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Immunomodulation of Intestinal Transplant With Allograft Irradiation and Simultaneous Donor Bone Marrow Infusion

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Passenger leukocytes, normal constituents of all organs, migrate after transplantation and produce persistent multilineage microchimerism, which modulates the recipient immune system and is suggested to have an important role in inducing graft acceptance after transplantation.^{1,2} Leukocytes in the intestine have been shown to have inferior tolerogenic qualities compared with bone marrow (BM) cells; in addition, leukocytes have a lineage profile predisposed to graft-versus-host disease (GVHD).³ We hypothesized that intestinal allograft alteration with ex-vivo irradiation will eliminate mature lymphoid elements from the graft without degrading the function of the epithelial components. Furthermore, we hypothesized that the replacement of irradiated graft passenger leukocytes with simultaneous donor BM infusion will improve the result of intestinal transplantation (SITx). This possibility was examined using the rat model of SITx.

METHODS

Inbred male Lewis (LEW, RT1^l) and Brown Norway (BN, RT1^b) rats were purchased from Harlan Sprague Dawley, Inc., (Indianapolis, IN) and used as donors and recipients, respectively. Small intestinal grafts were obtained from LEW donors and ex vivo irradiated from ¹³⁷Cs source before orthotopic SITx. Donor BM was obtained by flushing the tibias and femurs, and a total of 2.5×10^8 cells was intravenously infused into recipients on the day of SITx. FK 506 (1 mg/kg/d) was intramuscularly given to recipients on days 0–13, 20, and 27. Percentages of donor leukocytes were determined sequentially in recipient blood by flow cytometry using monoclonal antibodies specific for major histocompatibility complex (MHC) class I antigens on LEW and BN.

RESULTS

All recipients of unmodified intestinal grafts died of GVHD despite simultaneous donor BM infusion in some cases. Median survivals were 63 and 51 days with and without simultaneous donor BM infusion, respectively (n = 5 for each). Graft irradiation (10 Gy) effectively prevented GVHD, and median animal survival was prolonged to 92.5 days without BM infusion. Further prolongation was achieved by adjunct BM. All animals in this group survived for more than 100 days (n = 6). Percentages of donor cells in the peripheral blood correlated with animal survival. Donor phenotype cells remained at low levels (less than 2%) after transplantation of 10-Gy irradiated grafts, whereas they were consistently high in GVHD recipients of unaltered grafts. No major histopathological abnormalities caused by irradiation,

such as irradiation vasculopathy, were found in 10 Gy irradiated grafts 100 to 200 days after SITx.

DISCUSSION

Survival of graft passenger leukocytes was proved when donor leukocytes (microchimerism) were found in recipients whose allografts had been functioning for decades.^{1,2} The implication was that donor hematopoietic progenitor cells, including pluripotent stem cells, present in the transplanted organ had migrated and survived in the recipient. Before this finding, transplantation had been defined largely in terms of a unidirectional immune reaction, and this one-way paradigm had failed to elucidate numerous enigmatic observations, including the surprising clinical success of organ transplantation. The discovery of microchimerism has provided new insights into the immunological events following organ transplantation and prompted the development of new strategies.

In the field of SITx, GVHD has been a major concern since Monchik and Russell showed the development of GVHD in semiallogenic SITx using the parent-to-offspring F1 hybrid model.⁴ In fact, previous studies of intestinal graft modification have attempted to reduce the risk of GVHD using irradiation, antilymphocyte serum, or surgical manipulation. Based on the recent discovery of microchimerism and the current understanding of the dual immune reaction after organ transplantation (host-versus-graft [HVG] and graft-versus-host [GVH]), we have examined methods of intestinal graft conditioning and recipient immune modulation. This study shows the beneficial effects of graft irradiation and adjunctive donor BM infusion on graft survival. Preliminary results suggest that the superiority of this strategy is associated with the rapid replacement of graft lymphoid tissues with recipient leukocytes of the same lineages. Further study is currently underway to improve SITx from its present state of excessive mortality and morbidity.

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