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ELSEVIER

Prevention of T-Cell Activation by rhCTLA4-Ig and Anti-CD40L Monoclonal Antibody Results in Indefinite Islet Allograft Survival

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BLOCKADE of CD28/B7 and/or CD40/CD40L pathways has been employed with reasonable success to prevent organ allograft rejection.[1,2] Additionally, contemporaneous but not discrete use of rhCTLA4-Ig and anti-CD40L monoclonal antibody (MAb) has also been shown to prevent chronic rejection.[1,3] In the realm of cellular transplantation (Tx), it has been reported that the use of either rhCTLA4-Ig or anti-CD40L MAb alone resulted in prolongation but not indefinite survival of islet allografts.[4,5] Based on these observations, we hypothesized that combined transient perioperative blockade of both costimulatory pathways would result in indefinite islet allograft survival, thus allowing maintenance of euglycemia in a diabetic recipient without the need for exogenous insulin. This latter tenet was tested in an MHC-incompatible mouse model of islet cell Tx; the outcome of this novel study is presented herein.

MATERIALS AND METHODS

Animals and Islet Cell Tx

Inbred male DBA/2 (H-2^d) and C3H (H-2^k) mice were used as donors and recipients, respectively. On day 4, the recipients were rendered diabetic by a single injection of streptozocin (STZ; 300 mg/kg; IV). Animals with documented hyperglycemia received donor islets (~600) under the left kidney capsule and their blood glucose level was sequentially monitored thereafter. In selected long-term survivors, intraperitoneal glucose tolerance test (IPGTT) was also performed. In all recipients exhibiting indefinite islet allograft survival, the graft-bearing kidney was ultimately removed. Depending on the treatment protocol, the animals were divided into five groups (Table 1).

RESULTS AND DISCUSSION

In an untreated MHC-incompatible mouse strain combination, islet allografts were rejected within 19 days' post-Tx (Table 1; Group I). Comparable survival was also witnessed when recipients were treated with isotype-matched control antibodies (Table 1; Group II). In contrast, treatment with anti-CD40L MAb resulted in accelerated rejection of the graft with recurrence of hyperglycemia by day 13 post-Tx (Table 1; Group III). Interestingly, 5/7 (71%) of the recipients receiving rhCTLA4-Ig rejected their grafts within 26 days' post-Tx (Table 1, Group IV); only 2/7 (29%) recipients in the latter group had graft survivals of 54 and 66 days, respectively. Nevertheless, despite the latter observation, it was evident that discrete blockade of either CD28/B7 or CD40/CD40L pathways is not sufficient to prevent islet allograft rejection. On the contrary, indefinite islet allograft survival was witnessed in recipients treated perioperatively with both rhCTLA4-Ig and anti-CD40L MAb (Table 1; Group V). Selected recipients ($n = 3$) from this group when subjected to IPGTT at day 50 post-Tx reversed induced hyperglycemia within 30 minutes' postglucose infusion.

Additionally, removal of graft-bearing kidneys in these long-term survivors was followed by demonstrable elevation in blood glucose levels to >250 μg/dL, suggesting that the observed euglycemia was indeed maintained by transplanted islets and not by regeneration of the β cells in the native pancreata. Finally, these encouraging observations

Table 1. Islet Allograft Survival in DBA/2→C3H Recipients Treated With rhCTLA4-Ig and/or anti-CD40 MAb

Groups	*n*	Treatment	Graft Survival (d)
I	4	None	12, 13, 16, 19
II	4	Human IgG + hamster IgG	17, 20, 23, 24
III	6	anti-CD40L MAb*	13, 13, 13, 13, 13, 13
IV	7	rhCTLA4-Ig†	8, 12, 17, 24, 26, 54, 66
V‡	9	anti-CD40L MAb* rhCTLA4-Ig†	>81(‡), >90(†), >120(†), >180(†)

*250 μg/d on days 0, 2, and 4 post-Tx; i.m.
†200 μg/d on days 0, 2, 4, and 6 post-Tx; i.p.
‡Long-surviving animals were electively sacrificed at various times post-Tx to undertake both in vivo and in vitro analyses.

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have prompted us to suggest that clinical translation of this strategy would greatly improve survival of islets allotransplanted into insulin-dependent type I diabetics with long-term freedom from exogenous insulin.

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