



T Cell Depleted Haploidentical Bone Marrow Transplantation for the Treatment of Children with Severe Combined Immunodeficiency

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Abstract. Severe combined immunodeficiency (SCID) is fatal in early childhood if unrecognized and if not treated. The aim was to determine the efficacy of T cell depleted bone marrow transplantation (TCD BMT) in the treatment of children with SCID. Eleven children diagnosed with SCID received histocompatible related donor bone marrow transplantation – HRD BMT (group I). Thirty seven children diagnosed with SCID who did not have histocompatible donors were treated with TCD haploidentical parental bone marrow transplantation (BMT) (group II). TCD was performed by *in vitro* soybean lectin agglutination followed by E-rosette depletion. Patients were longitudinally assessed for the presence and function of T and B lymphocytes. In group I all children survived. The mean age of children in this group at the time of HRD BMT was 15.4 months. All surviving patients normalized their specific T cell function. Two out of 11 require treatment with intravenous immunoglobulin i.v. Ig. In group II 17 out of 37 (46%) children survived. At the time of TCD BMT the mean age of survivors was 7.5 months, vs. 11.4 months in patients who died. Death was caused most commonly by opportunistic infections, Epstein-Barr virus induced lymphoproliferative disease (EBV-LPD), and graft versus host disease (GvHD). Seventeen out of 17 surviving patients recovered normal numbers of CD3⁺ cells and antigen specific T cell function. Five out of 17 never recovered their B cell function and require i.v. Ig injections. Early diagnosis, prevention or treatment of opportunistic infections, and enhancement of immune recovery will be necessary to improve survival in patients with SCID treated with TCD BMT.

Key words: severe combined immunodeficiency (SCID); histocompatible bone marrow transplantation; T cell depleted haploidentical bone marrow transplantation.

Introduction

Severe combined immunodeficiency (SCID) is a heterogeneous group of rare genetic disorders, char-

acterized by markedly impaired cellular and humoral immune function. As a consequence SCID is associated with increased susceptibility to viral, bacterial, fungal, and protozoan infections, and a failure to thrive.

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Usually, infants born with SCID develop recurrent, life threatening infections at 3–6 months of age. These episodes often include opportunistic infections with *Pneumocystis carinii*, rotavirus, *Candida albicans* etc. Approximately one half of patients with SCID have evidence of graft versus host disease (GvHD), due to engraftment with maternal T lymphocytes which were transmitted to the fetus transplacentally.

SCID has many genetic causes. Over half of all SCID cases result from an X-linked recessive defect, the remaining cases can be caused by autosomal recessive defects. (Table 1). Recently, the molecular basis of X-linked SCID was determined to be mutations in the gene encoding the common γ subunit (γ c) of a number of cytokine receptors, including the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15²². Abnormal γ c chains may be expressed in the lymphocytes of as many as one third of patients with X-linked SCID²⁸, while the remainder probably have no γ c protein expression. Patients usually present normal number of B cells ($T-B^+$ phenotype), low serum immunoglobulins, lack of specific antibody response and lack of specific T cell blastogenesis³⁹.

In 1997 the novel occurrence of X-linked SCID in a Navajo Kindred with maternal mosaicism for a deleterious mutation within IL-2R γ was described²⁴.

Among autosomal recessive inheritance of SCID about 25% of cases are caused by genetic deficiency of the purine salvage enzyme adenosine deaminase (ADA). Multiple different mutations have now been identified¹⁴. Recently, adult onset of ADA deficiency has been described^{25, 36}.

Another 4% of cases are caused by a deficiency of the enzyme purine nucleoside phosphorylase (PNP)²⁶ and was also found to be an autosomal recessively heritable disease³⁴. Those patients with defects of purine degradation enzymes will have present with the phenotype of $T-B^-$, and in contrast to those with $T-B^+$ presentation will have normal or elevated numbers of natural killer cells (NK).

Another autosomal recessive inheritance of SCID is caused by the mutation of Janus kinase 3 (Jak3) gene^{18, 33}. Patients with Jak3 deficiency present with phenotype $T-B^+$, as is typical for X-linked SCID. Thus, the $T-B^+$ phenotype in a girl is likely to be due to Jak3 deficiency, while the same phenotype in boys can be due to either X-SCID or Jak 3 deficiency.

