Clinical and laboratory features of mesenchymal stromal cells in pediatric stem cell transplantation

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Part 2

Alternative donors

"In the field of observation, chance favors only the prepared mind".

- Louis Pasteur (1822-1895)

Chapter 2

Graft dysfunction and delayed immune reconstitution following haploidentical peripheral blood hematopoietic stem cell transplantation.

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Summary

For many children with life threatening hematological diseases hematopoietic stem cell transplantation (HSCT) is the only curative option. In children lacking a matched related or unrelated donor and with the certainty that, left untreated, death will ensue alternative donors must be sought. Haplo-identical peripheral blood stem cell transplantation (PBSCT) from a healthy parent is a feasible alternative. To reduce the risk of fatal graft versus host disease (GvHD) as a complication of transplant across major histocompatibility antigens, intense T cell depletion is required. Large numbers of purified, cytokine mobilized peripheral stem cells (the so called mega-dose concept) are required to compensate for the significantly increased risk of either graft failure or early rejection. In our unit, despite this approach, graft dysfunction has in a significant group of children proved problematic and, despite salvage attempts at re-transplantation, usually fatal. In children with hematological malignant disease, our overall relapse free survival is 41%. However, successful transplant outcome has been associated with considerable delays in immune re-constitution that can be implicated in subsequent viral reactivation. We are investigating new strategies to improve the outcome of haplo-identical PBSCT which may allow us to offer this form of treatment to more children requiring urgent HSCT.

Introduction

HSCT has been a successful modality of treatment for children with a wide range of disorders for almost forty years. HLA identical family donors can be found in up to 30% of recipients. The chance of finding a fully HLA identical, matched unrelated donor from the worldwide donor registry depends upon the recipients' ethnic background. Up to 70% of recipients of Caucasian origin will be successfully matched but this may be as low as 10% in those of non-Caucasian backgrounds.[1] The inclusion of one-locus mis-matched donors (or two locus mis-matched cord blood) has further increased the chance of finding a suitable donor.[2, 3] In order to procure such a donor considerable time, money and effort is required. Despite these advances, a significant number of children requiring HSCT do not have a suitable donor. For those children requiring urgent HSCT the time delay may preclude a successful outcome. As such, the advent of techniques to facilitate parents as donors has realized the potential that for every child there is a readily available donor. Early experience was associated with the frequent occurrence of GvHD, both acute and chronic, graft rejection and delayed immune reconstitution.[4] Aversa et al.[5] proved that, in the haplo-setting, rejection and GvHD risk could be reduced by supplementing markedly T cell depleted bone marrow with G-CSF mobilized, T-cell depleted peripheral blood stem cells. The mega-dose concept was described by Handgretinger et al.[6] utilizing magnetic cell sorting (CliniMACS®) and positive selection methodology. The CD34+ content of the graft was significantly enhanced and almost devoid of donor T-cells prior to infusion. In their experience, graft dysfunction was statistically related to the total CD34+ cell dose infused.

Although post transplant immune suppression such as CSA and Tacrolimus are unnecessary with intense T cell depletion, a significant delay in immune reconstitution post haplo-identical PBSCT exposes the child to reactivation of infections longer than is evident after an HLA matched HSCT.[7, 8]

In our patient population we have incurred a significant risk of graft dysfunction following haplo-identical PBSCT unrelated to the final dose of CD34+ stem cells administered. In this article we present our center's experience in children undergoing haplo-identical peripheral blood stem cell transplantation since 1998.

Patients and Methods

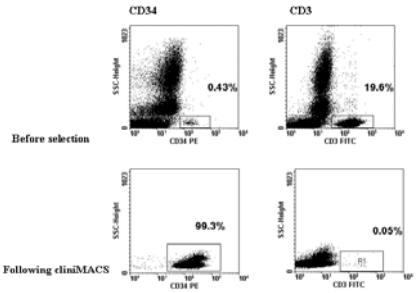
Since 1998, 18 children have undergone 23 haplo-identical PBSCT, 14 boys and 4 girls, aged between 1 and 18 years (median 8.5 years). Indication for transplant was malignancy (n=14, ALL: CR1 n=3; CR2 n=5; CR3 n=1; ANLL/2e ALL n=1; MDS n=2; MDS/ANLL n=1; CML n=1) and non-malignant conditions (n=4). All children with acute leukemia were, at the time of transplantation, in morphological remission. All donors were parents, in twelve instances the father (one father donated twice) and for ten transplants the mother.

