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Nonmyeloablative allogeneic bone marrow transplantation for treatment of childhood overlap syndrome and small vessel vasculitis

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Summary:

A 13-year-old Caucasian female with a systemic connective tissue disease (overlap syndrome with pulmonary vasculitis) underwent nonmyeloablative allogeneic BMT after failure of prolonged combination immunosuppressives to induce remission. The procedure also included cotransplantation of donor bone chips as a source of stromal cells. The unique protocol allowed good engraftment of hematopoietic (>95%) and bone core stromal cells (>60%). The patient was clinically improved, stable, and off all immunosuppressive medications 36 months post-transplant. To our knowledge, this is the first pediatric nonmyeloablative BMT with cotransplantation of stromal cells solely for treatment of an autoimmune disease.

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Autologous hematopoietic stem cell transplantation (AHSCT) is being used for severe life-threatening autoimmune diseases (ADs).[1] Beneficial responses to AHSCT have been attributed to the high levels of immunosuppressive conditioning.[2] It is unclear if AHSCT can cure AD as the recurrence rate is high.[3] Evidence that many experimental ADs are stem-cell-driven[4–6] suggests that an allograft may be curative.

Outcomes of patients with AD treated with SCT for coexisting malignancy tend to favor the use of allogeneic over autologous protocols. For example, in a recent review[7] regarding disease-free survival among 24 patients treated with allogeneic SCT, 21 survived the treatment and only two had relapsed AD (psoriasis and Crohn's) during a median 7-year follow-up. In comparison, among 24 patients treated with autologous SCT, although 23 had an initial improvement, 15 relapsed for AD within 3 years post transplant, including patients transplanted with positively selected CD34+ stem cells.

Autologous or allogeneic SCT is usually reserved for the very ill suffering from life-threatening AD. Reduced intensity or nonmyeloablative protocols are appealing because introduction of allogeneic cells can be accomplished while minimizing the transplant-related risks.[8] Nonmyeloablative protocols have been successful for treatment of some malignancies or genetic diseases, and although limited in numbers, case reports using such protocols on patients with AD are encouraging.[9–12] Our aim was to achieve an adequate level of engraftment without GVHD. Others[9,10] have suggested that there can be a beneficial role of limited GVHD in the form of graft *vs* autoimmunity.

Case report

Patient UP5 is a 15-year-old white female with overlap syndrome and small vessel vasculitis of lungs, diagnosed at All Children's Hospital in St Petersburg, Florida.[13] She was in good health until 7 years of age (1995) when she developed growth failure, fatigue, weakness, sinus congestion, and Raynaud's phenomenon. She was given nebulizers and short courses of adrenal steroids for atypical asthma and later started on nifedipine for mild hypertension and Raynaud's. She was referred to our Institute after two documented episodes of transient ischemic attack and temporary hemiparesis. In January 1998, pertinent exam findings were shotty lymph nodes, mild periungual telangiectasias, Raynaud's phenomenon, livido reticularis, and telangiectasias on the upper chest. Laboratory tests revealed a leukocytosis of 20×10^9/l and elevated platelet count (540×0^9/l), ESR 15 mm/h, and normal complement levels. ANA was 1:320, speckled, and anti-double-stranded (ds) DNA was borderline positive at 49 IU/ml (normal <29 IU/ml). She was negative for anti-Smith, U1RNP, SSA, SSB, ANCA, Scl-70, Jo-1, pm-scl, antiphospholipid, and antiglomerular basal membrane antibodies. Quantitative total serum immunoglobulin levels were normal. Chest CT revealed ill-defined peripheral nodules, and an open

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lung biopsy small vessel vasculitis as shown in Figure 1 and as described by Chamarthy *et al*.[13]

The decision for BMT was based on the presence of severe Cushing's syndrome and stunted growth, poor quality of life, profound adrenal steroid dependency, failure to induce remission with methotrexate and cyclophosphamide (CYT), and development of iatrogenic liver toxicity in August 2000. The treatment at the time of nonmyeloablative BMT included daily (8 mg p.o.) and monthly (25 mg/kg i.v.) methylprednisolone, IVIG (0.5 g/kg monthly), MTX (25 mg SQ weekly), nifedipine (30 mg p.o. twice daily), enalapril (2.5 mg p.o. twice daily), amitriptyline (50 mg p.o. once a day), celecoxib (100 mg p.o. twice daily), baby aspirin (81 mg p.o. once a day), and pamidronate (0.5 mg/kg i.v. monthly). She was also receiving medications for asthma symptoms.

