ELSEVIER

1912

Neonatal Bone Fragments for the Induction of Xenograft Acceptance: Transplantation of Xenogeneic Hematopoietic Stem Cells and Species-Specific Microenvironment for Hematopoiesis

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ALLOGENEIC chimerism and tolerance have been successfully achieved by infusing allogeneic bone marrow cells (BMC) into irradiated or neonatal recipients; however, engraftment of xenogeneic BMC using the same procedure has been difficult to achieve. One of the reasons for this difficulty was the lack of a species-specific hematopoietic microenvironment, such as donor stromal cells and species-specific growth factors. In order to overcome this problem, we applied bone fragments obtained from neonates (NBF) for induction of xenogeneic chimerism and subsequent xenograft acceptance. Results were compared to those of BMC.

MATERIALS AND METHODS

Inbred Lewis rats and outbled Syrian hamsters were used as recipients and donors, respectively. NBF were obtained from the tibias and femurs of hamster neonates (within 48 hours of the birth) and 15 to 25 pieces were implanted subcutaneously or under the kidney capsule of rat recipients. BMC (250×10^6) prepared from the tibias and femurs of 6 to 10-week-old hamsters were IV infused into Lewis rat recipients. All recipients received 4 Gy whole body irradiation (WBI, day 0), cyclophosphamide (CP) 15 mg/kg/d for 5 days (day −3 to 1), and oral form tacrolimus (TAC) 1 mg/kg/d for 30 days (day 0 to 30). On day 30, they were challenged with hamster heart transplantation (HTx). TAC (1 mg/kg/d) was given for 10 days after HTx, then xenografts were followed without any treatment. For comparison, BMC were IV infused into lethally irradiated (9.5 Gy) rat recipients, and recipients that survived for more than 30 days were challenged with hamster HTx.

RESULTS AND DISCUSSION

Combination treatment with WBI, CP, and TAC showed little effect on the survival of heart grafts transplanted on day 0 (Table 1, group 2) or 30 days later (group 3). Priming with BMC prolonged subsequent heart graft survival for only 2 days (group 4). When recipients were primed with NBF (group 6), heart graft survival was prolonged to a median of 19 days. Neither adult bone fragment (group 5) nor BMC infusion into lethally irradiated recipients (group 7) showed a similar effect. Implanted NBF in group 6 animals showed normal histopathology and hematopoiesis. This experiment demonstrates that NBF provides better protection for the subsequently transplanted heart xenograft compared to that by BMC infusion. NBF may provide both hematopoietic stem cells and a species-specific hematopoietic environment for the induction of xenogeneic chimerism.

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Table 1. Hamster Heart Graft Survival in Rat Recipients Pretreated With NBF or BMC Infusion

Group	Priming	Treatment*	Timing of HTx	n	Heart Graft Survival (d)	Median (d)	*P* Value*
1	No	No	D 0	6	3, 3, 3, 3, 4, 4	3.0	—
2	No	Yes	D 0	5	2†, 3†, 5, 5, 6	5.0	N.S.
3	No	Yes	D 30	3	4, 4, 5	4.0	<.05
4	BMC	Yes	D 30	8	4, 5, 5, 5, 5, 5, 6, 6	5.0	<.005
5	Adult bone fragments	Yes	D 30	2	5, 5	5.0	<.05
6	NBF	Yes	D 30	8	5, 6, 10, 19, 19, 20, 20, 23	19.0	<.005
7	BMC	9.5 Gy	D 30	6	3, 5, 6, 10, 12, 15	5.0	<.05

*Treatment protocol consisted of WBI 4 Gy (day 0), CP (orally) 15 mg/kg/d for 5 days (day −3 to 1), and oral form TAC (IM) 1 mg/kg/d for 40 days (day 0 to 40), except for animals in group 7, which received 9.5 Gy WBI on day 0. In groups 3 to 6, all animals survived for 30 days, whereas only 34.6% of animals in group 7 survived for 30 days after irradiation and bone marrow infusion.

*Mann-Whitney *U* test.

†Animal died with beating graft.

0041-1345/97/$17.00
PII S0041-1345(96)00546-5