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Research paper

Transplantation of CD6-depleted peripheral blood stem cells after DLA-haploidentical bone marrow transplantation contributes to engraftment and tolerance in a preclinical model of stem cell transplantation

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ABSTRACT

Human leukocyte antigen (HLA)-haploidentical stem cell transplantation is an opportunity for nearly all patients lacking an HLA matched stem cell donor. However, graft rejection and graft-versus-host disease (GvHD) as well as infectious complications still result in high treatment-related mortality.

Here, we used the dog as a preclinical model for the study of tolerance induction with the aim to optimize and to improve a clinical protocol of haploidentical stem cell transplantation. For this purpose CD6-depleted peripheral blood stem cells (PBSCs) were transfused 6 d after transplantation of unmodified bone marrow from dog leukocyte antigen (DLA)-haploidentical littermate donors in order to induce immune tolerance. Besides hematopoietic stem cells CD6-depleted PBSC contain, NK cells and a minority of suppressive CD8-positive cells that may suppress activated T lymphocytes. Recipients were conditioned with, cyclophosphamide and antithymocyte globulin (ATG) preceded by a transfusion of donor buffy coat and either 1, 2 or 3×3.3 Gy total body irradiation (TBI). Postgrafting immunosuppression was limited to 30 d of cyclosporine and methotrexate.

Abbreviations: ATG, antithymocyte globulin; BFU-E, burst forming unit with erythrocyte morphology; BM, bone marrow; BMT, bone marrow transplantation; CFU, colony forming unit; CFU-E, colony forming unit with erythrocyte morphology; CFU-G, colony forming unit with granulocyte morphology; CFU-GM, colony forming unit with granulocyte and macrophage morphology; CFU-GEMM, colony forming unit with granulocyte, erythrocyte, monocyte and macrophage morphology; CFU-M, colony forming unit with macrophage morphology; CY, cyclophosphamide; d, day(s); DBC, donor buffy coat; DLA, dog leukocyte antigen; DLT, donor lymphocyte transfusion; f, female; FACS, fluorescence activated cell sorting; GvHD, raft-versus-host-disease; GvL, graft-versus-leukemia; Gy, gray; HLA, human leukocyte antigen; h, hour(s); i.v., intravenously; kg, kilogram; MHC, major histocompatibility antigen; MLC, mixed lymphocyte culture; MNC, mononuclear cells; m, month(s); MTX, methotrexate; n.e., not evaluable; NK-cells, natural killer cells; p., post; rbC, baby rabbit complement; PBMCs, peripheral blood mononuclear cells; PBSCs, peripheral blood mobilized stem cells; RIC, reduced intensity conditioning; s.c., subcutaneously; SD, standard deviation; SGD, selective gut decontamination; TBI, total body irradiation; TCD, T-cell-depletion; TP, transplantation; yr, year(s).

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The additional administration of CD6-depleted PBSCs after unmodified marrow could not prevent GvHD, but it may improve engraftment and chimerism after conditioning with 2×3.3 Gy TBI. Reasons for incomplete suppression and possible improvements for clinical applications are discussed.

1. Introduction

Allogeneic stem cell transplantation may be curative for patients with otherwise refractory hematologic malignancies (Thomas, 1983). The aim of stem cell transplantation in the treatment of hematologic malignancies is twofold: induction of transplantation tolerance in the host-versus-graft as well as in the graft-versus-host direction while preserving a graft-versus-leukemia and graft-versus-tumour effect, respectively. (Adler and Turka, 2002). Transplantation tolerance can best be obtained with a graft from a human leukocyte antigen (HLA)-identical sibling (Goulmy, 1997; Szydlo et al., 1997). However the chance of having an HLA-identical sibling is only about 30% (Handgretinger and Lang, 2008; Petersdorf, 2008). The chance of having an HLA-matched unrelated donor is about 70–80%, a haplotype mismatched family donor can be found for almost every patient (Bethge et al., 2006; Copelan, 2006; Grewal et al., 2003). The advantage over cord blood transplantation is the availability of the donor for immunotherpay in the case of residual or relapsing disease.