Recently, a very rare mutation within autosomal genes encoding for T cell receptor signal transduction protein ZAP-70 has been demonstrated in SCID patients. Children present with the absence of peripheral CD8⁺ T cells but with the presence of CD4⁺ T cells,

Table 1. Different genotypes causing severe combined immunodeficiency (SCID)

X – linked (~ 50%)	Autosomal recessive
Common gamma chain (γ c) receptor subunit (IL-2, IL-4, IL-7, IL-9, IL-15) deficiency	Adenosine deaminase (ADA) deficiency (~25%) Janus kinase 3 (Jak3) deficiency ZAP-70 deficiency RAG-1/ RAG-2 deficiency MHC deficiency unknown mutation

which are unresponsive to T cell receptor (TCR) mediated stimuli^{4, 10, 38}. A phenotypically distinct form of autosomal recessive SCID has also been described among the Navajo Native American population^{15, 20}, but the molecular defect is unknown.

Some $T-B^-$ SCID patients have been found to carry a mutation of the recombinase activating gene 1 (RAG-1), RAG-2, or both³⁵. MHC class II deficiency (bare lymphocyte syndrome, BLS) is a rare primary deficiency disorder characterized by defective expression of MHC class II antigens¹². Affected patients are susceptible to severe and recurrent bacterial, viral, fungal and protozoal infections. BLS is due to mutations in either the CIITA, RF-X or RFXB genes, which regulate MHC expression. Recently, NONOYAMA et al.²³ concluded that the defective humoral immunity observed in BLS patients may be related to diminished expression of CD40 ligand on BLS T cells.

In many other cases of SCID the cause remains unknown.

The only effective treatment for SCID is a hematopoietic stem cell transplantation: bone marrow transplantation (BMT), or umbilical cord blood transplantation (CBT). The first successful allogeneic BMT for the patient with SCID was performed in 1968⁹. However, only 20% of children have an HLA identical sibling, and the chance of receiving a transplant from phenotypically matched unrelated donor (MUD) varies with the race of the patient, ranging from approximately 50% for Caucasian to less than 10% for ethnic minorities, and often requires waiting months to identify the donor and obtain the graft^{1, 2}. For SCID patients, without a sibling match or readily defined MUD source, the only choice is to be transplanted with HLA haploidentical bone marrow. By definition, each parent is haploidentical with the child, and therefore, BMT with parental marrow is a logical treatment for children without a matched donor.

One of the major problems of transplanting HLA nonidentical marrow is GvHD¹⁹. Even when using bone marrow from HLA-identical sibling there is a 25 to

15% chance of mild to moderate GvHD from donor derived T lymphocytes responding to recipient antigens. Elimination of immunocompetent T lymphocytes from the bone marrow with preservation of hematopoietic precursors prior to infusion, lowers the chance of GvHD development. In early 1980s clinical trials began and the first child successfully transplanted with parental, haploidentical BM was described in 1981³⁰. There is a greater than 90% chance to promptly identify a haploidentical donor within the family.

Several techniques of T cell depletion from non-identical bone marrow were described. T cells were removed from the donor's BM by antibody based methods (complement-mediated killing immunoadsorption, immunomagnetic separation) or by physical methods (lectin agglutination and sedimentation, erythrocyte rosette formation, counterflow centrifugation)^{3, 5, 6, 29, 41}.

Materials and Methods

Patients. Between November 1984 and December 1997, 56 children (33 boys and 23 girls) with severe combined deficiency were treated in the Division of Research Immunology and Bone Marrow Transplantation at Childrens Hospital Los Angeles. Children were diagnosed with primary deficiencies in T and B cell function, and classified as SCID according to the criteria of the World Health Organization⁴¹. Eight children with ADA deficiency were treated with polyethylene glycol-conjugated adenosine deaminase (PEG-ADA) enzyme replacement therapy, and 3 of them received gene therapy, using infusions of transduced autologous umbilical cord CD34⁺ cells^{16, 17}.

Of the 48 transplanted SCID patients, 11 had histocompatible siblings (23%), in which a match of 6/6 HLA-A, -B and -DR antigens was found, so they were treated with allogeneic, histocompatible bone marrow, (HRD BMT) (group I). The mean age of sibling donors (6 brothers and 5 sisters) was 6.2 years (range 2–13 years).

Thirty seven children, who did not have a histocompatible related donor, received T cell depleted haploidentical parental (28 maternal and 9 paternal) bone marrow transplant (TCD BMT) (group II). Two children in group II were patients with SCID caused by ADA deficiency, two were diagnosed with MHC class II deficiency (Bare lymphocyte syndrome, BLS).

