The Liver Graft as Trojan Horse—Multilineage Donor-Derived Hematopoiesis After Liver Transplantation: Case Report


ABSTRACT

Hematopoietic macrochimerism, which is rarely seen after orthotopic liver transplantation (OLT), has been linked to the development of graft versus host disease (GVHD). We report on a patient with GVHD after OLT in whom full engraftment of donor-derived, multilineage hematopoiesis occurred, indicating that the liver contains pluripotent hematopoietic progenitor cells (HPC) capable to restore hematopoiesis in recipients. Although preventing graft rejection, standard immunosuppressive therapy may be under certain immunological conditions not sufficient to prevent GVHD. Age-, disease-, and treatment-related variables might be critical determinants for the development of an effective alloreactive T-cell response leading to the establishment of full hematopoietic chimerism.

ACUTE and chronic GVHD represent a common complication of hematopoietic stem cell transplantation (HSCT). Donor-derived allo-reactive immune cells attack host tissues comprising skin, bile duct epithelium, gut and bone-marrow, thus leading to significant rates of morbidity and mortality. Recipients of liver grafts, however, are rarely affected by GVHD which usually leads to fatal outcome.1 Herein we present a patient who developed severe GVHD after liver transplantation accompanied by engraftment of liver-derived HPC leading to full donor derived hematopoiesis.

CASE REPORT AND METHODS

A 72-year-old male Caucasian underwent OLT for end stage cryptogenic liver cirrhosis with hepatocellular carcinoma, receiving a cadaveric graft from a 19-year-old male donor. Immunosuppressive prophylaxis consisted of basiliximab, tapered steroids, tacrolimus (switched to cyclosporine because of hyperglicemia) and mycophenolate mofetil. Three weeks post transplant severe pancoptnypenia occurred. The peripheral blood leukocyte count declined from 4.7 × 10⁹/L on day 20 to 0.2 × 10⁹/L on day 25 (normal range: 4.7 to 10.0 × 10⁹/L), with less than 0.1 G/L neutrophils and platelet count was <20 × 10⁹/L. The patient's body temperature increased to 38.3°C and treatment with lenoglatin 34 Mio. U/d (days 22-41), antibiotics and fluconazole was started. On day 23 the patient developed a maculopapular rash. The skin symptoms rapidly deteriorated (Fig 1) and watery diarrhea occurred. The clinical diagnosis of severe GVHD was made and confirmed by histological assessment of skin and colonic biopsies. Treatment with methylprednisolone IV 4-10 mg/kg BW (days 33-36) and alemtuzumab 20 mg IV on days 35 and 39 did not improve clinical signs. Ultimately, serum of a volunteer with anti-donor HLA-antibodies (anti-HLA-B7, titer 1:4) was administered intravenously on days 40 and 41 to deplete recipient-reactive donor T-cells. Peripheral blood leukocyte counts recovered to normal values at day 52 (88% neutrophils and myelocytes, 2% monocytes, no lymphocytes). Serological HLA typing was performed by routine serological methods using typing trays from Biotest (Biotest AG, Dreieich Germany) and Referece (Dynamo, Oslo, Norway) and revealed a 100% donor phenotype of peripheral blood mononuclear cells (MNC). The recipients phenotype was: HLA class I-antigens: A1, A29(19), B44(12), B57(17) Bw4, Cw6 and HLA class II-antigens: DRB1*07, DRB4, DQB1*02, DQB1*03. The donor genotype was: A*03, B*07 (class I) and DRB1*08, DRB1*15, DRB5. Chimerism was confirmed by STR typing, performed by routine PCR using sequence specific priming (PCR-SSP) reagents.

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HEMATOPOIETIC MACROCHIMERISM AFTER OLT

Fig 1. Graft versus host disease affecting the skin. Two weeks after OLT a maculo-papular skin rash occurred which deteriorated rapidly to bullae and desquamation. Histological analysis revealed severe GVHD.

(Genovision, Vienna, Austria). Buccal swabs and 10 mL blood samples were taken from the patient for the amplification of locus DSS1132 and 100 ng of genomic DNA, extracted with a commercial kit (NucleoSpin Blood, Macherey-Nagel, Vienna) from whole blood, was used.

HLA genotype and RH red blood cell antigens were analysed by BHD and RHCE PCR-SSP as described previously and were also found to be of donor origin (Fig 2A). Despite hematopoietic engraftment the patient developed septic multiorgan failure and died 45 days after OLT. A bone marrow biopsy showed a cellularity of 60% and erythropoiesis, myelomonopoiesis and megakaryopoiesis appeared normal (Fig 2A). Bone marrow MNC were assayed and analyzed for erythropoietic burst forming unit (BFU-E), granulocytic (CFU-G) and mononuclear (CFU-M) progenitor cells as described previously. Single colonies were picked and chimerism analysis by PCR revealed a complete donor genotype in all individual colonies tested, indicating full engraftment of HPC with multilineage differentiation potential from the donor liver in the recipients bone marrow (Fig 2C). At autopsy no evidence of extramedullary hematopoiesis was found.

