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Immunodulation of Intestinal Transplantation: Antilymphocyte Serum Donor Pretreatment vs. Ex Vivo Graft Irradiation

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WE HAVE SHOWN the beneficial effects of an immunomodulation strategy for small intestinal transplantation (SITx) by depleting mature lymphocytes from the graft and simultaneously infusing progenitor-rich donor bone marrow (BM) cells.[1] In this study we evaluated the relative benefit of leukocyte elimination between ex vivo graft irradiation versus donor pretreatment with antilymphocyte serum (ALS) in a model of fully allogenic rat SITx. Special attention was paid to their impact on early immune responses after SITx by analyzing the levels and lineages of chimerism as well as the cytokine up-regulation in recipient spleen and allografts.

METHODS

Orthotopic SITx was performed in Brown Norway (BN, $RT1^n$) to Lewis (LEW, $RT1^l$) rat strain combination using a short course of tacrolimus treatment (1.0 mg/kg/d, days 0 to 13, 20, and 27, IM). Three groups of recipients were studied: (1) controls without immunomodulation, (2) donor ALS pretreatment for 3 days (1.0 mL/d, IP) plus donor BM infusion (2.5×10^8 cells on day 0), and (3) ex vivo allograft irradiation (10 Gy) plus donor BM infusion. All recipients were followed for 150 days. Levels and lineages of chimerism were studied by flow cytometry. Cytokine mRNA levels were analyzed by RNase protection assay (RPA).

RESULTS

All recipients in this study survived for >150 days. The numbers of donor cells in recipient spleen and blood within 7 days after SITx were significantly greater among Group 1 than immunomodulated Group 2 and 3 recipients (Table 1). The decrease in donor cells among Group 2 hosts was mainly caused by the depletion of $\alpha\beta TCR^+$ cells, while the number of donor B cells was maintained (data not shown). In contrast, all lineages of donor cells were eliminated in Group 3, resulting in a low level of chimerism. Analysis of lymphocytes in graft mesenteric lymph nodes (MLN) showed prompt replacement of donor cells with recipient lymphocytes in immunomodulated Group 2 and 3 compared with Group 1. Immunomodulation with ex vivo graft irradiation resulted in a more rapid replacement of cells than ALS donor pretreatment. The percentage of donor cells in MLN 3 days after SITx was 50.5% in Group 1, whereas it was 20.8% and 2.5% for Group 2 and 3, respectively. mRNA levels for Th1-type cytokines (IL-2 and γ-IFN) were increased in spleen and graft MLN of Group 1 recipients early after SITx (day 1 to 7); however, these cytokines were significantly down-regulated in Group 2 and 3. Histopathological analysis of allografts at day 150 revealed the development of chronic rejection (lymphoid depletion, fibrotic changes of Peyer's patches and MLN, and existence of arteritis) in Group 1. These changes were completely prevented in immunomodulated Group 2 and 3 allografts.

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Table 1. Chimerism, Cytokine Activity, and Graft Histopathology After Small Intestinal Transplantation With Immunomodulation Using Donor ALS Pretreatment or Ex Vivo Graft Irradiation

	% of Donor Cells (Day 3)[a]			Cytokine mRNA Level (Day 1) (% GAPDH)[b]				Histopathology of the graft	
	Spleen T Cells	Spleen B Cells	MLN	Spleen IL-2	Spleen IFNγ	MLN IL-2	MLN IFNγ	Lymphoid Component	Arteritis
1. Control	4.6 ± 2.4	5.1 ± 0.6	50.5 ± 1.4	3.1 ± 0.2	1.9 ± 1.1	3.6 ± 1.1	3.0 ± 0.9	atrophy	+
2. ALS + BM	1.3 ± 0.4	4.5 ± 0.3	20.8 ± 1.1*	0.3 ± 0.1*	0.8 ± 0.8*	1.1 ± 0.1*	0.7 ± 0.5	well preserved	–
3. Rad + BM	0.1 ± 0.1*	0.8 ± 0.6*	2.5 ± 1.3*	1.4 ± 0.9*	0.9 ± 0.5*	0.8 ± 0.2*	0.1 ± 0.3*	well preserved	–

[a]determined by two-color flow cytometry using mAbs specific for MHC class I antigens on LEW and BN, and lineage specific mAbs R7.3 ($\alpha\beta TCR^+$ cells) and OX33 (B cells).
[b]mRNA levels were expressed as a ratio of cytokine/GAPDH.
*$P < .05$ vs control (ANOVA).

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CONCLUSIONS

Immunomodulation of SITx either by donor pretreatment with ALS or by ex vivo graft irradiation plus donor BM infusion improved intestinal allograft outcome by inhibiting the development of chronic rejection. Early down-regulation in allografts and recipient spleen of Th1-type cytokines may play a role in the immunomodulation strategy.

REFERENCES

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