Published in final edited form as:
Transplant Proc. 1995 February ; 27(1): 164–165.

Vascularized Bone Marrow Transplantation in Rats: Evidence for Amplification of Hematolymphoid Chimerism and Freedom From Graft-Versus-Host Reaction

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The persistence of donor-derived leukocytes, which are capable of proliferating, trafficking, and interacting within the recipient tissues, has been proposed as a biological mechanism which facilitates the induction of acquired tolerance after whole organ transplantation.[1] Although the details of the intrinsic mechanisms which underlie the bidirectional immune modulation are unknown, it is speculated that augmentation of chimerism will favor graft acceptance and may eventually lead to a drug-free existence.

Rat hind limb contains mature lymphocytes (regional lymph nodes), nonparenchymal tissue (skin and muscle), and vascularized bone marrow, which after transplantation provides a continuous supply of donor-derived progenitor cells. In the present study, we compared the level of chimerism induced experimentally by either vascularized bone marrow transplantation (VBMTx) using the rat hind limb allograft model, or else by infusion of either lymph node or bone marrow cells. Their influence on the incidence and severity of graft-versus-host disease (GVHD) was also assessed.

MATERIALS AND METHODS

Animals and Transplant Procedures

Male Lewis (LEW; *RT-1A*^{l}) and Brown Norway (BN; *RT-1A*^{n}) rats, weighing 200 to 300 g, purchased from Harlan Sprague-Dawley (Indianapolis, Ind), were used as donors and recipients, respectively.

Orthotopic transplantation of hind limb allografts was carried out according to a procedure described previously.[2] Briefly, osteo-synthesis was performed at the midfemur level, followed by end-to-end anastomosis of femoral vessels, repair of the sciatic nerve, approximation of thigh muscle, and skin closure.

Bone marrow cells were obtained by flushing the marrow cavities of donor femurs and tibias, whereas, lymph node cells were prepared by gentle teasing in RPMI (Gibco BRL, Grand Island, NY). Cell viability was determined by trypan blue exclusion which was routinely found to be >90%. Each recipient (BN) received via penile vein either 2.5×10^8 LEW bone marrow (BMTx) or lymph node (LNTx) cells.

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Immunosuppression

Recipients were continuously maintained on FK 506 (Tacrolimus. Prograf™, Fujisawa Pharmaceutical Co, Ltd, Osaka, Japan) with daily intramuscular injection of 1.0 mg/kg from days 0 to 13, followed by weekly injections during the follow-up period (100 days).

Flow Cytometric Analysis

Blood was taken from the recipients at various times after transplantation. Red blood cells were lysed by red cell lysis buffer (Sigma Chemical, St Louis, Mo) and lymphocytes were stained with MAb 163, which is specific for the *RT-1A*^{l} antigen on LEW, or with MAb 42, which is specific for the *RT-1A*^{n} antigen on BN (these antibodies were generously provided by Dr H.W. Kunz, Department of Pathology, UniverSity of Pittsburgh). A more detailed phenotype of chimeric cells was obtained by two-color flow cytometric analysis with MAbs specific for lymphocyte lineage markers. The following MAbs were purchased from Harlan Bioproducts for Science, Inc (Indianapolis, Ind): W3/25 (rat α-CD4 and α-macrophage), OX8 (rat α-CD8 and α-NK cells), and R73 (anti-$\alpha\beta$TCR), OX33 (rat α-B cells). Monoclonal antibody 3.2.3 (rat α-NK cells) was generously provided by Dr W. Chambers, (Pittsburgh Cancer Institute, Pittsburgh, Penn).

RESULTS

Recipient (BN) Survival and Incidence of GVHD

Lethal GVHD ensued in all animals that received mature immunocyte infusion (LNTx) with a median survival of 56.5 days (Table 1). None of the recipients treated with bone marrow cells (BMTx) demonstrated either clinical or histopathological evidence of GVHD and all survived for >100 days (Table 1). Interestingly, only 33.5% of VBMTx recipients developed moderate yet self-limiting GVHD between days 50 and 80 after transplantation, whereas the remaining animals were GVHD-free during the entire follow-up period of 100 days (Table 1).

Flow Cytometry

Very low levels of donor MHC class I-positive cells were detectable in recipient PBMC after infusion of either BM or lymph node cells (Table 2). The percentage of donor cells in the recipient PBMC was low (1% to 2%) during the first month after VBMTx; nevertheless, it gradually increased to a higher level (2% to 6%) and remained consistently so throughout the period of immunosuppression (100 days).

When chimeric donor cells were tested with lineage-specific markers, we were able to detect T cells ($\alpha\beta$TCR^{+}), B cells (OX33^{+}), and NK cells (3.2.3^{+}). Furthermore, in BMTx and VBMTx recipients, no specific lineage was found to be predominant; however, this was not true in recipients of LNTx, in whom donor T cells were found to be in abundance (Table 2).

Few donor MHC class II^{+} (L21.6^{+}) cells with dendritic morphology were found in the lymphoid tissues of BM-transplanted GVHD-free animals. On the contrary, GVHD-prone LNTx and VBMTx recipients exhibited a very high frequency of donor class II^{+} cells.

It is of interest to note that after complete withdrawal of FK 506 (100 days posttransplant), VBMTx recipients displayed clinical and histopathological signs of chronic rejection (ie, atrophic skin, muscle contracture, and reduced nociperception), an observation paralleled by disappearance of circulating donor-derived cells in the recipients.

DISCUSSION

This study demonstrates that VBMTx can achieve a significant amplification of donor chimeric cells without the concomitant induction of lethal GVHD. The augmentation of donor leukocyte chimerism was best achieved with VBMTx, and only 33% of these animals subsequently developed GVHD. On the contrary, despite moderate levels of chimerism, LNTx recipients developed lethal GVHD and died with a median survival of 56 days. These observations suggest that phenotypic characteristics rather than the level of donor-cell chimerism dictate the emergence of lethal GVHD.

Since multilineage macrochimerism induced by VBMTx was associated with fewer incidences of self-limiting GVHD, it is tempting to speculate that the trafficking of progenitor cells from the graft into the recipient and the subsequent establishment of mixed chimerism might be the basis for attenuation of GVH responses.[3] It is noteworthy that the induction and perpetuation of donor-cell chimerism was intimately associated with the well-being of the hind limb allograft, since termination of FK 506 therapy, which ushered in chronic rejection, closely shadowed the disappearance of donor-cell chimerism in the periphery. It remains to be ascertained if macrochimerism induced by VBMTx confers any functional advantage over microchimerism in achieving transplantation tolerance.

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Table 1

Animal Survival and Incidence of GVHD After Vascularized Bone Marrow (VBMTx) and Lymph Node (LNTx) and Bone Marrow (BMTx) Cell Transplant From LEW→BN

Types of Allografts	n	Animal Survival Median (days)	GVHD (%)	
			Incidence	Mortality
VBMTx	6	>100	33.5	0
LNTx	6	56.5	100	100
BMTx	6	>100	0	0

Table 2

Characteristics of Donor-Cell Chimerism After Vascularized Bone Marrow (VBMTx) and Lymph Node (LNTx) and Bone Marrow (BMTx) Cell Transplant From LEW→BN

Types of Allografts	Level of Chimerism	Characteristics of Chimeric Cells
VBMTx	1.2-6.7%	Multilineage
LNTx	1-2%	Predominantly T cell
BMTx	<1%	Multilineage