Importance of allogeneic T-cells for disease control after stem cell transplantation for high-risk Langerhans cell histiocytosis

Reduced intensity conditioning followed by allogeneic SCT (RIC-SCT) has recently emerged as promising new salvage option for children suffering from Langerhans cell histiocytosis (LCH) with risk organ involvement and failure to conventional therapy. We report on the posttransplant course of female toddler with high-risk LCH, who achieved complete remission after RIC-SCT, despite a posttransplant chimerism constellation, in which only the T-cell subset proved to be of donor origin in the long-term. We therefore suggest that allogeneic T-cells have played a crucial role in controlling disease activity in this patient and may exert the major curative effect after RIC-SCT for LCH.

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Introduction

Langerhans cell histiocytosis (LCH) is a rare disorder with a broad spectrum of clinical manifestation patterns and is characterised by a clonal proliferation of dendritic cells sharing phenotypic similarities with the Langerhans cells [1]. The most severe presentation pattern, formerly known as *Abt-Letterer-Siwe* disease, typically occurs in children under two years of age and is defined by a disseminated, multisystem involvement including one or more *risk organs*, i.e. hematopoietic system, spleen, liver and lungs. Within this group of patients a *high-risk*-subgroup has been identified, additionally characterised by treatment failure of conventional chemotherapeutic approaches. This high-risk patient cohort accounts for about 20% of all pediatric multisystem patients and has a poor survival rate of about 20%.[2, 3] Several salvage approaches, including escalation of chemotherapy, monotherapy with 2-CdA and CSA, as well as myeloablative SCT have not resulted in an improved outcome in this patient group.

Recently, the concept of a reduced intensity conditioning regimen followed by allogeneic SCT (RIC-SCT) has emerged and was recently considered a promising salvage approach by a study of nine patients, including the patient presented herein [4]. The particular posttransplant course of chimerism in the presented female toddler with achievement of complete remission of high risk LCH following RIC-SCT by only the T-cell lymphocyte subset being of donor origin in the long-term, prompted us to reflect more accurately on this matter. Possible explanations in the view of current pathophysiologic understandings of the disease and the potential role of allogeneic T-cells in disease control are discussed.

Case report

Patient presentation. The patient was the second child of non-consanguineous healthy Greek parents. At the age of 8 months she presented with persistent diarrhoea and a failure to thrive. Within the following 4 months the girl developed transfusion-dependent anemia (Hb 49 g/L) and thrombopenia (6.000 G/L), hepatosplenomegaly with ascites and liver dysfunction (albumin 12 g/L; _-GT 394 U/L; total bilirubin 151,7 µmol/L), together with refractory fever and a generalized maculopapulous rash. At age of 12 months skin biopsy revealed the histological diagnosis of Langerhans cell histiocytosis. Staging procedures showed involvement of both mastoids and severe hemophagocytosis was present in the bone marrow. The diagnosis of multisystem LCH with risk organ involvement was established and chemotherapy according to the LCH-1 protocol with prednisone, vinblastine and etoposide was introduced. After a short initial period of clinical improvement the general condition continuously deteriorated under chemotherapy. At the age of 15 months the girl was transferred to our transplant unit with treatment-refractory high risk LCH for allogeneic SCT. At this time disease manifestations were present as mentioned above, the extent of transfusion dependence was 2 transfusions per week for red cells and platelets, respectively.

Transplantation. Non-myeloablative conditioning consisted of six consecutive doses of fludarabine (30 mg/m^2 on days -8 to -3), one dose of melphalan (140 mg/m^2 on day -2) and five consecutive doses of MabCampath (anti-CD52-Ab, alemtuzumab) (0,2 mg/kg on day -7 to -3). The graft was unmanipulated bone marrow of her 5 years old, HLA-identical sister. The graft contained 16x10^6/kg CD34+ cells and 1x10^8/kg T-cells. Posttransplant GvHD and rejection prophylaxis consisted of MabCampath 0,2 mg/kg/d (day +1 to +5), cyclosporin A with a targeted level of 100-120 ng/mL (day -1 until +323), mycophenolate mofetil (day 0 until +84) and prednisone (day +1 until +271).

Donor lymphocyte infusions (DLI) due to decreasing donor chimerism were given on days +67 (1x10^7 CD3$^+$ cells/kg) and +180 (6x10^6 CD3$^+$cells/kg) in order to augment donor cell engraftment. Cells for DLI were obtained from lymphocyte apheresis of the donor.

Methods. Chimerism analysis of FACS-sorted cell populations was performed once weekly from day +7 until day +100 and every four weeks thereafter until day +500. Sorted CD45+ leucocytes included CD33+ monocytes, CD15+ granulocytes, CD3+/4+ and CD3+/8+ T cells, CD56+/CD3- NK cells and CD19+ B-cells.

FACS sorting of leucocyte subpopulations on the FACSVantage SE (BD) was performed after three-colour staining in a lyse-and-wash procedure. All cell types exceeding 1% of NC were targets for cell sorting. The number of target cells sorted for subsequent polymerase-chain reaction (PCR) ranged between 1000 and 15000. The purity of the sorted leucocyte fractions was generally >98%. For subsequent PCR, DNA was extracted from nucleated cells using the QIA amp Blood kit (Qiagen, Hilden, Germany). Recipient and donor DNA were tested prior to transplantation by a panel of seven highly polymorphic short tandem repeat (STR) markers to select an informative primer set suitable for the monitoring of chimerism during the post-transplant course. Upon amplification by PCR, the alleles were quantified by capillary electrophoresis and fluorescence-based quantification using the ABI Prism 310 Genetic Analyser (Applied Biosystems, Foster City, USA).