The nonmyeloablative BMT protocol was approved by the Institutional Review Board, and the parents and patient consented to the procedure. She was 42.5 kg (35 percentile for age), 128 cm ($\ll$3 percentile for age), and 1.2 m^2. The protocol included a conditioning regimen using fludarabine 25 mg/m^2 i.v. once a day for 5 days (day –7 to –3), CYT 50 mg/kg i.v. once a day for 2 days (day –3 and –2), and total body irradiation (200 cGy) (day –1). This regimen was tolerated well except for mild nausea. On day 0, freshly harvested HLA-matched (6/6) bone marrow cells from her mother were gravity-centrifuged to remove RBC due to an ABO incompatibility (A$\rightarrow$O) and infused to UP5 at 5×10^8/kg. Maternal bone chips were harvested during the bone marrow aspiration and surgically implanted next to the umbilicus as well as placed into the posterior iliac crest bilaterally after removal of small bone fragments from the patient with the same size Jamshidi needle. In addition, osteoblast-like cells (1.5×10^5), obtained from the donor's iliac crest and expanded *ex vivo* as described by El-Badri *et al*,[14] were infused i.v. on day +13.

Post BMT immunosuppressives included mycophenolate mofetil (MMF, 1250 mg/day divided twice daily), and cyclosporin (CSA, 6 mg/kg/day, divided twice daily) from day –1 to day 30, then gradually weaned off in January and August 2001, respectively. Methylprednisolone was given (6 mg twice daily) for 3 weeks after the BMT for fatigue and mild diarrhea, then gradually tapered down and discontinued in October 2002. IVIG was tapered off and discontinued in March, 2003. She stopped her asthma medications in October 2003.

Post transplant neutropenia was brief (Table 1) and platelet counts remained adequate. She received 1 U of pRBC on day +4 for an Hct of 22 prior to discharge home on day +9. She has not shown evidence of clinical GVHD or systemic infection over a 36-month follow-up period. The DLCO readings are returning to pretransplant levels after a transient decrease (Table 1).

She converted her blood group from O to A at 8 months post transplant without anemia. Donor chimerism measured by short tandem repeat (STR) analysis of peripheral blood mononuclear cells has been >90% donor phenotype since 12 months post transplant. A bone biopsy was performed in August 2001 (day +270). All traces of bone marrow were removed and the cells from the crushed bone were cultured for 11 days. The fibroblast-like cells (99%) were analyzed by STR and 66% were of donor origin.

Figure 1 Patient UP5 before and 3 years after bone marrow transplant. The inset depicts small vessel vasculitis in the lungs: on the left, H&E staining of a small pulmonary arteriole with concentric muscular thickening, endothelial cell swelling, and transmural infiltrate of lymphocytes. On the right Movat's pentachrome staining of a pulmonary vein with mural fibrosis and neovascularization indicating chronic changes.

