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Stem Cell Transfusion as a New Method for the Induction of Tolerance in Organ Transplantation

A. de Vries-van der Zwan, M.A. van der Pol, A.C. Besseling, L.P. de Waal, and C.J.P. Boog

A MAJOR goal in organ transplantation is the induction of donor-specific transplantation tolerance, without requiring chronic immunosuppressive therapy. Recently, we developed a murine transplantation model in which recipient mice were treated with a single dose of anti-CD3 and anti-CD4 monoclonal antibodies (MAbs) and low-dose total body irradiation (TBI). Transfusion of donor bone marrow cells across a full H-2 disparity resulted in induction of high levels of stable mixed chimerism and specific T-cell nonresponsiveness. Skin transplantation in chimeric animals performed 2 and 6 months after the conditioning regimen showed indefinite allograft survival as an indication for the development of classical transplantation tolerance.^{1,2}

It has been shown in several animal models that fetal liver cells can reconstitute the hematopoietic and lymphopoietic systems of lethally irradiated recipients, without evidence of graft-vs-host disease (GVHD).³ Furthermore, it has been shown that fetal liver is a rich source of stem cells. Therefore, we used allogeneic fetal liver cells as an experimental model for stem cell transfusion in our nonlethal conditioning protocol. The present study shows that stable multilineage chimerism and donor-specific transplantation tolerance developed across multiple histocompatibility barriers.

MATERIALS AND METHODS

Animals

C57BL/10 (H-2^b) (B10), B10.D2 (H-2^d), and B10.BR (H-2^k) mice were bred and maintained at our animal facility. H-2^{b/d} F1 hybrids were obtained by crossing B10 females with B10.D2 males. Recipients were used at 8 to 14 weeks of age.

Experimental Design

Fetal livers from 15 to 18-day-old B10.D2 or (B10 × B10.D2)F1 fetuses were pooled and with gentle disruption, a single cell suspension was prepared. Erythrocytes were lysed with an ammonium chloride buffer. 10.10⁶ cells were injected into low-dose irradiated (3 to 6 Gy TBI), anti-CD3 and anti-CD4-treated B10 recipients, as described before.² Two months later, tail skin grafting was performed. Each recipient mouse received a control B10 (syngeneic), a B10.D2 or (B10 × B10.D2)F1 (donor), and a B10.BR (third party) transplant. Second donor-type skin grafts were transplanted 4 months after the first skin grafts.

To evaluate chimerism, just before first skin transplantation and

4 months after conditioning, peripheral blood cells and mesenteric lymph node cells were stained with H-2D^d-FITC and H-2K^b-PE (Pharmingen, San Diego, Calif) and analyzed by two-color flow cytometry on a FACScan (Becton Dickinson, Mountain View, Calif). Lineage analysis of chimerism was performed 2 years after conditioning. R-Phycoerythrin (R-PE)-conjugated MAbs against T cells (Thy1.2), B cells (B220), granulocytes (GR-1), and macrophages (MAC-1) (all from Pharmingen) were used in double staining with anti-donor MAb (H-2D^d-FITC). Cytotoxicity assays were performed just before second skin grafting, as described previously.²

RESULTS AND DISCUSSION

B10 recipient mice were treated with anti-CD3 and anti-CD4 MAbs, 3 to 6 Gy TBI, and 10.10⁶ fully allogeneic B10.D2 fetal liver cells. This nonlethal protocol was well tolerated and there was no clinical evidence of GVHD. Engraftment of donor-derived cells occurred in almost all recipients treated with 4 to 6 Gy TBI (Fig 1). In order to maintain the host immune competence as much as possible, we succeeded in manipulating the level of chimerism by either lowering the irradiation dose (Fig 1) or lowering the dose of fetal liver cells (data not shown). FACS analysis revealed that the level of donor-type cells in mixed chimeras remained stable and that both cells of host and donor origin were present in lymphoid (T and B cell) and myeloid (granulocyte and macrophage) cell lineages. Donor-specific unresponsiveness in vitro was determined by cytotoxicity assays. Splenocytes from mixed chimeras were, just like bone marrow-transfused chimeras,^{1,2} functionally tolerant to host and donor-strain alloantigens but were immunocompetent because third-party target cells were lysed (data not shown).

Animals were tested for evidence of donor-specific toler-

From the Department of Transplantation Immunology, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Amsterdam, The Netherlands.

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Address reprint requests to Dr Claire J.P. Boog, Department of Transplantation Immunology, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands.

Fig 1. Percentages of donor-derived cells (H-2^d) in peripheral blood of B10 (H-2^b) recipients measured 4 months after conditioning with anti-T cell MAbs, 3 to 6 Gy TBI, and 10.10⁶ fully allogeneic B10.D2 fetal liver cells.

ance in vivo by skin transplantation. Permanent survival of both first and second donor skin grafts occurred in all chimeric recipients. Syngeneic grafts were also accepted, in contrast to third-party grafts which were rapidly rejected (Fig 2). Untreated B10 control mice rejected both B10.D2 and B10.BR grafts within 25 days. In addition, in the semiallogeneic donor-recipient combination an even lower dose of irradiation (3 Gy) was sufficient for engraftment of donor-type cells and subsequent tolerance induction. We suggest that sharing of MHC antigens between donor and recipient might play an important role in facilitating the development of chimerism and tolerance in organ transplantation.

Our study shows that fully allogeneic fetal liver cells can be used for induction of mixed chimerism and donor-

Fig 2. Graft survival of host (B10), donor (B10.D2), and third party (B10.BR) skin on mixed chimeras and control B10 mice.

specific tolerance in adult recipients. Also, when fetal liver cells were given to immunologically immature newborn mice, long-term survival of subsequent cardiac allografts has been shown.⁴

In conclusion, we have demonstrated that multilineage chimerism and permanent donor-specific tolerance can be induced by fetal liver stem cells across MHC barriers using a nonlethal preparative regimen. There was no clinical evidence for GVHD. We anticipate that our murine system can continue to be a useful model for improving human organ transplantation.

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