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Does intrathymic injection of alloantigen-presenting cells before islet allo-transplantation prolong graft survival?

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Abstract Current immunosuppressive agents have potentially dangerous side-effects, are non-specific and most are also diabetogenic. We investigated tolerance induction with intrathymic injection of purified antigen-presenting cells (APC) plus a single dose of anti-lymphocyte serum (ALS) intraperitoneally before allogeneic islet transplantation in the rat model WAG to Lewis ($RT1^u$ to $RT1^1$). Purified donor APC [non-parenchymal cells (NPC) or dendritic cells (DC)] were prepared from liver and spleen, respectively. Isograft function for more than 120 days proved that islet isolation, purification and transplantation procedures were adequate. A total of WAG DC (4×10^5) or NPC (2×10^6) in 20 µl were injected into both lobes of the thymus of 140–210 g Lewis recipients followed by a single injection of ALS. Three days later, diabetes was induced with streptozotocin (60 mg/kg). Four days later allogeneic islets were grafted into the liver by intraportal injection of 3000 WAG islets. Control animals ($n = 8$) received 20 µl saline intrathymically instead of APC. Graft function was assessed by blood glucose measurements with glucose levels above 15 mmol/l on 3 consecutive days defined as graft rejection. Animals given DC ($n = 9$) or NPC ($n = 8$) intrathymically plus 1 ml of ALS, rejected their grafts in an accelerated fashion with a median survival time (MST) of 3 days. However, control animals rejected their grafts with a MST of 7 days, but with two animals surviving for more than 2 months. In conclusion, intrathymic inoculation with purified APC plus a single dose of ALS did not prolong allogeneic islet graft function but induced accelerated rejection of the islet allografts.

Key words Intrathymic Inoculation · Antigen-presenting cells · Tolerance induction · Islet transplantation

Introduction

Induction of tolerance towards pancreatic islet tissue has been achieved by pretransplant intrathymic injections of donor cells under transient immunosuppression with antilymphocyte serum (ALS) followed by donor type islet grafts 7–14 days later [1–4]. Cells used for the induction inoculum includes islets, unpurified spleen cells and bone marrow cells. The cell component most common to these different inoculation suspensions could be antigen-presenting cells (APC) such as macrophages and dendritic cells (DC). We, therefore, investigated the effect of highly purified APC injected into both lobes of the thymus on the graft survival of strongly histoincompatible islet allografts in the WAG ($RT1^u$) → LEW ($RT1^l$) rat model.

Materials and methods

APC were purified from liver tissue by collagenase digestion followed by metrizamide gradients as previously described [5]. These non-parenchymal cell suspensions (NPC) contained over 60% Kupffer cells, 30% macrophages and some T + B cells, endothelium and very few hepatocytes as assessed by immunohistochemical staining techniques employing a range of monoclonal antibodies (Mabs) [6]. DC were purified from rat spleens by a modification of the method described by Klinkert [7]. Briefly, minced splenic tissue was gently massaged through a metal sieve. Mononuclear cells were obtained by BSA gradient centrifugation. Adherent cells were removed by overnight tissue culture methods followed by centrifugation over a 13.68% metrizamide gradient. DC with a purity of over 90% contaminated with some B cells were obtained as assessed by staining with Mabs. NPC and DC suspended in 20 µl were injected into both lobes of the thymus at a concentration of 1×10^6 and 5×10^5, respectively, followed by 1 ml i.p. ALS. Control animals received intrathymic Hanks solution. WAG islets were prepared by the method described by Hemke et al. [8]. Between 2500 to 3500 islets were grafted intraportally 7 days later into rats made diabetic by streptozotocin on day 4 post-intrathymic cell inoculum. Islet graft survival was assessed by daily blood glucose measurements.

Results and Discussion

Results of islet graft surival are summarised in Table 1. Control animals receiving Hanks solution intrathymically plus a single dose of 1 ml ALS i.p. (Accurate Scientific Corp. Westbury, USA) at the time of inoculation rejected the intraportal islet grafts with a median survival time (MST) of 7 days with two of six animals surviving for over 2 months. Animals receiving intrathymic inoculation with NPC plus ALS rejected the grafts within 2 days, similar to grafts rejected within 3 days after intrathymic DC inoculation. These results are in contrast to our previously published work where tolerance to cardiac grafts was induced by intrathymic NPC inoculation under transient immunosuppression with ALS [5]. Tolerance was tested in long-term survivors by donor-type skin grafts, which were rejected, though in a delayed fashion, but the donor-type stimulus did not seem to be strong enough to reject the heart grafts. A second donor-type heart graft was also not rejected, whereas a third-party heart graft was rejected in normal fashion (unpublished observation), clearly demonstrating that organ-specific tolerance was induced by NPC. Although islet tissue is very vulnerable to rejection, this accelerated rejection may be due to the effect of highly purified APC, rich in class II and negligible in class I molecules, introduced into the thymus. Oluwole et al. [9] have reported the induction of donor-specific unresponsiveness to rat cardiac allografts by intrathymic donor MHC class I antigens. In preliminary results of some pilot studies, they also showed that intrathymic inoculation with purified B cells caused graft rejection not different from control animals. Our results confirmed this observation, though with accelerated rejection, which may be due to the high content of highly purified APC in our inoculum. We have assessed the distribution and persistence of intrathymic inoculation of NPC and DC in a similar system, DA ($RT1^{av1}$) into WAG ($RT1^u$), and demonstrated donor chimerism for over 30 days under ALS treatment (A. Papalois, I.G.M. Brons, R.Y. Calne, manuscript submitted). Donor-type cells were mainly distributed at the cortico-medullary junction in close proximity to thymic interdigitating DC. Further studies on the importance of intrathymic donor MHC class I and class II antigens on tolerance induction are in progress.

Table 1 Allo-graft survival of intraportally transplanted islets 7 days after intrathymic antigen-presenting cells (APC) inoculation + 1 ml antilymphocyte serum (ALS) (*NPC* non-parenchymal, *DC* dendritic cells)

Intrathymic inoculum		Graft survival (days)	MST (days)
Hanks	$n = 6$	5, 5, 5, 9, > 60, > 60	7
NPC 2×10^6	$n = 8$	1, 1, 1, 1, 2, 2, 2, 3	2
DC 4×10^5	$n = 9$	1, 1, 1, 1, 3, 3, 3, 5, 5	3

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