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Effect of Tacrolimus and Splenectomy on Engraftment and GVHD After Bone Marrow Xenotransplantation in the Reciprocal Hamster to Rat Animal Models

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BONE marrow transplantation (BMTx) following lethal irradiation is an established modality to induce donor-specific tolerance in the rat to mouse xenogeneic combination.[1] The induction of donor-specific tolerance in this model is accompanied by a distinct absence of graft-versus-host disease (GVHD). The latter outcome, however, is not witnessed when BMTx is performed in hamster to rat strain combinations in which, despite splenectomy (Spx), the recipients invariably succumb to lethal GVHD.[2] The advent of tacrolimus (TCL, FK 506) as a potent immunosuppressive agent for the prevention and treatment of GVHD in allogeneic BMTx[3,4] prompted us to assess its efficacy on engraftment and GVHD after BMTx in the reciprocal hamster to rat xenograft combination.

MATERIALS AND METHODS

Animals. Syrian hamsters (100 to 120 g) and LEW rats (180 to 220 g) were used as donors and recipients, respectively.

Bone Marrow Transplantation. LEW recipients were splenectomized 7 days before BMTx and injected with 3×10^8 unfractionated hamster bone marrow cells (BMC) following irradiation (10.5 Gy). The hamster recipients received a similar quantity of LEW BMC and irradiation dose but were not subjected to Spx. Failure of BM to engraft was defined as recipient death within 14 days postinfusion without recovery of peripheral white blood cell count. Experimental groups are depicted in Table 1.

Tacrolimus. FK 506 (1 mg/kg/d; IM) was given daily to the recipients from days 0 to 30.

Detection of Chimerism. The presence of donor cells in the recipient's peripheral blood was determined by flow cytometry using LEW antihamster or hamster anti-LEW IgG antibodies, which were prepared by immunization of the appropriate animals with splenocytes. The IgG fraction was obtained by purification using gel chromatography. The primary antibody was biotinylated and PE-labeled streptavidin was used for its identification.

GVHD. Clinical cutaneous and/or hepatic manifestations of GVHD were confirmed by histopathological examination and immunostaining.

RESULTS AND DISCUSSION

Failure of engraftment (FE) was witnessed in all rats preconditioned with irradiation followed by hamster BMTx (Table 1, group II) with the addition of TCL having no beneficial effect (group III). Interestingly the use of a similar regimen in the reciprocal rat to hamster strain combination resulted in much lower FE and markedly prolonged animal survival with no evidence of GVHD at day 30 (group VIII). Splenectomy prior to irradiation and BMTx in the hamster to rat model also resulted in a much lower FE but with obvious manifestations of cutaneous

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Table 1. Failure of Engraftment (FE), GVHD, and Survival of Recipients After BMTx in the Reciprocal Hamster to Rat Xenograft Combination

Group	Treatment	n	FE (%)	GVHD (%) (at POD* 30)	Survival (days)		
					Max	Median	Min
A. Hamster to rat	I Radiation (Rad)† alone	5	100	N/A	9	8	6
	II Rad + BMTx‡	6	100	N/A	20	13	11
	III Rad + BMTx + TCL§	7	100	N/A	15	10	5
	IV Rad + BMTx + Spx	22	100	27	59	21	14
	V Rad + BMTx + Spx + TCL	10	0	0	44	40	34
B. Rat to hamster	VI Rad alone	5	100	N/A	8	8	6
	VII Rad + BMTx	39	49	28	112	20	7
	VIII Rad + BMTx + TCL	10	20	0	131	91	9

N/A = not applicable.
*Postoperative day.
†10.5 Gy.
‡3×10^8 unfractionated BM cells.
§1 mg/kg/d IM; 0-30 days.

GVHD (group IV). However, the administration of TCL to group IV animals resulted in complete abrogation of FE and of cutaneous GVHD, which markedly prolonged their survival. The animals in groups IV and V nevertheless succumbed to lethal GVHD, which was essentially limited to the liver.

In splenectomized TCL-treated rats, which enjoyed moderately prolonged survival (group V) following hamster BMTx, 80% of peripheral blood cells at 14 days postinfusion were of hamster origin. Interestingly, reconstitution with hamster RBCs was only possible in animals in group V. When tested in CDC assays, hamster serum (unlike that from naive LEW rats) had no detectable titers of antirat preformed antibodies, which may provide an explanation for the relative ease with which engraftment could be achieved in the rat to hamster strain combination. Taken together, these data support earlier observations that natural (preformed) antibodies are the main barrier to xenogeneic bone marrow engraftment.[5] Furthermore, Spx prior to BMTx across xenogeneic barriers enhances engraftment, an effect further supplemented by TCL, which also attenuates cutaneous (but not hepatic) manifestations of GVHD.

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