DISCUSSION

A minuscule number of donor derived hematopoietic cells can be found in peripheral blood shortly after liver transplantation resulting in a state of microchimerism (ie, <1% of donor cells). Patients with macrochimerism (>1% of donor derived hematopoietic cells) following OLT are prone to develop severe GvHD with an incidence of about 1%. Notably, it has been demonstrated that donor-derived HPC expressing the CD34 antigen are detectable in peripheral blood and bone-marrow of liver recipients up to several years after OLT. However, since CD34 antigens may also be expressed by endothelial cells detectable in peripheral blood as circulating endothelial cells, a long-term endothelial rather than hematopoietic chimerism cannot be excluded. The engraftment of donor-derived hematopoietic cells in the recipient’s bone-marrow requires a distinct immunological environment to allow foreign cells to survive and to proliferate. In allogeneic HSCT, these conditions are established by prior radiochemotherapy and immunosuppressive drugs before transplantation. In the case of GvHD after OLT, immunocompetent cells derived from the donor organ may attack and eliminate blood and bone-marrow cells of the recipient, allowing the colonization of the bone marrow by donor-derived, graft-resident HPC. To the best of our knowledge, only a single case of well documented full donor-type hematopoietic chimerism associated with GvHD has been reported so far. Notably, there are some striking similarities in comparison to our case: (1) Both patients received OLT for liver cirrhosis, a disease that per se is associated with an immunodeficient state; (2) in both cases, GvHD-induced bone marrow failure occurred 3-4 weeks after transplantation and both recovered with full donor-type hematopoiesis and a normal bone marrow cellularity; (3) no T-cell depleting agents for prophylactic immunosuppressive therapy were administered; (4) there was a substantial age difference (~50 years) between donor and recipient. In the case reported by Collins et al., a 65-year-old woman received a liver transplant from a 17-year-old male donor. In our case, the recipient was 72 years old and the sex-matched donor was 19 years old. Indeed, the risk of GvHD was found to be 10-fold higher if the donor is more than 40 years younger than the recipient.

We hypothesize that immunosuppressive prophylaxis might exert different effects on host and recipient immune cells in elderly recipients of grafts from substantially younger donors. In our patient, the switch from tacrolimus to cyclosporine might have additionally affected the regulatory T-cell (Treg) compartment of both, recipient and donor, as it preferentially inhibits Treg function by interfering with calcineurin-dependent IL-2 production, which is critical for Treg proliferation and survival. This might have tipped the balance to an exaggerated T-cell response without inhibitory Treg control, mediated by donor-derived alloreactive effector T-cells. Alloreactivity of donor-derived T-cells might have further been facilitated by the fact that the diversity of the T-cell receptor repertoire diminishes with age and is substantially exhausted in elderly subjects. In addition, loss of the co-stimulatory signal CD28, lower levels of Treg and a diminished B-cell function could be found as indicators of an impaired immune function in the elderly.

After transplantation of other solid organs (ie, kidney) appearance of GvHD has not been described so far. This difference is most likely due to the fact that the liver contains large numbers of different T-cell populations. In fact, approximately 1-3 x 10^6 lymphocytes per kg body weight of the recipient are delivered with a donor liver. Moreover, the composition of lymphocytes residing in the liver is completely different from peripheral blood and bone marrow, with an inversed ratio of CD4 and CD8 T-cells, and abundant T-cells with uncommon phenotypes, e.g. CD4+CD8+, CD4−CD8− and many γδT-cells.

Furthermore, recipients of liver grafts are often immunocompromised due to their underlying disease (eg, liver cirrhosis). Thus, a large number of immunocompetent
Fig 2. (A) DNA-typing of DRB1,3,4,5* low resolution (23 PCR-SSP reactions) and RHCE (17 PCR-SSP reactions) of the recipient, donor and post-transplantation DNA. Whereas the original recipient’s DNA clearly types as DRB1*07 and RhCcEe (upper lane), the donor’s DNA shows DRB1*08, 15 and RHCcEe (middle lane). The recipient’s post-transplant DNA types exactly as the donor’s DNA for HLA, only reaction 3 of the RHCE typing-block gives a weak positive signal indicated by a white asterisk, pointing to the presence of a RHCcEe/RHCcEe chimerism (lower lane). (B) A bone-marrow biopsy after recovering from GvHD induced pancytopenia revealed a normal cellularity and all hematopoietic cell lineages were present including megakaryocyte. (C) Genotyping of single hematopoietic colonies grown from bone-marrow cells showed all colonies to be donor derived. R: recipient; D: donor.

REFERENCES
2. Rozman P, Dono T, Gassani C. Differentiation of autologous ABO, RHD, RHCE, KEL, JK, and FY blood group genotypes by analysis of peripheral blood samples of patients who have recently received multiple transfusions. Transfusion. 2000;40:936–942.


