Arch Immunol Ther Exp, 2005, 53, 180–184
PL ISSN 0004-069X

Received: 2004.12.14
Accepted: 2005.02.04
Published: 2005.04.15

The influence of immune system stimulation on encapsulated islet graft survival

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Source of support: self financing

Summary

Introduction: The aim of this study was to determine the influence activating of the recipient immune system on the function of microencapsulated islet xenografts.

Materials and Methods: The skin of WAG or Fisher rats and WAG free or encapsulated (APA) Langerhans islets were transplanted to healthy or to streptozotocin diabetic BALB/c mice. Skin grafts were performed following the method of Billingham and Medawar. Rat islets were isolated from pancreas by the Lacy and Kostianovsy method and encapsulated with calcium alginate-poly-L-lysine-alginate according to the 3-step coating method of Sun.

Results: The transplantation of encapsulated WAG islets, despite activation of the host immune system, restored euglycemia for over 180±100 days. A subsequent skin graft taken from the same donor was rejected in the second set mode, but euglycemia persisted. In diabetic recipients, impaired immune response was corrected by successful encapsulated islet transplantation. In diabetic mice, strong stimulation with 2-fold skin transplantation induced primary non-function of grafted islets despite their encapsulation.

Conclusions: The survival of an islet xenograft depends on the level of activation of the recipient immune system. The immune response of diabetic mice was impaired, but increased after post-transplant restitution of euglycemia. Microencapsulation sufficiently protected grafted islets, and remission of diabetes was preserved. However, after strong specific or non-specific stimulation of the host immune system, non-function of xenografted islets developed despite their encapsulation. Therefore, islet graft recipients should avoid procedures which could stimulate their immune systems. If absolutely necessary, the graft should be protected by exogenous insulin therapy at that time.

Key words: islet xenograft • islet encapsulation • type I diabetes mellitus

Full-text PDF: http://www.aite-online/pdf/vol_53/no_2/7156.pdf

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INTRODUCTION

The survival of islet allografts depends on the degree of activation of the host immune system[8]. A reduction in specific alloantigen responsiveness leads to a prolongation of islet allograft survival, whereas stimulation may provoke graft rejection[5]. The effect of specific and non-specific xenogeneic stimulation is less recognized. The aim of this study was to evaluate the relationship between the activity of the recipient's immune system and the function of a microencapsulated Langerhans islet xenograft. In these experiments the host immune system was stimulated by xenotransplantation of free or encapsulated islets and of skin grafts, or impaired by evocation of chemical diabetes.

MATERIALS AND METHODS

WAG and Fisher rats were used as islet and skin donors. BALB/c recipient mice were rendered diabetic by a single intravenous injection of 165 mg/kg b.w. streptozotocin. Only animals maintaining a postprandial blood glucose level of more than 400 mg/dl were used as graft recipients. Islet graft rejection was defined as a recurrence on two successive days of glycemia greater than 250 mg/dl.

Recipients were divided in the following groups: 1) Control group ASS: 8 healthy mice allografted twice with CH3 mouse skin; 2) Control group SS: 8 healthy mice xenografted twice with WAG rat skin; 3) Control group IS: 8 healthy mice transplanted intraperitoneally (i.p.) with 1000 free islets followed by WAG rat skin; 4) Control group ES: 8 healthy mice transplanted i.p. with 1000 encapsulated islets; 5) Group DSS: 20 diabetic mice transplanted twice with WAG rat skin; 6) Group DI: 7 diabetic mice transplanted i.p. with 1000 free islets; 7) Group DE: 26 diabetic mice transplanted i.p. with 1000 encapsulated islets; 8) Group DES: 13 diabetic mice euglycemic after encapsulated WAG rat islet transplantation, grafted with islet donor skin; 9) Group DSSI: 6 diabetic mice, grafted twice with WAG rat skin followed by free WAG islet transplantation; 10) Group DSSE: 8 diabetic mice, grafted twice with WAG rat skin followed by free encapsulated WAG islets transplantation; and 11) Group DFSSE: 6 diabetic mice, grafted twice with Fisher rat skin and thereafter with encapsulated WAG islets. Comparison between groups was based on their mean skin survival times.

