Published in final edited form as:

Transplant Proc. 1999 ; 31(1-2): 1242–1243.

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

C. Rasteilini, A. Salam, R. Kuddus, A. Aitouche, V. Subbotin, M. Braun, R. Leach, R. Peach, J.J. Fung, T.E. Starzl, and A.S. Rao

Section of Cellular Transplantation, Thomas E. Starzl Transplantation Institute, and the Departments of Surgery, and Pathology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, and Bristol-Myers Squibb, Princeton, New Jersey

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 $> 250 \mu\text{g/dL}$, 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 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.

References

1. Larsen CP, Elwood ET, Alexander DZ, et al. *Nature* 1996;381:434. [PubMed: 8632801]
2. Kirk AD, Harlan DM, Armstrong NN, et al. *Proc Natl Acad Sci USA* 1997;94:8789. [PubMed: 9238056]
3. Sun H, Subbotin V, Chen C, et al. *Transplantation* 1997;64:1828.
4. Kenyon, N.; Fernandez, L.; Masetti, M., et al. Presented at the 24th Annual Scientific Meeting of the American Society of Transplant Surgeons; May 13–15, 1998;
5. Levisetti MG, Padrid PA, Szot GL, et al. *J Immunol* 1997;159:5187. [PubMed: 9548454]

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 μ g/d on days 0, 2, and 4 post-Tx; i.m.

[†] 200 μ g/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.