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

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

Experimental Design

Fetal livers from 15 to 18-day-old BlO.D2 or (BlO x BlO.D2)F1 fetuses were pooled and with gentle disruption, a single cell suspension was prepared. Erythrocytes were lysed with an ammonium chloride buffer. 10^7 cells were injected into low-dose irradiated (3 to 6 Gy TBI), anti-CD3 and anti-CD4-treated BlO recipients, as described before.² Two months later, tail skin grafting was performed. Each recipient mouse received a control BlO (syngeneic), a BlO.D2 or (BlO x BlO.D2)F1 (donor), and a BlO.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-2Dd-FITC and H-2Kb-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 Phycocerythrin (R PE) conjugated MAbs against T cells (Thyl.2), B cells (B220), granulocytes (GR-1), and macrophages (MAC-1) (all from Pharmingen) were used in double staining with anti-donor MAb (H-2Dd-FITC). Cytotoxicity assays were performed just before second skin grafting, as described previously.²

RESULTS AND DISCUSSION

BlO recipient mice were treated with anti-CD3 and anti-CD4 MAbs, 3 to 6 Gy TBI, and 10^6 fully allogeneic BlO.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-
Fig 1. Percentages of donor-derived cells (H-2d) in peripheral blood of B10 (H-2b) recipients measured 4 months after conditioning with anti-T cell MAbs, 3 to 6 Gy TBI, and 10 x 10^6 fully allogeneic B10.D2 fetal liver cells.

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

References