Cross Correction Following Haemopoietic Stem Cell Transplant for Purine Nucleoside Phosphorylase Deficiency: Engrafted Donor-Derived White Blood Cells Provide Enzyme to Residual Enzyme-Deficient Recipient Cells

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Abstract Purine nucleoside phosphorylase (PNP) deficiency is an immunodeficiency disorder characterized by recurrent infections, failure to thrive and neurologic symptomatology. While enzyme replacement therapy (ERT) is a therapeutic option for adenine deaminase (ADA) deficiency, a similar disorder, this is not available for PNP deficiency, and bone marrow transplant (BMT) is the only treatment option. Moreover, even with BMT, improvement of neurological deficits is not definite. We describe a 16-month-old boy who underwent BMT for PNP deficiency which resulted not only in freedom from infections but also in neurological improvement and autologous T-cell recovery.

Pre-transplant, this child had severe lymphopenia with recurrent infections and psychomotor retardation. Post-transplant, in the presence of mixed chimerism, he had normal lymphocyte count, including presence of recipient T cells and neurological improvement. The re-emergence of recipient T cells, when there were virtually no such cells pre-transplant, and the neurological improvement are indicative of improvement of the enzyme deficiency in tissues which remain genetically enzyme depleted.

These defects are not directly corrected by BMT, but are due to delivery of the missing enzyme by the transplanted tissue. In this aspect, transplantation in PNP deficiency is similar to transplantation in other inborn errors of

metabolism where the engrafted donor cells deliver enzyme and restore function to deficient tissues. This further lends support to the recommendations that BMT should be the favoured treatment option in disorders like ADA deficiency or Hurler syndrome, where, even though ERT is available, it is limited by inability to correct the central nervous system defects.

Introduction

Purine nucleoside phosphorylase (PNP) is an extremely rare metabolic disorder. It has been reported in less than 50 patients in the world and is among the rare causes of severe combined immunodeficiency (SCID). Adenine deaminase (ADA) deficiency, on the other hand, has an overall incidence of about 1 in 100,000 live births and accounts for about 20% of cases of SCID. Left untreated, these patients usually die in the first year of life, often succumbing to infections. Up to two-thirds of patients with PNP deficiency also have neurologic abnormalities (Hirshhorn and Canotti 2007; Hershfield and Mitchell 2001).

Both PNP and ADA are key enzymes in the purine salvage pathway which is vital for the removal of metabolites of DNA breakdown. Lack of these enzymes allows intracellular accumulation of such metabolites which are particularly toxic to immature lymphoid cells, leading to lymphopenia and impaired cell-mediated immunity (Hirshhorn and Canotti 2007; Hershfield and Mitchell 2001). Bone marrow transplant (BMT) is an effective treatment option for PNP deficiency, though it may not always be “curative” for neurological defects (Hallett et al. 1999; Myers et al. 2004).

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Case Report

The 16-month-old boy mentioned in this study was the first child of unrelated parents with no significant family history. He was referred with a history of recurrent chest infections starting from the age of 3 months associated with failure to thrive and delayed motor development. He had two hospital admissions in the previous 12 months with bilateral pneumonias and an episode of severe herpes stomatitis. Initial investigations were as follows: haemoglobin 12.0 g/dL, total leucocyte count $2.6 \times 10^9/L$, platelets $345 \times 10^9/L$ with significant neutropenia (0.1×10^9) and profound lymphopenia. (Lymphocytes $0.41 \times 10^9/L$, CD3 $0.12 \times 10^9/L$, CD4 $0.05 \times 10^9/L$, CD8 $0.06 \times 10^9/L$, CD19 $0.06 \times 10^9/L$, CD56 $0.15 \times 10^9/L$) Further investigations ruled out bone marrow failure syndromes and ADA deficiency was excluded by demonstration of normal ADA activity [130 nmol/mg/Hb/h (range: 40–100)]. Subsequent investigations showed a very low uric acid concentration in blood, a complete absence of PNP activity in red blood cell lysate [PNP RBC concentration of 0 nmol/mg/Hb/h (normal: 3,000–7,000)] and increased urine levels of inosine, guanosine and their deoxy forms, thus confirming PNP deficiency. [Urine inosine: 2.77 mmol/L (0.00–0.001), guanosine: 1.379 mmol/L (0.00–0.001), deoxyinosine: 1.062 mmol/L (0.000–0.001), deoxyguanosine: 0.702 mmol/L (0.000–0.001)].

