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CGD associated with cytochrome B deficiency: granulocyte function tests 25 months after bone marrow transplantation

M M Manzionna*, D De Mattia*, R Fumarulo**, G Torlontano***, F Schettini*

*Institute of Clinical and Preventive Pediatrics, University of Bari, Italy
**Institute of General Pathology, University of Bari, Italy
***BMT Centre, Division of Hematology, University of Chieti, Italy

We report a case of a boy, who is now 14 years old, with Chronic Granulomatous Disease (CGD) and who underwent successful bone marrow transplantation (BMT) on the 7th December 1985 in Pescara Centre (4.7×10^8 nucleated cells/Kg) from his 9 year old histocompatible heterozygous sister (HLA identical and mixed lymphocyte reaction negative) after a preconditioning regimen of Busulfan 3.25 mg/Kg/day for 4 days, followed by cyclophosphamide 50 mg/Kg/day for 4 days. The child received cyclosporin i.v. from -1 day to $+12$ days (5 mg/Kg/day) and orally to 6 months (12.5 – 10.0 mg/Kg/day). Oral Acyclovir was administered for the prevention of Herpes virus infection from -1 day to $+120$ days. The patient was protected by an L. A. F. isolator and by our decontamination protocol [1, 7, 9].

The clinical course of the transplantation presented no complications. At the time of the transplantation the boy was free from infection and remained so throughout the course of the transplant. He was engrafted promptly with PMN counts normalizing on the day 27th and there was a complete reversal of the neutrophil function defect.

Before transplantation the child had recurrent pyogenic infections due to Staphylococcus aureus and Candida albicans (recurrent suppurative cervical lymphoadenopathy, liver abscess and pneumonia). His polymorphonuclear cells (PMN) were defective in tests of Nitro blue tetrazolium (NBT) reduction and O_2^- generation. No cytochrome b could be detected in sonicated cells in dithionite reduced-oxidized spectra [7].

Twenty-five months after BMT the boy continues to be free from infection with normalisation of the total leukocyte-count and C3 and Ig levels, all of which were persistently raised before the graft. In fact NBT is positive for zymosan ($49 \pm 2\%$) and for PMA ($56 \pm 2\%$) (Fig. 1, Table). O_2^- generation with PMA is 3.4 nmol/min/10^6 cells and with OPZ is 0.4 nmol/min/10^6 cells. Chemi luminescence with PMA is 1090 mV/min/10^6 cells and with OPZ is 290 mV/min/10^6 cells

