Tolerance to Skin and Vascularized Cardiac Allografts Using Mixed Chimerism

P.M. Markus, G. Selvaggi, X. Cai, T.E. Starzl, and H.D. Röher

IN recent years a number of methods have been developed to induce donor-specific transplantation tolerance in adult recipients using bone marrow transplantation.[1-4] One approach, using a mixture of syngeneic and allogeneic bone marrow to prepare allogeneic chimeras (A + B − A) has been demonstrated to be effective in achieving long-term donor-specific transplantation tolerance to alloantigens in mice.[5] We adapted the model of mixed chimerism to the rat to investigate the response of mixed allogeneic chimeras to skin and primarily vascularized organ grafts that are limited in mice due to technical difficulties.

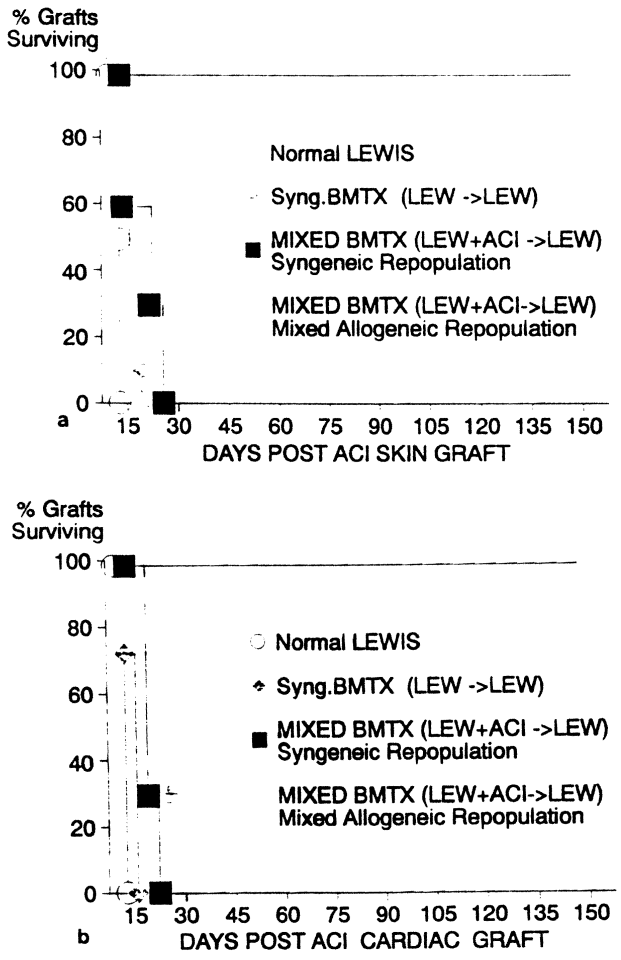

Fig 1. Life table survival of ACI skin **(A)** or cardiac **(B)** grafts in naive, syngeneic, reconstituted, and mixed chimeras. Four weeks after bone marrow transplantation, each animal received an ACI, LEW, and BN skin, or an ACI cardiac graft (only donor type ACI grafts are presented). Animals that received mixed bone marrow yet repopulated syngeneically were analyzed separately (MIXED BMTX, syngeneic repopulation). Each group consisted of six to 10 animals.

MATERIALS AND METHODS

Bone Marrow Transplantation

LEW recipient rats (225 to 250 g) were lethally irradiated with 1000 rad total body irradiation and reconstituted with a mixture of syngeneic LEW (7×10^6 cells/recipients) and allogeneic ACI (43×10^6 cells/recipient) T cell–depleted bone marrow (LEW + ACI-LEW). T-cell depletion (TCD) was performed using magnetic polymer beads (Dynabeads M4501, Bioproducts for Science, Indianapolis, Ind) coated with monoclonal antibody (MAb) OX-19 (anti-CD5). Flow cytometric analyses of spleen controls were routinely performed to assess successful depletion of OX-19$^+$ T cells. Syngeneic reconstituted controls received 50×10^6 TCD syngeneic bone marrow cells (LEW-LEW).

Assessment of Chimerism

Four weeks after reconstitution with mixed allogeneic bone marrow, animals were assessed for the presence of allogeneic donor type ACI T cells. Strain-specific MAb for the RT1.A major histocompatibility complex (MHC) class I antigen was used as primary biotinylated antibody.

Organ Grafting

Full thickness skin grafts of donor (ACI), third party (BN), and host type (LEW) were performed using a modified method of Billingham.[6] A heterotopic abdominal heart transplant model was used for vascularized grafts.

RESULTS

Survival of Mixed Allogeneic Reconstituted Rats

Recipient survival after transplantation of the mixed bone marrow inoculum was 96% at 150 days. Thirty seven percent of all animals (19 of 52) repopulated as mixed chimeras with detectable ACI donor type peripheral blood lymphocytes. The remainder repopulated syngeneically,

From the Department of Surgery, University of Düsseldorf, Düsseldorf, Germany (P.M.M., H.D.R.), and the Department of Surgery, Division of Transplantation Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania (G.S., X.C., T.E.S.).

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Address reprint requests to Peter M. Markus, MD, Department of Surgery, University of Düsseldorf, Moorenstrasse 5, 4000 Düsseldorf 1, Germany.

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despite the mixed ACI + LEW bone marrow transplants. Subsequently, the animals that repopulated as mixed chimeras were used for further investigation (1) to assess the percentage of chimerism and (2) for transplantation experiments with skin and cardiac iso- and allografts.

Assessment of Chimerism

Animals that repopulated as mixed chimeras were evaluated at 1, 3, and 5 months after reconstitution for the percentage of chimerism. The percentage of donor type allogeneic ACI cells varied from 0.5 to 82% and this was maintained throughout the study.

Survival of Skin and Cardiac Grafts in Mixed Allogeneic Reconstituted Rats

Although syngeneic repopulated animals rejected donor (ACI) and third-party (BN) skin grafts, all chimeric animals accepted host (LEW) (data not shown) and donor type (ACI) (Fig 1a and b) skin and cardiac grafts, but rejected third-party (BN) grafts at the same time as naive controls (data not shown). The level of chimerism did not influence the survival of skin or cardiac grafts. All donor type skin and cardiac grafts survived more than 150 days without histologic evidence of chronic rejection. Animals that repopulated syngeneically without detectable chimerism uniformly rejected ACI and BN skin and cardiac grafts.

DISCUSSION

We report a model that achieves stable mixed allogeneic chimerism using transplantation of a mixture of TCD allogeneic and TCD syngeneic bone marrow. Transplantation tolerance was specific for skin and also vascularized cardiac grafts across an MHC and non-MHC barrier (ACI-LEW). The model is still limited by a high number of rats rejecting the allogeneic bone marrow inoculum and repopulating syngeneically. Neither increasing the total number of allogeneic cells nor increasing the number of totally transplanted cells up to 200×10^6 cells/recipient (data not shown) promoted the acceptance of donor type ACI marrow. It is well recognized that TCD of the syngeneic component of the mixed bone marrow inoculum is critical in order to allow engraftment of the allogeneic bone marrow cells when mixed allogeneic reconstitution is performed.[7] One could argue that TCD using OX-19–coated magnetic beads is inefficient in removing all syngeneic $CD\text{-}5^+$ T cells, which then prevents engraftment of the ACI bone marrow component. It is also possible that the rat exhibits a greater level of alloresistance to engraftment that we have found in mouse-to-rat and rat-to-mouse transplantation experiments (data not shown).

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