Skin grafts were performed following the method of Billingham and Medawar[3]. After removal of the plasters, on the seventh (first-set grafts) or on the fifth (second-set grafts) day, the grafts were scored by visual and tactile inspection until necrosis was considered complete and confirmed histologically. The length of second skin graft survival served as a criterion of the degree of activation of the host immune system. Rat islets were isolated from pancreas by the Lacy and Kostianovsky[9] method and purified by density gradient centrifugation on Histopaque. The islets were cultured at 37^0C in RPMI 1640 in a humidified atmosphere with 5% CO_2 and then encapsulated using an electrostatic droplet generator with calcium alginate-poly-L-lysine-alginate (Sigma) according to the 3-step coating method of Sun[13]. In brief, islets suspended in 1.5% sodium alginate were collected by air extrusion into 1.1% $CaCl_2$. Encapsulated in this way (APA), the islets were washed several times with 0.1% CHES and suspended in 0.05% poly-L-lysine (Sigma), washed again with 0.9% saline and suspended in 0.15% sodium alginate. After washing in saline they were put into 0.05 mM sodium citrate and finely washed with 0.9% saline and culture medium. The microcapsules were approximately 500 μm in diameter and regular in shape[14].

After culture in RPMI 1064 medium (in an atmosphere of 5% CO_2 at 37^0C), the identity and *in vitro* viability of the encapsulated and free islets were confirmed by dithizone staining[6] and 1000 free or encapsulated islets were implanted into the peritoneal cavities of streptozotocin-diabetic mice. Results are given as the mean±SD. The Student's and Mann-Whitney tests were used to evaluate the statistical significance of differences. P values <0.05 were considered as significant.

RESULTS

Graft survival times are presented on Table 1.

Healthy mice

Skin allografts (group ASS) survived longer (8.4±0.5 days and 6.4±0.5 days; $p<0.04$) than skin xenografts (group SS: 7.8±0.9 days and 5.5±0.8 days; $p<0.06$). The second-set phenomenon developed in both groups: the survival times of the second grafts were shorter ($p<0.001$) than those of the first. In recipients of free (group IS) and of encapsulated (group ES) islets, the survival of subsequent skin grafts (5.4±0.4 days in group IS and 6.0±0.5 days in group ES) did not differ significantly ($p>0.15$) from that of the second skin graft in the SS group.

Diabetic mice

In group DSS, survival of the first skin xenografts (8.5±0.5 days) was non-significantly longer ($p<0.06$)

Table 1. Graft survival in experimental groups

Group	Donor	Recipient BALB/c mice	Survival time (mean days±SD)		
			1st graft	2nd graft[a]	3rd graft[b]
1. ASS	C3H mice	8 healthy mice	skin; 8.4±0.5	skin; 6.4±0.5	
2. SS	WAG rats	8 healthy mice	skin; 7.8±0.9	skin; 5.5±0.8	
3. IS	WAG rats	8 healthy mice	free islets	skin; 5.4±0.4	
4. ES	WAG rats	8 healthy mice	WAG encapsulated islets	skin; 6.0±0.5	
5. DSS	WAG rats	20 diabetic mice	skin; 8.5±0.5	skin; 7.3±0.4	
6. DI	WAG rats	7 diabetic mice	free WAG islets; euglycemic for 6.7±3.5		
7. DE	WAG rats	26 diabetic mice	encapsulated islets; 16 mice euglycemic during the whole period of observation >186±100 days; 10 mice for 42±22 days		
8. DES	WAG rats	13 diabetic mice	encapsulated islets; euglycemic during the whole period of observation; 51.4±2.1	skin; 5.7±0.5	
9. DSSI	WAG rats	6 diabetic mice	skin; 8.5±0.6	skin; 7.2±0.4	encapsulated islets; all recipients developed primary graft non-function
10. DSSE	WAG rats	8 diabetic mice	skin; 8.5±0.5	skin; 7.2±0.4	encapsulated islets; 2 recipients euglycemic (for 8 and 171 days). 6 developed primary graft non-function
11.DFSSE	Fisher rats	6 BALB/c diabetic mice	skin; 9.0 ±0	skin; 9.0 ±0	encapsulated islets; 1 recipient euglycemic for 9 days; 5 developed primary graft non-function

[a] 30 days after the 1st graft; [b] 8 days after the 2nd graft.

than in healthy mice. The second-set phenomenon appeared ($p<0.001$), but the second xenografts were rejected later ($p<0.05$), after 7.3±0.4 days, than in healthy recipients.

Free islet grafts (group DI) were rejected after 6.7±3.5 days.

After i.p. transplantation of encapsulated islets, 16 out of the 26 group DE mice remained euglycemic during the whole period of observation (up to 355 days, mean: 180±100 days). However, 10 recipients relapsed into hyperglycemia after 42±22 days.

In diabetic recipients of encapsulated islets (group DES), post-transplant restoration of glycemia improved immune system function. In consequence, subsequent skin xenografts were rejected in the second-set mode after 5.7±0.5 days, sooner ($p<0.05$) than the second grafts in group DSS. However, encapsulated islets survived and maintained euglycemia during the whole period of observation (up to 54 days, mean: 51.4±2.1 days).

