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Effects of Peritransplant Administration of Hematopoietic Growth Factors on the Development of Chronic Allograft Rejection

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CHRONIC allograft rejection (CR) remains as the major obstacle to successful organ transplantation. We have previously shown that persistence of donor multilineage hematolymphoid cells after transplantation correlates with the resistance to CR.[1–3] We hypothesized in this study that perioperative administration of hematopoietic growth factors will be able to augment donor chimerism and therefore ameliorate the development of CR. This hypothesis was examined using a rat heart transplantation model with simultaneous donor bone marrow (BM) infusion.

METHODS

Female Brown-Norway (BN, $RT1^n$) rats received heterotopic heart grafts from male Lewis (LEW, $RT1^1$) donors. Recipients also received intravenous infusion of donor BM (2.5×10^8) harvested from the tibias and femurs on the day of transplantation and were treated with intramuscular tacrolimus (1.5 mg/kg per day, Fujisawa Pharmaceutical Co., Ltd, Osaka, Japan) on days 0 to 13, 20, and 27. Six groups of animals were examined using four different hematopoietic growth factors, including rh-granulocyte colony stimulating factor (G-CSF, Amgen, Thousand Oaks, Calif), rh-granulocyte microphage colony stimulating factor (GM-CSF, Immunex, Seattle, Wash), rh-Flt-3 ligand (Immunex) and rh-IL-6 mutein (ImClone System Inc., Somerville, NJ) (Table 1). Levels of chimerism were semiquantitatively analyzed by polymerase chain reaction and Southern hybridization using rat sex determining region-Y (SRY) specific oligonucleotide primers[4] in sequential blood samples and recipient tissues at sacrifice on day 100 after transplantation. Heart allografts were further histopathologically analyzed for the severity of CR.

RESULTS

Donor BM infusion alone significantly increased male DNA concentration in the blood for the first 2 months after grafting. Additional treatment with growth factors did not show further significant augmentation of blood chimerism. At day 100, donor male DNA was not detected in the peripheral blood of any recipient, but it was found more frequently in recipient tissues such as the skin, native heart, and spleen. Level and frequency of chimerism were significantly increased in recipients with adjunctive BM infusion and further augmented by Flt-3 ligand and IL-6 treatment (Table 1). Histopathologic analysis of heart allografts at day 100 revealed the significant amelioration of overall inflammation in BM-infused recipients compared to uninfused controls (Table 1). Evaluation of arterial changes demonstrated the significant improvement of the degree of obliterative arteriopathy in BM-infused recipients treated with G-CSF and IL-6 (Table 1). Additionally, when samples from all cardiac allografts of six groups were pooled and the histopathologic changes were correlated with the pooled

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Table 1. Effects of Adjunctive Donor Bone Marrow Infusion and Perioperative Administration of Hematopoietic Growth Factors on Levels of Chimerism and Histopathologic Changes of Chronic Allograft Rejection

Group	Transplantation	Growth Factor	Dose (kg/d)	n	OA grade	Inflammation Score	Chimerism* ($\times 10^{-3}$%)
1	HTX	None	NA	7	0.94 ± 0.33	2.71 ± 0.36	0.69 ± 0.42
2	HTX + BM	None	NA	8	0.98 ± 0.14	1.38 ± 0.26†	21.8 ± 9.22†
3	HTX + BM	G-CSF	200 μg	5	0.38 ± 0.09¶	0.63 ± 0.13‡	116 ± 39.8‡
4	HTX + BM	GM-CSF	200 μg	5	0.48 ± 0.14	0.94 ± 0.37‡	19.2 ± 9.79†
5	HTX + BM	Flt-3 ligand	200 μg	6	0.57 ± 0.10	0.75 ± 0.11‡	109 ± 40.5‡¶
6	HTX + BM	IL-6	500 μg	6	0.44 ± 0.10¶	0.92 ± 0.24‡	87.7 ± 30.5‡¶

All values are mean ± SE.
*In native heart at 100 days after transplantation.
P values vs HTX (group 1): † <.001; ‡ <.0001; vs HTX + BM (group 2): ¶ <.05.
Abbreviation: OA: obliterative arteriopathy.

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levels of chimerism in the native hearts of these same animals, significant inverse correlation was found between the severity of CR (inflammation score and arterial changes) and levels of chimerism.

DISCUSSION

It was determined in this study whether there was a correlation between the quantity and localization of chimerism and severity of CR using histopathologic endpoints and PCR analysis of Y-chromosome in sex-mismatched transplantation. Results demonstrate that CR was reduced in inverse proportion to the amount of detectable chimerism, which in turn was greater in animals given adjunct BM and postoperative growth factor therapy. Differences among growth factors in increasing chimerism and preventing CR were not significant, but chimerism tended to be greater with Flt-3 ligand and IL-6. Trends of reduced CR were seen with G-CSF and IL-6 treatment. G-CSF and GM-CSF already have been widely used in clinical bone marrow transplant recipients and after cancer chemotherapy. The administration of G-CSF and GM-CSF long after transplantation to stable organ recipients has not increased the risk of either rejection of GVHD. This study demonstrates that perioperative hematopoietic growth factor treatment is effective in enhancing the level of chimerism and improving histopathologic abnormalities associated with CR. Preclinical laboratory studies under controlled circumstances will be doubly important to further improve the method to use growth factors for CR.

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