In four cases both parents donated. HLA typing was carried out using standard serological methods for HLA A and B loci and high resolution typing for HLA DR loci. Peripheral blood stem cells were mobilized using granulocyte colony stimulating factor (G-CSF Neupogen® Amgen) at a dose of 1.0 IU / kg for a minimum of five days. Stem cells were harvested using continuous flow centrifugation at day 5. In addition day 6 harvests were carried out in order to reach a total CD34 cell dose of 10 x 10^6/kg until 2001, and thereafter 20 x 10^6 / kg recipient's body weight. CD34 positive cell selection was carried out using an automated CliniMACS® protocol according to standard published procedures (Fig. 1). The total CD34+ cells infused ranged from 6.5 to 31.0 x 10^6/kg (median 18.9).

Patients were conditioned according to their underlying disease. Children with ALL received Etoposide i.v. (350 mg/m^2/day, on day –9 and –8), Cyclophosphamide i.v. (60 mg/kg/day, on day –5 and –4), and total body irradiation either in one or two fractions depending upon age (7-12 Gy). Conditioning for ANLL was identical other than Etoposide was omitted. Busulphan i.v. (3.2 mg/kg/day, from day –9 to -6) together with Cyclophosphamide (60 mg/kg/day, on day –4 and –5) and Melphalan 140 mg/m^2 on day –1, was administered to children with MDS. Busulphan i.v. (3.2 mg/kg /day, from day –10 to –7) and Cyclophosphamide (50 mg/kg/day, from day-5 to day –2) was standard conditioning in children with non- malignant conditions.

In patients with rejection or primary non-engraftment further attempt at transplant was undertaken using either OKT3 (from day –1 until day +18) 0.1 mg/kg (maximum dose 5 mg) or Fludarabine (30 mg/day, day –5 to day –1) and Campath IH (0.2 mg/day, day –5 to day –2).

Figure 1. FACS analysis of apheresis product before and after CliniMACS® positive selection



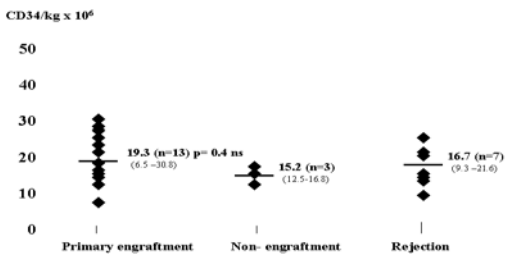
A picture of a FACS analysis of the stem cell product, before and after CliniMACS® magnetic bead separation. G-CSF mobilized, peripheral blood stem cell product is rich in CD34+ and CD3+ cells as demonstrated in the pre-selection analysis. Fatal GvHD would complicate infusion of such a product in the haplo-identical setting. CliniMACS® treatment effectively enriches the stem cell product by positive selection of CD34+ cells and virtually eliminates the CD3+ T cell population.

Results

Sustained engraftment, defined as a persistent absolute neutrophils count > 0.5 x 10^9/l, was observed in 13 patients at a median of 17.0 days (range 13 –26). One of these patients required G-CSF support from day +24 despite a documented 100% donor chimerism, possibly in light of a concurrent adenoviral infection. He later underwent a stem cell boost from the same donor at day +58 and is presently undergoing follow-up to assess his response. Three grafts failed and seven others, although showing initial hematological recovery, rapidly lost their donor cells. Cell doses administered in those with a sustained engraftment (n=13) ranged from 6.5 to 31.0 x 10^6/kg (median 19.3), in cases with non-engraftment (n=3) 12.5-16.8 (median 15.2) and in children with early rejection (n=7) 9.3 –21.6 x 10^6/kg (median 16.7). There was no statistical difference (p=0.4) between the CD34+ cell dose infused and outcome. (Fig. 2)
In patients transplanted for malignancy (n=14) seven are presently alive albeit two of these are +2 months and it is too early to say whether they will remain disease free. Of the truly evaluable patients the overall disease free survival is 42% (Fig. 3) at follow

up of 1.5 to 6 years. Three children who engrafted relapsed (21%). However, five children (35%) developed graft dysfunction of whom, only one survived. Of the remaining four children all but one had documented engraftment following further re-transplantation, but all died from infection.

Figure 2. Scattergram of CD34 cell dose infused and engraftment outcome.



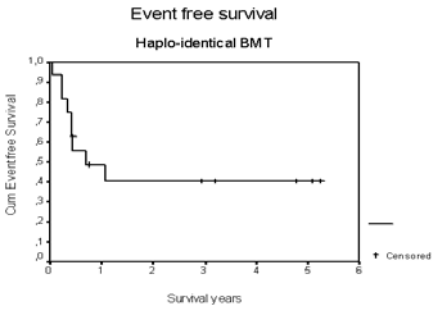
Scattergram of CD34+ cell dose infused in children undergoing haplo-identical PBSCT between 1998 and 2003 related to engraftment. The range of CD34+ cells infused varies considerably, and result from variations in donor mobilization and the weight of the recipient. However, there is no statistical difference between the number of CD34+ cells infused per kg body weight of the recipient and episodes of early rejection and /or engraftment potential. (95% CI: -7.703 to 2.938)

Overall graft dysfunction occurred in 6/18 patients (33%). No acute or chronic GvHD was documented.