Posttransplant course. The conditioning regimen was well tolerated and no major transplant-related toxicities or infections were seen during the posttransplant clinical course, except the appearance of WHO grade 1 mucositis. Primary engraftment, defined as an absolute neutrophil count above 0.5x10^9/L (500/µL) and evidence of donor chimerism, was seen on day +21. T-cells rose above 100/µL on day +102. A slow, but constant decrease of LCH disease activity was clinically reflected by the achievement of transfusion independence of blood products (day +30), regression of hepatosplenomegaly (day +42) and remission of fever (day +33). After initial engraftment, lineage-specific chimerism was characterised by a decline of donor allelic signals in all analysed cell subsets except T-cells, despite performance of two donor lymphocyte infusions. Clinically, no LCH activity was verifiable anymore from

day +60 post SCT and also the developmental delay caused by the severe illness was gradually caught up by the child. At the same time, hemophagocytic activity, as well as the high proportion of CD1a positive cells in the bone marrow regressed.

Despite the good clinical condition of the patient a routine x-ray performed at day +300 revealed an isolated LCH reactivation in the bones (vault, clavicle), which promptly responded to a short course of chemotherapy with prednisone and vinblastine for three months. 725 days post SCT she is off any therapy and in good clinical condition without evidence of LCH disease activity.

Discussion

The posttransplant clinical course in the presented case appears particularly interesting in synopsis with the development of lineage specific chimerism. To date, the general pathogenesis of LCH still remains poorly understood. It is assumed that LCH is a disease primarily caused and promoted by clonal proliferation of dendritic cells bearing a Langerhans cell phenotype.[5-7] Accordingly, the eradication of this pathogenic clone, or the establishment of a donor-derived dendritic cell lineage by means of SCT would suggest curative potential. Despite the fact that exclusively the T-cell subset was of donor genotype and all the other cell-subsets, including monocytes were of recipient origin, long-term remission of systemic LCH was achieved in this critically ill child. As the conditioning regimen was non-myelobalative and myeloid hematopoietic reconstitution was autologous, a potential eradication of the causative cell clone by the conditioning regimen seems rather unlikely in this case. We therefore suggest that allogeneic T-cells have played a crucial role in controlling disease activity in this patient. It can be presumed that in LCH, unlike in true malignant diseases, not an eradication of the pathologic cell clone (i.e. LCH cell clone), but rather a strong immunomodulating influence, probably mainly exerted by allogeneic T-cells (*graft versus histiocytosis* effect), may incorporate a curative potential. An *in vitro* study, which revealed a dysfunctional cross-talk between T-cells and LCH cells with resulting abundant cytokine secretion as a critical pathophysiological promoter of disease activity, may support this hypothesis.[8] On the other hand, the adoption of anti-CD52 antibody (alemtuzumab) in the conditioning regimen may also enable to directly target LCH cells, as they have recently been reported to aberrantly express the CD52 antigen on their cell surface.[9]

In conclusion, the underlying salutary effects of non-myeloablative SCT in LCH may basically emerge from the highly immunosuppressive and -modulating properties of the donor immune cells (particularly allogeneic T-cells) and of the conditioning regimen (particularly fludarabine and anti-CD52-antibody) on a deranged host immune system. Further research, particularly focused on the role of T-cells in LCH pathogenesis and in the posttransplant setting, may help to improve our knowledge and the therapeutic approaches to this still enigmatic disease.

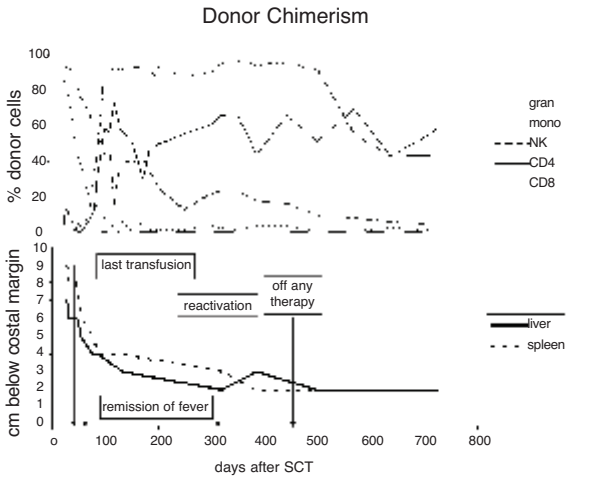

Figure 1. Posttransplant donor chimerism in synopsis with the posttransplant disease course.

M. Steiner,[1] S. Matthes-Martin,[1] A. Attarbaschi,[1] A. Lawitschka,[1] M. Minkov,[1] E. Mittheisz,[1] G. Fritsch,[2] T. Lion,[2] A. Zoubek[1] and H. Gadner[1]

[1]St. Anna Children's Hospital, Vienna, Austria
[2]Children's Cancer Research Institute, Vienna, Austria

Correspondence: Helmut Gadner, MD
St. Anna Children's Hospital Kinderspitalgasse 6
A-1090 Vienna, Austria
Fax: +43-1-40170-70 Tel: +43-1-40170-250

E-mail: helmut.gadner@stanna.at

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