Table 1 Data on CBC, flow cytometry, and DLCO over time

Date[a]	*WBC*[b]	*CCD4:CD8*	*%CD19*	*%NK*	*Plt*[b]	*DLCO*[c]
−33 mo	20.7 (11.0)	1.3	26	6	536	65
−15 mo	14.3 (15.5)	0.8	9	3	672	60
−3 mo	11.4 (12.8)	0.6	2	8	563	55*
−1 week	15.3 (16.0)	0.2	1	6	341	ND
0	7.5 (0.3)	3.5	<1	1	434	ND
1 week	0.3 (Nd)	3.3	2	6	30	ND
2 week	2.0 (9.0)	3.1	1	40	76	ND
1 mo	12.2 (11.0)	1.8	<1	52	83	48
3 mo	8.3 (17.3)	0.3	1	4	184	52
12 mo	8.3 (41.0)	0.8	19	3	319	45
30 mo	11.2 (36.3)	0.8	22	2	473	63

[a]−33 months (mo) is the time of first visit (January 1998), and 0 the time of BMT (November 2000).
[b]WBC (including % lymphocytes in parenthesis) and platelets (plt) $\times 10^9$/l. Flow cytometry data using BD FACSCalibur and fluorochrome-labeled specific antibodies. Normals for % lymphocyte, 25–33; CD4:CD8, 1.1–2.1; % CD19, 3.5–24, % NK, 3–11.
[c]Percent predicted DLCO corrected for Hgb (ml/min/mmHg); *after 6-monthly doses of intravenous CYT; ND = not done.

The peripheral blood lymphocyte profile showed an improvement of the abnormal CD4 : CD8 ratio and B lymphocyte (CD19) and NK percentiles that were seen during the first month post transplant. In June 2003, 3 months after stopping of IVIG, the serum IgG, IgM, and IgA immunoglobulin levels, specific antibody titers to tetanus, and lymphocyte proliferation assays with concanavalin-A and the recall antigen candida were in the normal reference range and were similar to pretransplant values.

She resolved the cushingoid features (Figure 1), and grew 17.7 cm in the last 3 years. She is now a full-time student in a regular classroom, but continues to have occasional periods of increased fatigue with transient and mild

Gottron-like rash, which has decreased in frequency over the last 3 years. Current medications are baby aspirin, leuprolide acetate, and somatropin.

Discussion

UP5 represents the first pediatric case who was given nonmyeloablative BMT using fully matched allogeneic hematopoietic and stromal cells for the treatment of a systemic autoimmune disease. The procedure was well tolerated and resulted in a high level of engraftment without GVHD. She now exhibits more than 90% donor phenotype per STR analysis of peripheral blood mononuclear cells and has converted her blood group from O to A. Analysis of her fibroblasts cultured from bone showed 66% donor cells. At 3 years after BMT, UP5 shows striking improvement allowing discontinuation of all immunosuppressive medications, including daily steroid therapy, while preserving lung functions. Whether her chimeric state will result in a permanent halt of her disease process and reverse pathological changes in the lungs remains to be seen.

Successful outcomes using a less-intense preparative regimen for stem cell transplantation for treatment of relapsed malignancy combined with severe psoriasis have been previously reported.[9,11] An analogy between the concept of 'graft *vs* autoimmunity' and 'graft *vs* leukemia' effects of GVHD was suggested.[9] Case reports from Oyama *et al*[12] and Marmont *et al*[10] have pioneered applications of nonmyeloablative stem cell tranplantation solely for autoimmune diseases. Both cases were males with intractable Evans' syndrome receiving HLA-matched stem cells.

Nonmyeloablative MHC-matched HSC transplant protocols have reduced risk for transplant-related mortality and morbidity compared to total myeloablative protocols. A high degree of chimerism may be important but the threshold of donor chimerism sufficient to achieve a useful arrest of a given autoimmune condition remains to be determined. In preclinical studies,[15] stromal cells appeared to promote donor engraftment and immune recovery. Although it remains to be proven, transplantation of bone chips could be more efficacious by providing precursor cells a natural, three-dimensional scaffold environment to facilitate *in vivo* expansion. Furthermore, the capacity of stromal cells to differentiate into parenchymal cells[16] may contribute to tissue healing in target organs although this has not been apparent yet in our patient's pulmonary function tests. Thus, nonmyeloablative allogeneic stem cell transplantation is a promising approach for treatment of life-threatening autoimmune diseases. Further studies are needed to determine the mechanisms involved.

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