In long-term survivors, immune recovery was substantially delayed and no detectable T-cell recovery was evident until 5-6 months post transplant. Functional T and B cell recovery was not evident until considerably later. In contrast, signs of NK activity were detectable as early as one-month post PBSCT. Reactivation of CMV was evident in two children post transplant and herpes zoster in one other child. A total of five patients developed adenovirus infection. In 4/5 this was following second attempt transplantation either with OKT3 or Fludarabine/ Campath IH conditioning. One patient developed adenoviral reactivation (defined as a plasma DNA load > 10^3 copies/mL) shortly after engraftment. He remains under anti-viral treatment with no evident dissemination. One child developed immune mediated thrombocytopenia,

intra-cranial hemorrhage and PCP following steroids and emergency splenectomy, three months after successful engraftment for a secondary leukemia. Four years later, he remains well with no clinically apparent neurological problems and in complete remission.

Figure 3. Kaplan Meyer survial curve of children undergoing haploidentcial PBSCT for malignant hematological disease (n=12)



Kaplan Meyer survival of the first twelve children having undergone haplo-identical PBSCT for underlying hematological malignancies. The DFS is 41% with a follow up of 1.5 to 6 years. This is comparable to published data but the high levels of rejection compounded the success of the procedure.

Discussion

Our results are comparable to the reported literature in relation to overall and disease free survival, especially in pediatric patients with underlying hematological malignancies.[9, 10] The overall survival rates are especially encouraging considering that these children would have certainly died from their initial disease without the transplant procedure. The lack of an otherwise suitable donor was no impediment to hematopoietic stem cell transplantation. Although it is generally reported that the problems of engraftment have been overcome in haplo-identical PBSCT, all centers undertaking this procedure have occasional patients with a dysfunctional graft.[11-13] However, even though some failures may be explained by differences in immune

suppression given pre-transplant and lower doses of CD34+ cells infused,[14, 15] there is no doubt that not all patients receiving mega doses of CD34+ cells are guaranteed engraftment.[16]

Our relatively high levels of graft dysfunction cannot be explained by either low numbers of CD34+ cells infused nor insufficient pre-transplant immune suppression. Despite the introduction of mega doses of stem cells, we are neither able to predict nor overcome graft dysfunction based purely upon the final CD34+ cell count administered, as described by other authors. [6, 17] We routinely administer anti-thymocyte globulin (ATG) to all patients pre-haplo-identical transplant as standard therapy. Children with hematological conditions undergo standard myelo-ablative conditioning, inclusive of total body irradiation, albeit as single fraction rather than fractionated radiotherapy.
As graft dysfunction has reduced the success of our haplo-identical transplants we consider this to be a higher risk procedure than perhaps other centers with less lethal graft failure. Second attempt grafts in our unit have been complicated by lethal infections, mainly adenovirus, despite documented engraftment. It is hoped that with the recent introduction of PCR-monitoring and pre-emptive treatment this, as well as other viral infections, may be controlled. However, in light of the poor immune recovery, especially specific T cell function, the control of infection may yet prove difficult.[8, 11]

We observed a substantial number of viral infections in our patient population but they did not differ substantially from other T cell depleted HSCT's. Delayed recovery of adaptive immunity results from the degree of HLA disparity, the low numbers of T cells infused in the graft and the use of ATG which may inhibit T cell expansion.[12] Pediatric studies show that the median time to normal T and B cell numbers and proliferative responses was 7-8 months and our patients have similar reconstitution profiles.[8] Initial T-cell recovery is of oligoclonal memory T-cells as a results of expansion of peripheral T-cells from the graft. Later, at approximately 6 months, naïve T-cells are recognizable presumably resulting from donor precursor cells emigrating from the thymus.[12] In the pediatric setting immune recovery may be more rapid than in adults because of intact thymic function and because it is feasible to infuse higher doses of

CD34+ cells which enhance the recovery of naïve T-cells.[8] The relatively low numbers of serious infections occurring in our patients with a sustained graft reflects this observation, together with the documented rapid neutrophil recovery.

