DURABILITY OF DONOR-SPECIFIC AND ORGAN-SPECIFIC HEART TRANSPLANT TOLERANCE INDUCED BY INTRATHYMIC PRETREATMENT WITH ALLOGENEIC SPLEEN CELLS

Permanent acceptance of an experimental cardiac allograft can be achieved in the rat by pretreating the recipient with antilymphocyte serum and intrathymic donor lymphocytes. We investigated the durability and specificity of the tolerance produced by this pretreatment in a rat model of heterotopic heart transplantation with Lewis-Brown Norway donors and Lewis recipients. Pretreated Lewis rats received 1 ml antilymphocyte serum intraperitoneally and 5×10^7 Lewis-Brown Norway splenocytes intrathymically, followed 21 days later by Lewis-Brown Norway cardiac transplantation. The first Lewis-Brown Norway cardiac allograft survived long term (mean 140 days) in pretreated recipients who were given no subsequent immunosuppression. After 60 days with a beating Lewis-Brown Norway allograft, tolerant Lewis recipients underwent a second cardiac allograft with either a Lewis-Brown Norway heart or a third-party Wistar-Furth heart. The second Lewis-Brown Norway cardiac allograft was not rejected (mean survival 76 days), but that from the third-party Wistar-Furth donor was rejected in a normal fashion (mean survival 10.4 days). The presence of second grafts did not affect survival of first grafts. Tolerant Lewis recipients of two Lewis-Brown Norway heart grafts underwent subsequent transplantation with Lewis-Brown Norway skin. Skin allograft survival in this group (mean 8.4 days) was not different from that in Lewis recipients without pretreatment. Rejection of skin grafts had no effect on the heart grafts. These data suggest that tolerance to cardiac allografts produced by intrathymic pretreatment is durable and extends to a second heart graft from a genetically identical donor. Tolerant rats reject third-party hearts and primary donor skin grafts normally, and tolerance to previously placed heart grafts is not abrogated by this rejection. Non-major histocompatibility complex skin antigens not present on cardiac cells may account for the tissue specificity of the tolerance produced by intrathymic treatment in this model. (J THORAC CARDIOVASC SURG 1996;111:429-31)

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Organ transplantation is effective treatment for patients with end-stage organ failure. Success of heart transplantation between genetically disparate individuals, however, is currently dependent on long-term use of nonspecific immunosuppressive agents that cause significant morbidity and mortal-

ity. The induction of donor-specific tolerance without the need for long-term nonspecific immunosuppression has long been a goal of transplantation research. In previous experiments, we and others showed that permanent acceptance of a heterotopic experimental cardiac allograft can be achieved in the rat by pretreating the recipient with antilymphocyte serum (ALS) and donor-specific lymphocytes given intrathymically.¹⁻⁴ A report by Nakafusa and colleagues,⁵ however, suggested that this method fails to induce tolerance to renal and skin allografts. Major histocompatibility complex (MHC) antigens as well as other antigens were involved in the differential rejection of various organ allografts.⁶ In these studies, we investigated the durability and specificity of the graft tolerance produced by intra-

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Table I. Survival of cardiac and skin grafts in Lewis rats

Pretreatment	First graft	Second graft	Third graft	Survivals (days)	Mean survival (days)
None	LBN heart	None	None	7, 7, 8, 8, 9	7.8
None	WF heart	None	None	8, 8, 9, 10, 10	9
None	LBN skin	None	None	6, 7, 8, 8, 9	7.6
ALS and IT LBN cells	LBN heart	LBN heart	None	>100, >100, >100, >200, >200	140*
				>60, >60, >60, >100, >100	76*
		WF heart	LBN skin	7, 8, 8, 9, 10	8.4
			None	>100, >100, >150, >150, >150 8, 10, 10, 11, 13	130* 10.4

IT, Intrathymic.

* $p < 0.001$ vs control (unpaired two-tailed t test).

thymic pretreatment in an experimental model of allotransplantation.

Materials and methods

Animals. Mature male Lewis, Lewis-Brown Norway (LBN), and Wistar-Furth (WF) rats were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, Ind.). Animals were acquired without viral infestation and were kept in an environment free of virus in the animal facility at Medical College of Pennsylvania Hospital. All animals were handled in a humane fashion and in accord with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Experimental design. In these experiments, Lewis rats were recipients, LBN rats were donors of splenocytes, hearts, and skin, and WF rats were third-party heart donors. Lewis recipients were pretreated by intrathymic inoculation with LBN splenocytes plus ALS injected intraperitoneally. LBN-to-Lewis cervical heterotopic heart transplantation was performed 21 days later. After 60 days with a beating cervical LBN heart, recipient animals were divided into two groups. In group A, tolerant Lewis recipients underwent a second, intraabdominal cardiac allograft with an LBN donor heart, then underwent subsequent transplantation with LBN skin after 60 days with two hearts. In group B, tolerant Lewis recipients underwent a second, intraabdominal cardiac allograft with a third-party WF donor heart. Control heart transplantation with an LBN or WF donor and full-thickness skin transplantation with an LBN donor were carried out in untreated Lewis rats. Each group included five subjects.