In diabetic recipients strongly stimulated by 2-fold specific (groups DSSI and DSSE) and non-specific (DFSSE) skin grafts, subsequent transplantation of encapsulated islets did not restore euglycemia despite encapsulation because primary graft non--function[1] developed.

DISCUSSION

It is well documented that non-specific and specific stimulation of the recipient immune system influences islet survival[5, 8]. To obtain more information on this subject, appropriate experiments were performed in that the host immune system was stimulated by skin and islet xenotransplants or impaired by evocation of experimental diabetes. The changes in the recipients' immune activity were assessed by the appearance of second-set mode skin graft rejection, the length of the skin graft survival time, and the duration of post-transplant glycemia. WAG rather then Fisher rats were used as donors because the antigenic differences between Fisher rats and BALB/c mice were evidently small: skin graft survival was longer in the DFSSE group than in the WAG

group, and the second-set phenomenon did not appear. This dependence of rat skin xenograft survival time on the strain of mice-recipient, especially immunosuppressed, has already been documented[7].

In experiments on the development of the second-set phenomenon, encapsulated and free islets were grafted. As the immunoprotective effect of microcapsules depends on the kind of alginate used and on a capsule production technique appropriate to assess their value, control groups were included (groups DE and DES). The results confirmed previous reports[11, 12, 13, 16] that our method of microencapsulation was satisfactory. Islets encapsulated in this way and xenografted i.p. to diabetic recipients restored host euglycemia. However, the immune protection provided by APA capsules is not absolute, as some cytokines small enough to cross their walls may enter the capsule and damage the grafted islets[18].

The second-set phenomenon occurred in the ASS, SS, IS, and ES groups. It was triggered not only by skin (group SS), but also by free (group IS) and encapsulated (group ES) islet xenografts. The development of the second-set skin graft phenomenon in euglycemic diabetic mice (group DES) grafted with encapsulated islets proves that not only free, but also encapsulated islets release antigens, stimulating the host immune system[14]. In these groups the second-set phenomenon was most probably initiated by indirect presentation of soluble factors released from the graft. In group ES their recognition was delayed by the length of time needed for the soluble graft antigen to diffuse through the capsular wall.

Because islet endothelial cells are almost completely disrupted during collagenase digestion by the donor's pancreas and, grafted i.p., do not have any connection with the host vasculature, they are considered as "cellular" transplants[15].

After islet transplantation, three kinds of reaction followed depending on procedure used: xenograft acceptance (e.g. in groups DE and DES), xenograft rejection (as in group DI), and primary islet xenograft non-function[1] (in groups DSSE and DFSSE).

The degree of acceptance of transplanted islets by diabetic animals may be assessed by their influence on host hyperglycemia. Diabetic impairment of the immune system was manifested by a prolongation of skin xenograft survival (group DSS). This was corrected after transplantation of encapsulated islets (group DES), with the reappearance of euglycemia. In both groups the second-set phenomenon developed. Free islets transplanted to a naive host (group DI) restored euglycemia only for a few days, then they were rejected and hyperglycemia reappeared. Only encapsulated islet xenografts (group DE) were accepted and induced life-long euglycemia. Encapsulation protected them from rejection even despite correction of hyperglycemia and specific stimulation of the host immune system strong enough to provoke second-set skin graft rejection (group DES).

Stimulation, if strong enough, can provoke macrophages to destroy even encapsulated islets[4, 10], as happened most probably in the cases of groups DSSI, DSSE, and DFSSE. Host and donor intra-islet macrophages, activated non-specifically in consequence of 2-fold skin transplantation, secreted cytokines which damaged encapsulated islets[4, 10, 18, 19] and provoked primary non-function of subsequently transplanted encapsulated grafts. Only in one mouse did life-long euglycemia develop. The results of these experiments confirm that changes in immune system activity influence even encapsulated graft function.

The different responses to skin and islet grafts in group DFSSE recipients depend on the different mechanisms of the immune reactions involved: specific and time-consuming in the case of skin, non-specific and immediate after islet transplantation.

In conclusion, the survival of islet xenografts depends on the level of activation of the recipient immune system. The immune response in diabetic mice was weaker than that in healthy animals. It increased after post-transplant restitution of euglycemia. Microencapsulation sufficiently protected the grafted islets and the remission of diabetes was preserved. However, after strong specific or non-specific stimulation of the host immune system, non-function of xenografted islets developed despite their encapsulation. Therefore islet graft recipients should avoid procedures which could stimulate their immune system. In case of absolute necessity, the graft should be protected by exogenous insulin therapy at that time[17].

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