Conclusion

Haplo-identical stem cell transplantation using highly enriched CD34+ cells is a feasible solution for those children requiring HSCT, who otherwise have no suitable donor. Technological advances have made transplantation across major HLA-antigen barriers possible without the induction of fatal GvHD. Delays in immune reconstitution, despite good myeloid engraftment, are significantly greater than in the HLA matched allogeneic transplant setting, but mirror immune-reconstitution in T-cell depleted bone marrow transplantations. Infections have been no worse than in those children undergoing mis-matched unrelated allogeneic transplantation.
In our experience, non-engraftment and rejection cannot always be prevented by mega-doses of CD34+ stem cells. Attempts at re-transplanting children with graft dysfunction have resulted in significant morbidity and mortality.
Apart from relapse, graft dysfunction and delayed immune reconstitution remain significant barriers to the application of this treatment to many more children, thus overcoming the considerable time delays and expense of complicated donor searches. New strategies are presently being employed in our unit to combat these problems, namely the role of co-transplantation of expanded donor mesenchymal stem cells. These pluripotent stem cells, known to support hematopoiesis, may have an as yet undetermined role in improving the clinical outcome of children undergoing haplo-identical PBSCT.[18]

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References

1. Beatty PG, Mori M, Milford E. Impact of racial genetic polymorphisms on the probability of finding an HLA matched donor. Transplantation 1995; 60: 778-783.
2. Casper J, Cannita B, Truitt R, et al. Unrelated bone marrow donor transplants for children with leukemia or myelodysplasia. Blood 1995; 85: 2354-2363.
3. Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes of 562 recipients of placental-blood transplants from unrelated donors. The New Eng J Med 1998; 339: 1565-1577.
4. Rowe JM, Lazarus HM. Genetically haploidentical stem cell transplantation for acute leukemia. Bone Marrow Transplant 2001; 27: 669-676.
5. Aversa F, Tabalio A, Terenzi A, et al. Successful engraftment of T-cell depleted haploidentical "three loci" incompatible transplants in leukemia patients by addition of recombinant human granulocyte colony-stimulating factor mobilized peripheral blood progenitor cells to bone marrow inoculum. Blood 1994; 84: 3948-3955.
6. Handgretinger R, Klingebiel T, Lang P, et al. Megadose transplantation of purified peripheral blood CD34+ progenitor cells from HLA mismatched parental donors in children. Bone Marrow Transplant 2001; 27: 777-783.
7. Kook H, Goldma F, Padley D, et al. Reconstruction of the immune system after unrelated or partially matched T-cell depleted bone marrow transplantation in children: immunophenotypic analysis and factors affecting the speed of recovery. Blood 1996; 88: 1089-1097.
8. Eyrich M, Lang P, Shangara L, et al. A prospective analysis of the pattern of immune reconstitution in a pediatric cohort following transplantation of positively selected human leucocyte antigen-disparate haematopoietic stem cells from parental donors. British Journal of Haematology 2001. 114: 422-432.
9. Lang P, Handgretinger R, Niethammer D, et al. Transplantation of highly purified CD34+ progenitor cells from unrelated donors in pediatric leukemia. Blood 2003; 101: 1630-1636.

10. Klingebiel T, Handgretinger R, Lang P, et al. Haploidentical transplant for acute lymphoblastic leukemia in childhood. Blood Reviews 2004; 18: 181-192.
11. Bornhauser M, Platzbecker U, Theuser C, et al. CD34+-enriched peripheral blood progenitor cells from unrelated donors for allografting of adult patients: high risk of graft failure, infection and relapse despite donor lymphocyte add-back. British Journal of Haematology 2002; 118: 1095-1103.
12. Veys P. The role of haploidentical stem cell transplantation in the management of children with hematological disorders. British Journal of Haematology 2003; 123: 193-206.
13. Platzbecker U, Ehninger G, Bornhauser M. Allogeneic transplantation of CD34+ selected hematopoietic cells – clinical problems and current challenges. Leuk Lymphoma 2004: 45: 447-453.
14. McDonough CH, Jacobson DA, Vogelsang GB, et al. High incidence of graft failure in children receiving CD34+ augmented elutriated allografts for non-malignant disease. Bone Marrow Transplant; 2003; 31: 1073-1080.
15. Lang P, Handgretinger R, Griel J, et al. Transplantation of CD34+ enriched allografts in children with nonmalignant disease: does graft manipulation necessarily result in high incidence of graft failure. Bone Marrow Transplant; 2004; 33: 125-126.
16. Passweg JR, Kuhne T, Gregor M , et al. Increased stem cell dose, as obtained using currently available technology, may not be sufficient for engraftment of haploidentical stem cell transplantation. Bone Marrow Transplant 2000; 26: 1033-1036.
17. Martelli MF , Reisner Y. Haploidenical "mega dose" CD34+ cell transplants for patients with acute leukemia. Leukemia 2002; 16: 404-405.
18. Maitra B, Szekely E, Gjini K, et al. Human mesenchymal stem cells support unrelated donor hematopoietic stem cells and suppress T cell activation. Bone Marrow Transplant 2004; 33: 597-604.