In some of the most recently diagnosed patients, the type of their immunodeficiency was defined by genetic analysis. Five children were confirmed as having X-linked SCID, and one ZAP-70 deficiency.

Table 2. Phenotype of SCID patients transplanted at Childrens Hospital LA (n = 48)

Phenotype	Number of survivors/ total BMT
T ⁻ B ⁺	18/27 (67%)
T ⁻ B ⁻	6/12 (50%)
T ⁺ B ⁺	3/8 (38%)
T ⁺ B ⁻	1/1 (100%)

Table 3. Survival rates of SCID patients who received parental haploidentical T cell depleted bone marrow transplants according to type of BMT conditioning

	Number of survivors/number of patients transplanted
Busulfan Cyclophosphamide/+/- ATG	14/31 (45%)
Cyclophosphamide/ATG	3/6 (50%)
TBI/VP16	0/1 (0%) (as a second BMT)

Doses and administration of ATG, Cyclophosphamide, Busulfan, VP16 and TBI are described in the text (Materials and Methods).

The phenotype of all transplanted children with SCID is presented in Table 2.

Longterm survival was assessed, and patients were longitudinally evaluated for the presence and function of T and B lymphocytes.

Ten children out of 11 who received histocompatible bone marrow transplant (group I) were preconditioned with anti-thymocyte globulin (ATG) and one with Busulfan/Cyclophosphamide/ATG. ATG (Atgam, Pharmacia and Upjohn, Kalamazoo, MI) was given at the dose of 20 mg/kg in 4 daily doses every other day, Cyclophosphamide (Cytosan, Mead Johnson, NY) 50 mg/kg/dose in 4 doses over 4 days, and Busulfan (Myleran, Glaxo Wellcome Inc. Research Triangle Park, NC) at total dose of 600 mg/m² divided into 16 doses over 4 days.

Type of pre-BMT conditioning regimen for TCD haploidentical transplants (group II) is presented in Table 3. Doses of ATG, Cyclophosphamide and Busulfan as above, and VP16 (Ve Pesid, Bristol Lab., NY) was given at 60 mg/kg as a single dose. Total body irradiation (TBI) was given at 1200 cCy divided into 6 doses over 3 days.

T cell depletion. The bone marrow was T depleted *in vitro* by fractionation with soybean agglutinin (SBA) and sheep red blood cells (SRBC), as previously described³⁰, with some modifications.

The modified procedure for large-scale purification of BM hematopoietic progenitors is presented in Fig. 1.