A decision to perform a reduced intensity conditioned BMT was made on the basis of the boy's poor health. In July 2008, he was conditioned with Fludarabine ($30 \text{ mg/m}^2 \times 5 \text{ days}$), Melphalan $140 \text{ mg/m}^2 \times 1 \text{ day}$ and Alemtuzumab (1 mg/kg in five divided doses) and proceeded to a unrelated donor BMT, matched at HLA class I (HLA-A, -B and -C) and class II (HLA-DRB1 and -DQB1). Graft versus host disease prophylaxis was given

with ciclosporin and Mycophenolate mofetil. Initially there was a good donor cell engraftment with normal T-cell numbers and CD8 count with the majority of the T cells in the peripheral blood being of donor origin, as would be expected. However, his CD4 count remained low and he never achieved B-cell engraftment. With time, the whole blood chimerism fell with concomitant autologous reconstitution. In spite of withdrawal of immune suppression, he eventually lost the graft (Fig. 1). As a result, it was decided to perform a second transplant. This was necessary to achieve B-cell engraftment and to ensure permanence of T-cell numbers. The child had a second BMT from the same donor in July 2010. On this occasion, he was conditioned with Fludarabine (150 mg/m^2), Treosulfan (42 g/m^2) and Alemtuzumab (1 mg/kg).

A year after the second transplant, he has normal lymphocyte subsets (Fig. 1) and is free from infections. Moreover, the second transplant has been associated with improvement in his neurological function. He is now able to walk independently and his speech continues to improve. His peripheral blood shows mixed yet stable chimerism which includes apart from the donor cells also recipient T cells (Fig. 2). The emergence of recipient T cells – where there were virtually no such cells prior to the first transplant – is indicative of correction of the enzyme insufficiency in the recipient immune system, implying that the donor cells are acting as an enzyme delivery system. This allows “detoxification” of recipient T cells and leads to their reconstitution. This is comparable to immune recovery with enzyme replacement therapy (ERT) in ADA deficiency with pegylated adenine deaminase (PEG-ADA) (Hershfield 1995).

With PEG-ADA, even though cellular uptake of the enzyme is not significant, maintaining plasma ADA levels >100-fold normal levels leads to a reduction in

Fig. 1 Serial variation in total lymphocyte count and subsets over 2 years; note the marked lymphopenia. Arrows represent points of the two transplants

Fig. 2 Post first transplant when total PBL chimerism has dropped to nearly 30%, T-cell chimerism (CD3) is maintained at 90%, thus there is minimal autologous recovery. On the other hand, post second transplant, while PBL chimerism is near 60% (and thus there are

adequate cells to deliver the enzyme), CD3 chimerism has been maintained at 60%, indicating the remaining T cells being autologous. (*TL* total lymphocytes, *PBL* peripheral blood leucocytes)

extracellular adenosine and deoxyadenosine levels and subsequent normalization of intracellular levels through maintenance of equilibrium between intra- and extracellular compartments (Booth and Gaspar 2009). It is likely that in our patient, circulating blood cells provided the PNP enzyme and thus led to similar compartment shift of the metabolites.

Discussion

Untreated, both ADA and PNP deficiencies are fatal in childhood. ERT with PEG-ADA has been considered a viable therapeutic option in ADA deficiency while specific enzyme replacement is not available for PNP deficiency. In the former, it allows metabolic detoxification and thus enables immune function, though this can wane with time. Similar pharmacological ERT is also given in lysosomal storage disorders (LSDs) such as mucopolysaccharidosis (MPS) type I (Hurler, Hurler/Scheie and Scheie syndromes), but even then BMT offers a curative option. While it can be argued that BMT procedure, although curative, has its inherent risks of treatment-related morbidity and mortality, ERT has its own limitations. First, there is a risk of an allogeneic antibody response to pharmacological enzyme by the host immune system to which it is a foreign protein. The immune response is more frequently problematic in individuals who produce no protein at all than in individuals who make nonfunctioning enzyme. Such alloantibody responses to enzyme are not found after BMT and this might limit the utility of the ERT (Wynn et al. 2009a, b). Second, the presence of the blood–brain

barrier (BBB) limits enzyme delivery to the central nervous system (CNS), whilst after BMT the bone marrow-derived stem cells can differentiate into blood monocytes that can migrate across the BBB and further differentiate into microglial cells. Microglia of donor origin have the potential to produce therapeutic amounts of the missing enzyme and thus deliver it in the long term to the CNS (Asheuer et al. 2004; Priller et al. 2001; Desnick and Schuchman 2002). For this reason, BMT is the preferred therapeutic modality where there is CNS involvement as a consequence of an inherited deficiency (de Ru et al. 2011). Moreover, there is evidence that metabolic correction and clearance of stored substrate is improved following BMT compared with ERT (Hoogerbrugge et al. 1995). This will however vary between diseases depending upon the secretion of deficient enzyme by engrafted leucocytes.

In this report, we demonstrate correction of disease manifestations of PNP deficiency by delivery of enzyme to tissues which remain genetically enzyme deficient. Residual autologous lymphocyte production is resumed in parallel to allogeneic donor-derived haematopoiesis and lymphopoiesis. This was associated with neurological function improvement. We propose that in this aspect transplantation of PNP deficiency is similar to transplantation in other inborn errors of metabolism such as Hurler syndrome where the engrafted donor blood cells deliver enzyme and restore function to tissues that remain genetically deficient in that enzyme. This can also be extended to ADA deficiency with CNS involvement as in the similar disorder of PNP deficiency, we clearly demonstrate correction of tissues that are not directly corrected by transplanted tissue.

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