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Identification of Donor Hematopoietic Progenitor Cells After Allogeneic Liver Transplantation

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THE PERSISTENCE of multilineage microchimerism after solid organ transplantation suggests long-lasting engraftment of donor hematopoietic progenitor cells (HPCs) originated from the transplanted organs. This study describes a method developed to identify donor HPCs in the recipient bone marrow and reports preliminary results obtained after allogeneic liver transplantation.

METHODS

Inbred male Lewis (LEW) and Brown Norway (BN) rats were used for experiments. Plastic dish nonadherent bone marrow cells (BMC) were prepared and cultured with 2% PWM-SCM for 6 days in a fibrin clot culture system to detect colony-forming units in culture (CFU-C). Clusters (10 to 50 cells per aggregate) and colonies (>50 cells per aggregate) in cultures were counted as CFU-C. LEW-derived CFU-C was identified by in situ immunohistochemical staining of cultured cells in the fibrin clot, using a standard three-step avidin-biotin complex method with monoclonal antibody L21-6, which recognizes the class II MHC antigens of LEW but not BN rats.

RESULTS

CFU-C generated from 5×10^4 naive LEW BMC was 54 ± 18, of which $39 \pm 9\%$ (21 ± 7) was composed of L21-6 strong positive cells, including macrophages and dendritic-like cells. CFU-C induced from 5×10^4 normal BN BMC was 43 ± 4, and none of CFU-C contained L21-6^+ cells. When normal LEW and BN BMC were mixed at various ratios (1:10, 1:100, 1:1000), L21-6^+ CFU-C derived from the mixture were 0.2 ± 0.4, 2.3 ± 1.4, and 21.3 ± 5.7, respectively, and correlated to the number of LEW BMC contained in the culture. Finally, the engraftment of donor HPCs in transplant recipients was examined in a LEW to BN liver transplantation model, in which rejection was controlled with a short course of tacrolimus treatment (1 mg/kg for 14 days). L21-6^+ CFU-C in BN recipient BMC (5×10^5) obtained 14 and 30 days after LEW liver transplantation were 1.0 ± 0.6 and 0.3 ± 0.3, respectively. The frequency of donor HPCs in the bone marrow of these recipients is estimated as 0.1% to 0.5%, according to L21-6^+ CFU-C counts obtained in the previous mixed culture experiment.

CONCLUSION

This study provides the strong evidence that HPCs in the liver graft were able to engraft into the recipient bone marrow after transplantation. The role of donor HPCs in maintaining microchimerism and donor-specific graft acceptance seen in this model is currently under investigation.

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