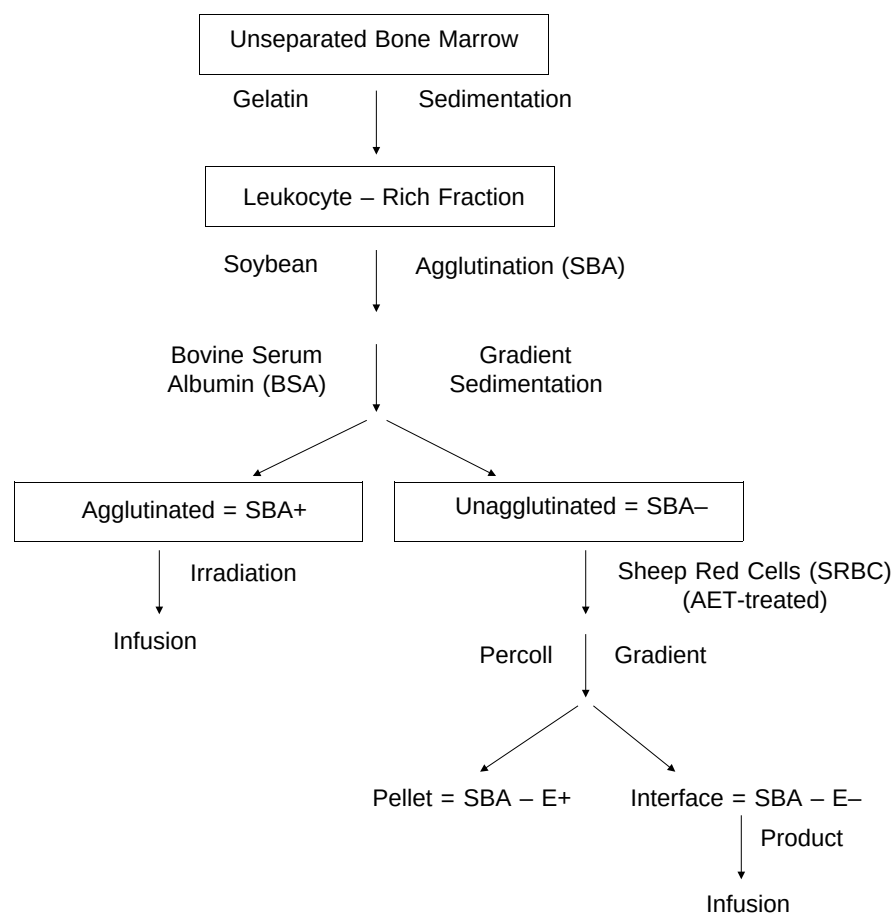


Fig. 1. T cell depletion. Schema of the large-scale purification of bone marrow hematopoietic precursors. SRBC – sheep red blood cells, SBA – soybean agglutinin, AET – 2-aminoethylisothiuronium bromide, E+ – cells forming rosettes with SRBC, E– – cells not forming rosettes with SRBC

Parental BM, (800–1100 ml) was harvested under general anesthesia by multiple aspirates from the posterior iliac crests, and collected in 200 ml of RPMI 1640 medium with 20–30 000 units of preservative free heparin. The heparinized BM was filtered through a 200 μ m mesh wire screen, was mixed with 3% gelatin (J. T. Baker) in the transfer bags (Baxter). The transfer bags were hung with ports pointed upright in an open plasma extractor and the suspensions were allowed to settle for about 1 h at room temperature. The red blood cell (RBC) depleted, leukocyte-rich plasma fraction was expressed into the 1000 ml attached bag, washed twice in 50 ml conical tubes (Falcon) with PBS, pH 7.4 (BioWhittaker) containing penicillin/streptomycin (pen/strep, Gemini Bio-Products, Inc.) and twice with PBS containing 1% human serum albumin (HSA) (Baxter, Glendale, Ca). Tubes were changed at the time of pellet consolidation to eliminate fat. After another two washes with PBS without HSA, the cell concentration was adjusted to $125 \times 10^6/\text{ml}$ and the aliquots of 6 ml of cell suspension was allowed to agglutinate for

1–3 min in room temperature with an equal volume of SBA (Vector Laboratories) (2 mg/ml) in sterile T 75 flasks (Falcon). The agglutinated mixture was then layered on top of 35 ml of 5% bovine serum albumin in PBS in 50 ml conical polystyrene tubes. After 10–20 min at room temperature, the agglutinated cells sedimented while the other cells remained at the interface with the albumin solution. The top and bottom fractions were transferred to separate 50 ml conical tubes. The cells were then resuspended in 30 ml of 0.2 mol/l D-galactose (J. T. Baker, Phillipsburg, NJ) in PBS. After 10 min the cells were centrifuged at 400 g for 5 min, collected, washed once with D-galactose and twice with Medium 199 (BioWhittaker).

At this point of the procedure, the SBA⁺ fraction was irradiated (Dose 5400 Rads, Cesium-137 Gamma-cell, Atomic Energy of Canada Ltd., Ottawa, Canada) and infused to the patient. The SBA[–], unagglutinated cell fraction was mixed in a 50 ml polypropylene ($5 \times 10^6/\text{ml}$, 20 ml) with 2-aminoethylisothiuronium bromide (AET; Sigma) treated SRBC (2%, 20 ml) and

5 ml absorbed fetal calf serum, spun down at 400g for 10 min and incubated on ice for 1 h. After incubation, gently resuspended pellet was spun for 30 min at 2000 rpm in 60% Percoll gradient. The interface containing T lymphocyte depleted population of cells was washed twice PBS, then twice with lactated ringers solution (LRS, Baxter) with HSA and pen/strep, then another two times with LRS with HSA, without pen/strep. The final product, after taking an aliquot for cell count, viability, and sterility testing, was resuspended in 25 ml of LRS with HSA and then gently aspirated into a 60 ml syringe.

The TCD bone marrow was infused over 1–2 h via a central venous catheter into the patient. Usually a dose of 7.5×10^7 /kg of nucleated cells was given. The 1.5–2.5 log reduction in T cell content was achieved, and the patients received $<1 \times 10^6$ T cells/kg.

In one case, because of the mother's availability and scheduling problems, T cell depleted BM was viably frozen, and infused after thawing 2 months later.

