Infantile osteopetrosis; bone marrow transplantation from a cousin donor

G Malcolm Taylor, Simon P Dearden, Andrew M Will, David I K Evans, Richard F Stevens, Sybil Simon, Maurice Super, Gillian Morrell, William D Fergusson, Ian H Brown

Abstract

The successful correction of infantile osteopetrosis in an Asian child by bone marrow transplantation (BMT) from an HLA-A,B matched cousin donor is reported. Retrospective HLA molecular analysis revealed that patient and donor were incompatible for HLA-DPB1. Donor type cells detected in the patient after transplantation indicate successful engraftment. The patient is currently alive and well.

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other neurosensory defects. The probability of a child with infantile malignant osteopetrosis surviving to 6 years of age is about 30%, and most untreated children die within the first decade.¹

The only currently effective long term treatment of infantile malignant osteopetrosis is allogeneic bone marrow transplantation (BMT).^{2,3} In one series, six out of nine children receiving HLA identical BMT exhibited disease-free survival, but age was an important factor in prognosis. In this paper we report on the successful correction of infantile malignant osteopetrosis in the daughter of consanguineous Asian parents by allogeneic BMT from a cousin donor.

Osteopetrosis is an inherited skeletal disease in which defective bone resorption by osteoclasts leads to excessive bone deposition.¹ The disease occurs in two basic forms: a relatively mild autosomal dominant adult form, and a more severe autosomal recessive infant form. Infantile malignant osteopetrosis is characterised by increased skeletal mass, extra-medullary haemopoiesis, obstruction of the cranial foramina leading to increased intracranial pressure, optic atrophy, blindness, and

Methods

Blood from the patient and relatives was obtained in preservative-free heparin. Viable lymphocytes were separated by standard methods. Genomic DNA for molecular analysis was extracted from fresh or frozen blood samples, or from lymphoid cell lines. Serological HLA typing was carried out by the National Institutes of Health microlymphocytotoxicity technique. Lymphoid cell lines were prepared by immortalising lymphocytes

Immunogenetics
Laboratory, St Marys
Hospital, Hathersage
Road, Manchester
M13 0JH
G M Taylor
S P Dearden
R F Stevens
W D Fergusson

Departments of
Haematology and
Clinical Genetics,
Royal Manchester
Children's Hospital,
Manchester
A M Will
D I K Evans
S Simon
M Super

Regional Blood
Transfusion Centre,
Manchester
G Morrell

Child Development
Centre, Burnley
Health Care Trust,
Burnley
I H Brown

Correspondence to:
Dr Taylor.

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Figure 1 Pedigree of extended family of the patient. Generation number is shown in Roman type (I, II, etc), and individual number in Arabic (1, 2, etc). The proband (VI.12) is cross hatched and the donor (VII.2) is marked with an asterisk.

using the B95-8 strain of Epstein-Barr virus.⁴ Mixed lymphocyte cultures were carried out as previously described.⁵

HLA class II alleles were typed by restriction fragment length polymorphism analysis.⁶ Typing of HLA-DPB1 and DRB1 alleles was carried out by the reverse polymerase chain reaction – sequence specific oligonucleotide technique.⁷ Analysis of engraftment after transplant was carried out by the single strand conformation polymorphism (SSCP) method of Orita *et al.*⁸

Results

The girl was born at 38 weeks' gestation to 24 year old Asian parents who were first cousins. Her mother previously had two miscarriages but no other liveborn children. No problems were encountered with the child during the perinatal period, but at 5 weeks of age she was admitted to hospital with feeding difficulties, upper respiratory tract infection, failure to thrive, hepatosplenomegaly, and hypocalcaemia. Her serum calcium was 1.76 mmol/l, and alkaline phosphatase was 1165 IU/l. Radiography revealed densely sclerotic bones with irregular epiphyses typical of osteopetrosis. Her blood picture was leucoerythroblastic, with a haemoglobin concentration of 83 g/l, and a leucocyte count of $14.6 \times 10^9/l$. The blood film showed teardrop red cells and fragmentation, with 1% normoblasts.

The girl was HLA incompatible with both parents, so a bone marrow donor search among relatives was instigated. Pedigree analysis (fig 1) and HLA haplotype assignment in 27 relatives showed that she shared her A26, B8/A9, B35 haplotype with two cousins (VII.1 and VII.2). Mixed lymphocyte cultures between the proband and her cousin VII.2 showed that she reacted weakly in the host-versus-graft (HvG; 6%), and graft-versus-host (GvH; 1%) direction. By comparison, she was strongly reactive to VII.1. VII.2 was selected as the bone marrow donor.

The patient (4.9 kg) was prepared for BMT at 6 months of age by conditioning with busulphan (4 mg/kg/day) for four days, followed by cyclophosphamide (50 mg/kg/day) for four days. Methotrexate was used for prophylaxis for graft-versus-host disease. The transplant consisted of 3×10^8 cells/kg of non-lymphocyte depleted bone marrow. The post-transplant course was complicated by severe perineal dermatitis due to cyclophosphamide and pyrexias, which were treated with piperacillin, cefuroxime, flucloxacillin, and metronidazole at different times. The absolute neutrophil count reached $0.5 \times 10^9/l$ by day 26, and the spleen became impalpable by day 38 after transplant. At eight months after transplant, the bones showed markedly improved density on x ray examination, with normal appearance of the metaphyses and epiphyses. At eight years after transplant, the patient's intelligence is normal, and apart from a squint and poorly developed teeth, her physical development is on the 75th centile for both height and weight. The blood count,

Figure 2 SSCP analysis of DNA from blood samples from the proband at 3.8 (1) and 4.5 (2) years after BMT, compared with the proband before transplantation and the donor. Note the presence of bands in both of the samples from the proband after BMT, which are present in the donor, but absent from the sample taken before BMT.

blood film, and radiographic appearances are also normal.

Retrospective HLA molecular typing showed that the patient (before BMT) and donor were identical for DRB1, DQA1, and DQB1, but mismatched for one DPB1 allele. Before BMT the girl was homozygous for DPB1*0401, and her donor cousin (VII.2) heterozygous DPB1*0401/*1301. SSCP analysis on blood samples obtained from the index case 3.8 and 4.5 years after transplantation (fig 2) show a DPB1 band pattern which is the same as donor VII.2, indicating that the patient successfully engrafted with DPB1 mismatched donor bone marrow.

Discussion

We describe the successful correction of infantile osteopetrosis in an Asian child by

BMT using a cousin donor. An extensive family search for a matched donor was instigated because evidence of consanguinity suggested restricted HLA heterogeneity. Bone marrow from the HLA compatible cousin donor fully corrected the osteopetrosis, and the patient remains in good health eight years after transplant. The weak mixed lymphocyte culture reaction between the patient and donor in the HvG direction before transplantation was found to be due to DPB1 incompatibility. Long term engraftment is evident from the results of SSCP analysis of the patient four years after BMT, and the absence of GvH disease indicates that the DPB1 mismatch presented no significant clinical problem.

The presence of significant numbers of Asian immigrants in this region practising first cousin marriage⁹ may be a factor in the frequency of osteopetrosis. One of the most important criteria for the success of BMT in infantile malignant osteopetrosis is early diagnosis and identification of a suitably matched donor.¹ As an HLA matched sibling donor may not be available, and the results of cross racial unrelated donor transplants are poor, the only realistic option is to search for a matched donor in the extended family. The limited

genetic heterogeneity in some Asian families significantly increases the chance of finding an HLA matched donor.

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