Preparation of donor spleen cells. Whole spleens were harvested from donor rats and disrupted by means of a tissue homogenizer. Erythrocytes were lysed with ammonium chloride. Remaining leukocytes were washed with RPMI 1640 medium (Sigma Chemical Co., St. Louis, Mo.). Viability of cells was evaluated by trypan-blue dye exclusion.

Intrathymic inoculation of cellular alloantigen. On the day before intrathymic treatment, animals received 1 ml ALS intraperitoneally. The next day, after induction of methoxyflurane (metofane) inhalation anesthesia, the thy-

mus of the recipient rat was exposed by a partial medial sternotomy and 2.5×10^7 donor splenocytes in a volume of 0.05 ml RPMI 1640 medium were injected into both lobes of the recipient thymus under direct vision.

Heart transplantation. All procedures were done with the subjects under general anesthesia. Heterotopic cervical cardiac transplants were performed with a suture technique, anastomosing the donor aorta and pulmonary artery to the recipient's internal carotid artery and jugular vein, respectively.⁷ Heterotopic abdominal heart transplantation was performed with the modified technique of Ono and Lindsey,⁸ with the donor aorta and pulmonary artery anastomosed end-to-side to the recipient's abdominal aorta and inferior vena cava, respectively. Cardiac allograft survival was determined by daily palpation and, if results of palpation were indeterminate, by direct visualization. Rejection was marked by the complete absence of ventricular contractions and was confirmed histologically.

Skin grafting. Skin grafting was performed according to the method of Thornburg and associates.⁹ Full-thickness skin was harvested and cleansed of subcutaneous tissue. The graft, measuring 3×3 cm, was sutured onto the flank of the recipient animal. Dressings were removed on postgrafting day 5, and grafts were inspected daily. Hair loss, epidermal breakdown, and ulceration were used as signs of rejection. More than 50% necrosis of the grafted surface area was considered complete rejection.

Histopathology. Heart allografts were fixed in 10% buffered formalin, paraffin-embedded, sectioned, and stained with hematoxylin and eosin. Slides were examined by light microscopy.

Statistical analysis. Comparison between groups was done with an unpaired, two-tailed Student's t test.

Results

Results of allograft survivals are summarized in Table I. Intrathymic inoculation of LBN splenocytes in conjunction with a single intraperitoneal dose of ALS produced essentially permanent (mean 140 days) tolerance to LBN hearts in Lewis recipients. This survival was significantly different ($p < 0.01$) from that in untreated Lewis recipients (mean 7.8 days).

Tolerant Lewis rats with surviving LBN cardiac grafts (>60 days) did not reject either heart when

rechallenged with a second LBN heart, with second graft survival longer than 60 days (mean 76 days, $p < 0.01$ vs control), but they did reject a third-party WF cardiac allograft in a normal fashion (mean 10.4 days). Rejection of third-party donor grafts did not affect the survival of the LBN heart grafts in pretreated animals.

Tolerant Lewis recipients bearing two beating LBN hearts normally rejected skin allografts (mean 8.4 days) from the same donor strain (LBN). Rejection of the skin graft had no effect on the heart grafts.

Discussion

Donor-specific unresponsiveness in the absence of immunosuppressive agents is a goal in heart and other organ transplantation. Intrathymic inoculation with donor splenocyte alloantigens is effective in producing tolerance to cardiac allografts. In the experiments reported here, tolerance to cardiac allografts was not affected by rechallenge with grafts from either the donor strain or a third-party strain. These data show that intrathymic pretreatment can produce specific tolerance to a cardiac allograft and that this tolerance is permanent.

Several potential mechanisms could be responsible for generating the tolerance observed in our model. One likely possibility is clonal deletion of alloreactive T cells. Clonal deletion is an important mechanism in immunologic development.² The injection of cells bearing alloantigens into the thymus changes the thymic microenvironment. ALS eliminates mature T cells, and the subsequent development of new T cells in the altered thymus now takes place in the presence of foreign antigen, which is reinterpreted as a self-antigen. Clones reactive to the alloantigen might therefore be eliminated, producing clonal deletion and thus tolerance to a subsequent graft. Our findings are consistent with this mechanism because subsequent donor strain grafts are not rejected but third-party grafts are. This reaction rules out a nonspecific suppressor mechanism.

These experiments also demonstrated that intrathymic injection of splenocyte alloantigens induced a state of donor-specific tolerance that allowed indefinite cardiac allograft survival but failed to produce prolonged skin allograft survival in the same recipient animals. These results suggest that non-MHC antigens or special tissue antigens exist on the skin and are not expressed either on the splenocytes injected into thymic environment or on

cardiac cells. If these antigens are not introduced into the thymus, they cannot take part in thymic recognition events that produce tolerance to tissue antigens. Whatever the mechanism of tolerance induction in this model, it appears that such non-MHC antigens or special skin antigens may account for the tissue specificity of the tolerance produced by intrathymic pretreatments.

These studies show that tolerance induced to a cardiac allograft by intrathymic pretreatment is durable and specific for donor strain and tissue type (heart vs skin). Intrathymic pretreatment thus appears to produce a permanent alteration in immune response to donor tissue. These studies also suggest that non-MHC antigens or special antigens may account for the tissue specificity of the tolerance produced by intrathymic treatment in allotransplantation.

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