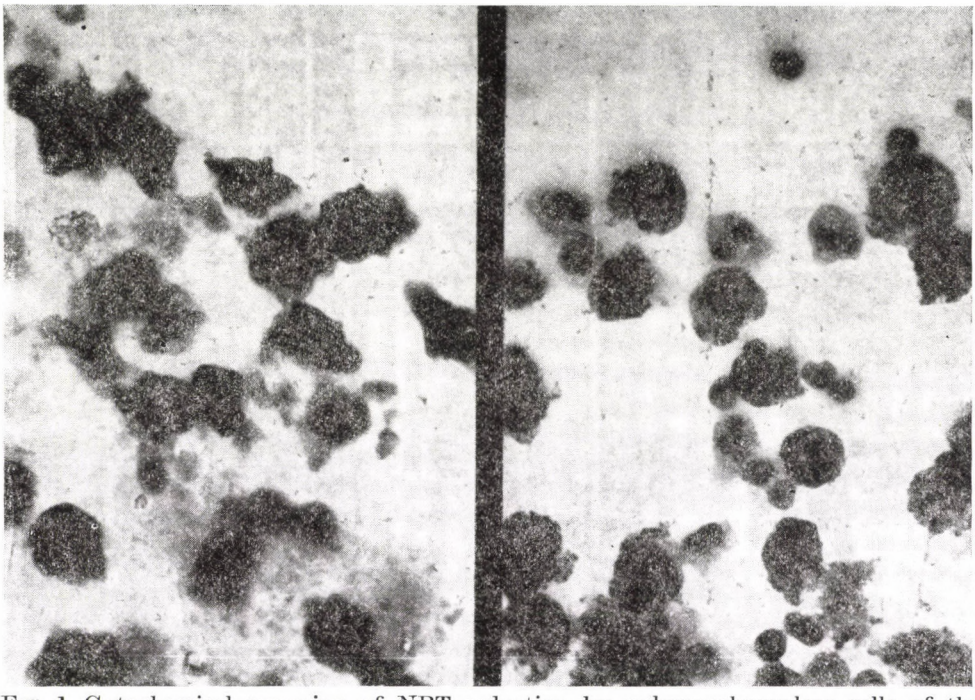

FIG. 1. Cytochemical screening of NBT reduction by polymorphonuclear cells of the patient was performed using phorbol myristate acetate (PMA, 1 μg/ml) or opsonized Zymosan (OPZ, 1 mg/ml) as stimulants

TABLE

Polymorphonuclear functions from patient before and after BMT as compared with donor and father cell values

	Patient before BMT	Patient after BMT	Donor (sister)	Father
NBT (% positive)				
Endotoxin	1±0.03*	43±4	ND**	ND**
Zymosan	ND**	49±2	50±2	93±2
PMA	ND**	56±2	62±1	98±2
O_2^- generation (nmol/min/10^6 cells)				
Zymosan	0	0.4	0.32	0.76
PMA	0	3.4	4.76	6.4
Chemiluminescence (mV/min/10^6 cells)				
Zymosan	ND**	290	263	500
PMA	ND**	1090	980	1630

* Values are mean ± SEM
** ND = Not Done

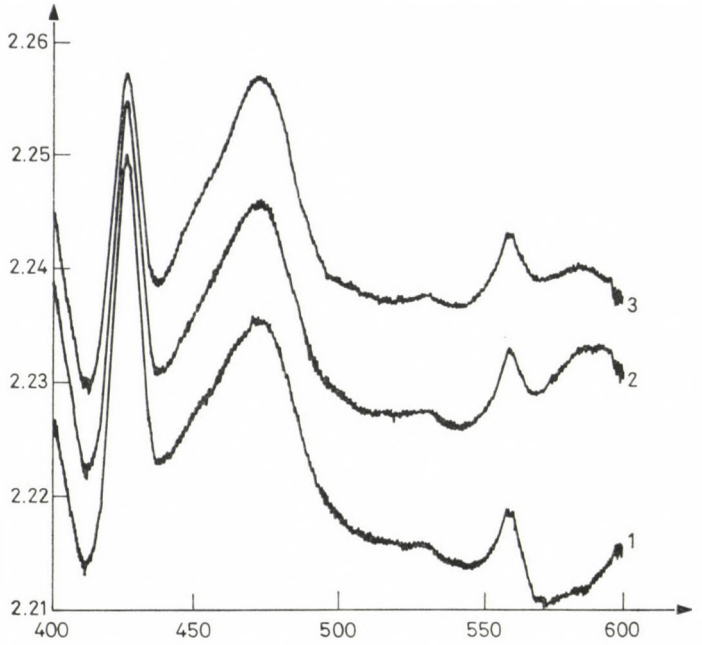

FIG. 2. Reduced minus oxidized spectra of neutrophil homogenate from the patient (1) from the father (2), from the donor (3)

(Table). Cytochrome b spectra show peaks at 428, 530, 558 nm (Fig. 2). There was mosaicism of NBT positive and negative cells similar to that of the marrow donor. Complete chimerism was documented by prompt cell markers and chromosome pattern.

Using the above intensive conditioning regimen developed by us (G. T.) for transplantation of thalassemia complete neutrophil engraftment was achieved in CGD with cytochrome b deficiency [1, 9]. Earlier attempts of BMT for CGD by bone marrow from related [3, 4, 5, 6] or unrelated [2] donors have failed either because of slow loss of the graft and gradual deterioration in neutrophil function or because of lethal infections and/or GvH disease.

Because engraftment is retained and GvH absent after 25 months, BMT in CGD from a related donor using the above technique for myeloablation and immunosuppression could be undertaken widely.

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