Immunological evaluation. Immunological testing, T and B cell numbers and function, was performed at 3 month intervals, and later yearly, whenever patient's peripheral samples were available. Numbers of T, B and NK cells were quantitated by direct immunofluorescence and flow cytometry, using anti-CD3, -CD4, -CD8, -CD56, -FITC and -PE conjugated monoclonal antibody (FACScan, Becton Dickinson, San Jose, CA). The number of newly formed CD4⁺ CD45RA⁺ cells was evaluated by 3 color analysis, using CD4-PerCP conjugated antibody (Becton Dickinson, San Jose)^{21, 32}. T cell function was monitored by *in vitro* blastogenesis assay, incorporation of H³-thymidine during 3 days culturing the cells in response to mitogens (PHA, Difco) and 6 days in the presence of specific bacterial and fungal antigens (Tetanus Toxoid, Connaught Lab. Ltd., Ontario, and Candida, Bayers Corp.) and three herpes viruses (CMV, HSV, VZV, UCHSC, Denver, Colorado) with/without exogenous IL-2 (Chiron Corp., CA) and IL-7 (Biosource Intern., Camarillo, CA) by standard method⁸. Most recently, evaluation of CD40L expression on T cells after *in vitro* PMA and ionomycin stimulation was performed. Function of B cells, i.e. production of specific antibodies against tetanus toxoid (Wyeth Lab. Inc., PA) and polyribose phosphate (PRP, NIAIB Pneumococcal Reference Lab., NY) was tested by ELISA standard method.

Results

In group I, children after HRD BMT, there was 100% survival. The mean age of the children at the time of transplantation was 15.4 months.

Table 4. Proportion of survivors after haploidentical T cell depleted bone marrow transplantation for SCID (n = 37)

	Number of survivors/number of total BMT
Boys	12/21 (57%)
Girls	5/16 (31%)
Total	17/37 (46%)

Table 5. Gender and age distribution of SCID patients treated with bone marrow transplantation

	HAPLO TCD BMT		HISTO BMT
	survivors	nonsurvivors	
Boys	12	9	6
Girls	5	11	5
Total	17	20	11
Age at BMT (months)	7.5 (range 1–16)	11.4 (range 3–40)	15.4 (range 1–108)

HAPLO TCD BMT – haploidentical T cell depleted transplant (n = 37).
HISTO – histocompatible transplant (n = 11).

In group II, children after TCD BMT, 17 out of 37 survived (46%); 12 out of 21 boys (57%) and 5 out of 16 girls (31%) (Table 4). At the time of transplantation the mean age of children who survived was 7.5 months, vs. 11.4 months for patients who died. Death was caused by opportunistic infections (in 9), Epstein-Barr virus induced lymphoproliferative disease (EBV-LPD) (in 8), and GvHD (in 3).

All together, out of 27 boys with SCID 18 survived (67%), among them, 6 out of 6 after histocompatible related BMT, and 12 out of 21 after TCD BMT. Out of the 21 girls with SCID, 10 survived (48%) with all 5 who received histocompatible related BMT, and 5 out of 16 who received TCD BMT survived (Table 5).

Distribution of the age and the gender of the parental donors of the haploidentical bone marrow for TCD was analyzed. In the group of children who survived TCD BMT, 3 out of 17 parents were fathers (18%) with a mean age of 30 years. In the group of the children who died after TCD BMT, 6 out of 20 were fathers (30%) with the mean age of 23 years. The mean age of the female donors, i.e. the mothers (14 in each group), was identical in both groups (27 years).

The age, gender and the relation to the recipient of histocompatible related bone marrow was analyzed. All the donors were 6/6 HLA-histocompatible siblings, 6 brothers and 5 sisters, with the mean age of 6.2 years (range 2 to 13 years). Out of 11 children with SCID, transplanted with HRD bone marrow, 6 received the bone marrow from male donor (mean age 4.6 years) and 5 from female donor (mean age 8 years). Three

Table 6. Frequency of graft versus host disease (GvHD) in SCID patients post bone marrow transplantation

Histocompatible related BMT	3/11 (27%)
Haploidentical T cell depleted BMT	8/17 (47%)

Table 7. Recovery of B cell function post bone marrow transplantation for SCID

	Number of children who require i.v. Ig/total survivors (%)
T cell depleted haploidentical BMT	5/17 (29)
Related histocompatible BMT	1/11 (9)

children out of 11 received the bone marrow from the donor of the opposite gender.

We found that there was a higher incidence of GvHD in patients who received TCD haploidentical BMT (Table 6).

In group I, children after HRD BMT, all children (11/11) normalized their specific T cell function. However, one out of eleven requires treatment with i.v. Ig.

