Brief Definitive Report

LONG-TERM RESIDENCE OF A GRAFT IS AN INSUFFICIENT STIMULUS FOR THE INDUCTION OF TOLERANCE

Investigating the Role of Cyclosporine in Class I-disparate Heart Grafts in the Rat.

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We (1-4) and others (5-7) have previously demonstrated the ability of a short period of Cyclosporine A (CyA) treatment to produce prolonged, stable, donor-specific allograft tolerance in rats. An understanding of the mechanism of induction of this tolerance is clearly a prerequisite for its application to man. It is known that continued antigen presence (8, 9) is essential for the maintenance of this state. The recent availability of congenic rat strains (10, 11) has enabled us to address the issue of the role of the MHC antigens versus the role of the CyA treatment in the induction of tolerance. By using congenic, class I-incompatible rat strains, we have been able to analyze the influence of the long-term residence of heart grafts on the immunological status of the recipients, with and without CyA treatment.

Materials and Methods

Rat Strains. Four levels of mismatch at the MHC (full, major, class II, and class I) were studied. This was achieved using DA (RT1.AaBa) rats and the following PVG congenics: PVG (RT1.AcBc), PVG-RT1a (RT1.AaBa), and PVG-RT1r1(RT1.AaBc), in the combinations as detailed in Table I. WAGS(RT1.AuBu) were used as third-party donors. All were males; donors weighed 100-150 g; recipients 200-250 g. All rats were obtained from Olac Ltd., Bicester, Oxon, England.

Heart Grafts. Accessory cervical heart transplantation was performed by the technique described by Heron (12). The aorta and pulmonary artery of the donor heart were anastomosed to the common carotid artery and external jugular vein of the recipient, respectively, using an external cuff technique. The transplanted hearts were checked by daily inspection and palpation. Rejection was diagnosed by the cessation of heartbeat and confirmed in all cases by histological examination.

Skin Grafts. Full-thickness skins were grafted onto recipients bearing functioning heart grafts for >100 d. Skins were raised from the ventral abdominal wall of donors, trimmed of fat, sutured onto recipient flank beds prepared with meticulous hemostasis using continuous 6/0 silk, and bandaged with elastoplast. Dressings were removed on day 5. No further treatment was given. Rejection was said to have occurred when >50% of the graft surface had become raised, necrotic, or scabby.

Immunosuppression. CyA (gift from Sandoz Ltd., Basel, Switzerland) was administered intramuscularly in olive oil at a dose of 15 mg/kg/d for 17 d according to the intermittent treatment schedule (3) (treatment days 0-6, 21-27, 42-44). This schedule was previously shown to be effective in maintaing CyA blood levels >300 ng/mL for 60 d, and in so doing, tolerance to heart grafts could be achieved in a very high percentage of cases.

Experimental Design. 20 hearts were grafted across each of the levels of mismatch studied (full, major, class II, class I). These were subdivided at each level into untreated controls (n = 10), and CyA-treated animals (n = 10). As a test for tolerance, long-term survivors with functioning heart grafts >100 d, were grafted with donor-specific, as well as third-party (WAG) skins.

Results and Discussion

The role of MHC antigens in the induction of tolerance to heart grafts was investigated. The data from Table I, groups 1a, 2a, and 3a, demonstrate that a class II incompatibility whether alone (median survival time [MST] 13.9 d) or in combination with a class I (MST, 6.3 d), or class I and minor incompatibility (MST, 7.4 d), causes the heart graft to be rejected. With CyA treatment however, tolerance was successfully induced in these groups. This was confirmed by the ability to accept donor-specific skin grafts for at least the length of the observation period of 50 d, whereas third-party skin (WAG) was rejected in near normal time (MST, 9.7 d). The critical observation, however, is in group 4 of Table I where hearts are grafted across a class I barrier only. Under these circumstances, even without CyA, all the heart grafts survive indefinitely. However, subsequent donor-specific skin grafts are rejected (MST, 20.6 d) demonstrating that despite the long-term residence of the heart graft, systemic tolerance fails to develop. Is the failure to develop tolerance in this group due to a lack of appropriate antigenic stimulation or a lack of CyA? Group 4b demonstrates that when these class I–incompatible heart grafts are treated with CyA, despite this being unnecessary to retain the graft, such treatment now induces allograft tolerance, as demonstrated by donor skin acceptance. Since the only difference between these two groups is the CyA treatment, it is concluded that CyA plays an active part in the induction of allograft tolerance where the prolonged presence of a heart graft alone failed to do so.

TABLE I

The Induction of Tolerance by CyA Across Various Levels of MHC Mismatch in the Rat

Group	Donor	Recipient	Mismatch	Cy Rx	HG median survival (n = 10)	SG median survival on LTS (n = 10)	
						DS	TP
					d	*d*	
1a	DA	PVG	Major and minor	–	7	–	–
1b	DA	PVG	Major and minor	Yes	100	50	10
2a	PGV-RT1a	PVG	Major	–	6	–	–
2b	PGV-RT1a	PVG	Major	Yes	100	50	9
3a	PVG-RT1r1	PVG-RT1a	Class II	–	12	–	–
3b	PVG-RT1r1	PVG-RT1a	Class II	Yes	100	50	10
4a	PVG-RT1r1	PVG	Class I	–	100	20	10
4b	PVG-RT1r1	PVG	Class I	Yes	100	50	10

Heterotopic heart grafts (HG) were performed across four levels of MHC mismatch in the rat; full (major and minor), major (classes I and II), class II, and class I. Each group was subdivided into a no treatment and CyA treatment (CyA Rx) subgroup of 10 animals each. Long-term survivors (LTS) with functioning heart grafts >100 d were tested for systemic tolerance by grafting with donor-specific (DS) and third-party (TP) skins. Tolerance was confirmed by donor-specific skin graft (SG) survival >50 d.

The term "tolerance" has been used to describe a variety of unresponsive states. The criteria used here for the definition of tolerance is specific, systemic, nonresponsiveness to donor strain antigens as demonstrated by the failure of the animal to reject a skin graft of donor origin. In this study, we were able to generate long term survivors (LTS; defined as recipients with functioning heart grafts >100 d) in five groups, of which four received CyA treatment (groups 1b, 2b, 3b, 4b), and one did not (group 4a). We demonstrate, however, that only those groups treated with CyA developed specific, systemic tolerance (Table I, groups 1b, 2b, 3b, 4b). These results appear to differ from the studies of Pescovitz et al. (13), who looked at the effect of class II antigen matching on renal allograft survival in miniature swine. However, it is appreciated that both the organ graft studied, and the experimental model used, were different.

Summary

This study demonstrates that the induction of tolerance is possible across a class I only antigenic barrier that fails to produce heart graft rejection. However, the long-term residence alone of such a graft per se, does not necessarily lead to the establishment of systemic tolerance in the recipient. The important finding in this study with regard to the biology of allograft tolerance, is that while the class I antigen provides the stimulus, its presence alone is not sufficient for the induction of tolerance; indeed, the action of the Cyclosporine A (CyA) is a necessary adjunct to its induction.

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