However, HLA haploidentity is a barrier to haematopoietic stem cell transplantation: graft rejection, severe GvHD and life-threatening infections are the hurdles (Powles et al., 1983; Schattenberg and Dolstra, 2005). Depletion of T cells (TCD) from the donor bone marrow decreases the incidence and the severity of GvHD (Rodt et al., 1979), but it is associated with an increased risk of graft rejection and in cases of malignancy risk of disease relapse (Apperley et al., 1988; Horowitz et al., 1990; Martin et al., 1988). Most conditioning regimen for stem cell transplantation include total body irradiation (TBI) and cyclophosphamide (Thomas et al., 1975). In HLA-haploidentical stem cell transplantation, more intensive conditioning regimen failed to improve survival, and both graft failure and GvHD remained problems (Henslee-Downey, 2001). Therefore, new treatment protocols are needed to reduce toxicity of HLA-haploidentical stem cell transplantation while preserving activity to infection and malignancy.

Large numbers of purified CD34+ stem cells can overcome the HLA-barrier of haploidentical transplantation and induce tolerance (Aversa et al., 1997; Bachar-Lustig et al., 1995). However with depletion of T cells an increased rate of infections and recurrence of malignancy in advanced cases remain problems. In HLA-haploidentical transplantation graft-versus-leukemia activity was observed in cases with transplants of donors with non-cross reactive groups of killer inhibitory receptors (KIR) of NK cells (Ruggeri et al., 2002). Besides their graft-versus-leukemia activity NK cells have a potent suppressive effect on activated lymphocytes. CD6 antibodies have been used for the depletion of T cells (Soiffer et al., 1997a,b, 2001). It was noted that this antibody did not deplete NK cells. This observation and the finding that a minority of CD8 positive cells were retained after CD6-depletion encouraged us to study the transfusion of CD6-depleted, mobilized blood stem cells (PBSC) after transplantation of unmodified marrow transplantation for modulation of host-versus-graft as well as graft-versus-host reaction. Clinical results have been promising including patients with advanced disease (Kolb et al., 2003).

In order to improve the induction of tolerance and chimerism, we used the dog as preclinical model. In accordance with clinical protocols, we studied the immunomodulatory effect of CD6-depleted PBSCs in the canine model of DLA-haploidentical bone marrow transplantation after conditioning with fractionated total body irradiation in combination with cyclophosphamide. The rationale of the study was to find out, whether the total dose of total body irradiation could be reduced without an increased rate of graft rejection and whether GvHD could be modified.

2. Materials and methods

2.1. Animals

All animals were adult outbred beagles of both sexes, raised in the kennels of the Helmholtz Zentrum München (Neuherberg, Germany). The dogs were regularly dewormed and vaccinated against leptospirosis, canine hepatitis, canine distemper and parvovirosis. Dog leukocyte antigen (DLA)-typing was performed by microsatellite analysis of the canine MHC I and MHC II locus, and by mixed lymphocyte cultures (MLC) as described elsewhere (Weber et al., 2003). DLA-typing always included parents and offspring. All animal experiments were in compliance with protocols approved by the Institutional Animal Care and Use Committee and the Committee for Animal Protection of the Government of Upper Bavaria.

3. Bone marrow harvest

Bone marrow (BM) was harvested under general anaesthesia by aspiration from both humeri and femurs and the iliac crest with a total volume of 300-500 ml and kept in heparinized Earle's Media 199 (Gibco®, Invitrogen, Karlsruhe, Germany) for processing and transplantation. The percentage of mononuclear cells in the bone marrow was analyzed in a bone marrow differential slide and the total number of bone marrow mononuclear cells (BM-MNCs) was calculated by correcting the total number of cells by