In group II, all children who survived TCD BMT (17/17), recovered their cellular immune function, they normalized CD3⁺ cell numbers and T cell antigen specific T cell function (data not shown) however there was a delay in immune reconstitution when compared with children from group I.

Five out of 17 TCD BMT survivors never recovered their B lymphocytes and they require i.v. Ig injections (Table 7).

Discussion

Histocompatible bone marrow transplantation is the treatment of choice for the patients with severe combined immunodeficiency. We report 100% longterm (3 to 13 years) survival, in patients with a histocompatible sibling donor. Unfortunately, three quarters of the patients lack an HLA-identical sibling. We report 46% long-term survival in our patients with SCID lacking histocompatible sibling and transplanted with parental TCD BM.

The described procedure for T depletion for the large scale purification of BM is a manual, time consuming but effective method. There are 4 major steps in the procedure. The first is removal of red blood cells by gravity sedimentation in gelatin, yielding a leukocyte-rich fraction which contains 68–76% of the original BM nucleated cells. The second is agglutination of BM cells with SBA and differential sedimentation of

agglutinated cells (SBA⁺). This step removes 70–90% of the BM nucleated cells, which include B cells, monocytes, and helper T cells²⁹, as well as most of the polymorphonuclear leukocytes. The average cell recovery in the unagglutinated SBA[−] cell fraction is about 17%³¹ of the nucleated cells in the original leukocyte-rich fraction. The third step is removal of E-rosette forming T cells from the SBA[−] fraction by centrifugation over Percoll gradient. In this step residual bands and polymorphonuclear leukocytes are also removed, together with the rosetted cells, during centrifugation. The average recovery of non-rosetting cells (SBA[−] E[−]) from the SBA[−] cell fraction is about 30%³¹.

The infusion of the SBA⁺ fraction of separated leukocytes provides saturation of the reticulo-endothelial system, so the SBA[−] E[−] cells are not compromised by phagocytosis.

There are several reports on the outcome of TCD and HRD BMT from other centers. In the European survey, published in 1990⁷ T cell depleted HLA-non-identical BMT from related donor was associated with a 52% long-term survival.

In a British study, published in 1977, DICKINSON et al.³, reported a success rate of 50% in 9 out of 18 children with SCID transplanted with TCD (Campath 1M plus fresh donor complement).

Recently, in 1998, HADDAD et al.¹³ published the outcome of TCD HLA-nonidentical related BMT performed in 18 European centers on 193 patients with SCID. One hundred sixteen of 193 patients survived (60%) with the evidence of engraftment 6 months post BMT.

One of the most important factors affecting the outcome of bone marrow transplantation in patients with severe combined immunodeficiency is the presence of pre-existing infections prior to BMT. Children who survived, were younger than the children who died after TCD BMT (7.5 vs. 11.4 months). The younger age reflected earlier diagnosis and fewer debilitating infections. Thus the importance of early diagnosis, prevention and treatment of infections while awaiting BMT must be emphasized. BMT should be considered an urgent priority since delay increases the risk of infection.

Following bone marrow transplantation, recipients are characterized by a varying period of immunoincompetence. This is due to a lack of sustained transfer of donor antigen specific immunity, the recapitulation of immunological ontogeny and reduction of thymic function. Additionally, T cell depletion delays reconstitution because of removal of donor mature T cells. The additional important factor, especially in the case

of HLA mismatched transplants, is the presence of acute and/or chronic GvHD and anti-GvHD therapies²⁷ which inhibit lymphopoiesis.

SMALL et al.³⁷ concluded from the studies of TCD HLA-identical BMT in 170 patients with different, mostly malignant diseases, including 41 children, that delayed immune reconstitution following TCD BMT was associated with older age and graft rejection prophyllaxis rather than T-depletion alone.

In most cases, sustained antibody production and antigen specific T cell response is not present following transplantation unless the recipients are reimmunized or have a viral reinfection following BMT¹¹. In order to evaluate a post transplant specific immune response, the recipients have been immunized with protein, tetanus toxoid (TT). This is usually done around day +100 post BMT, as by that time most of the patients recover their T cells. More recently, experimental vaccination with $\emptyset$ X-174 has been used as an antigen to which the patient and the donor have never been exposed, in order to test antibody protection against encapsulated organisms.

Early diagnosis, prevention or treatment of opportunistic infections, and enhancement of immune recovery will be necessary to improve survival in patients with SCID treated with TCD-BMT.

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