytes. At autopsy, aspergillus pneumonia was detected in the right lower lobe and bronchopneumonia in the left lower lobe. In addition, there was a diffuse interstitial infiltrate of cytotoxic donor lymphocytes. These lymphocytes represented an overwhelming response of EBV-specific cytotoxic donor T cells in the face of the EBV load. Acute trapping of these lymphocytes in the lungs contributed to the diffusion capacity blockage with the result that the patient could not be adequately oxygenated by mechanical ventilation and ultimately died. ARDS has also been described in patients receiving donor T cells to control EBV-associated LPD.[11] It may be speculated whether earlier administration of dexamethasone, a higher dose or the use of low-dose cyclosporine could have delayed the overwhelming CD8 response. In addition, rituximab may have helped to reduce the EBV load before transplantation in order to reduce the risk of overwhelming T cell expansion post BMT.

In conclusion, in a patient with SCID and rapidly progressing LPD and HLH triggered by an overwhelming EBV infection, we recommend reduced dose chemotherapy and stem cell transplantation from an EBV-positive donor. With this regimen, we achieved rapid and effective eradication of LPD and HLH. However, an explosive expansion of $CD8^+$ lymphocytes and the resulting interstitial infiltration may exacerbate pulmonal insufficiency.

References

1 Migliorati R, Castaldo A, Russo S *et al*. Treatment of EBV-induced lymphoproliferative disorder with epipodophyllotoxin VP16–213. *Acta Paediatr* 1994; **83**: 1322–1325.
2 Okano M, Gross TG. Epstein–Barr virus-associated hemophagocytic syndrome and fatal infectious mononucleosis. *Am J Hematol* 1996; **53**: 111–115.
3 Seideman K, Tiemann M, Henze G *et al*. Therapy for non-Hodgkin lymphoma in children with primary immunodeficiency; analysis of 19 patients from the BFM trials. *Med Pediatr Oncol* 1999; **33**: 536–544.
4 Milpied N, Vasseur B, Parquet N *et al*. Humanized anti-CD 20 monoclonal antibody (Rituximab) in post transplant B-lymphoproliferative disorder: a retrospective analysis on 32 patients. *Ann Oncol* 2000; **11**: 113–116.
5 Seemayer TA, Gross TG, Egeler RM *et al*. X-linked lymphoproliferative disease: twenty-five years after discovery. *Pediatr Res* 1995; **28**: 471–478.
6 Henter JI, Arico M, Egeler RM *et al*. HLH-94: a treatment protocol for hemophagocytic lymphohistiocytosis. *Med Pediatr Oncol* 1997; **28**: 342–347.
7 Imashuku S, Hibi S, Ohara T *et al*. Effective control of Epstein–Barr virus-related hemophagocytic lymphohistiocytosis with immunochemotherapy. *Blood* 1999; **93**: 1869–1874.
8 O'Reilly RJ, Friedrich W, Small TN. Hematopoietic cell transplantation for immunodeficiency diseases. In: Thomas ED, Blume KG, Forman S (eds). *Hematopoietic Cell Transplantation*. Blackwell Science: Malden, 1999, pp 1154–1172.
9 Kuehnle I, Huls MH, Liu Z *et al*. CD20 monoclonal antibody (rituximab) for therapy of Epstein–Barr virus lymphoma after hemopoietic stem-cell transplantation. *Blood* 2000; **95**: 1502–1505.
10 Rappeport JM. Hematopoietic cell transplantation for granulocyte and macrophage disorders. In: Thomas ED, Blume KG, Forman S (eds). *Hematopoietic Cell Transplantation*. Blackwell Science: Malden, 1999, pp 1195–1203.
11 Papadopoulos EB, Landanyi M, Emanuel D *et al*. Infusions of donor leukocytes to treat Epstein–Barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. *New Engl J Med* 1994; **330**: 1185–1191.