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The capsular overgrowth on microencapsulated pancreatic islet grafts in streptozotocin and autoimmune diabetic rats

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Abstract This study investigates whether capsular overgrowth on alginate-polylysine microencapsulated islets is influenced by (1) the presence of islet tissue, (2) MHC incompatibility between donor and recipient, or (3) the presence of autoimmune diabetes. Encapsulated Albino Oxford (AO, $n = 6$, isografts) and Lewis ($n = 6$, allografts) rat islets, and encapsulated human islets ($n = 5$, xenografts) were implanted intraperitoneally into streptozotocin-diabetic AO rats. Also, encapsulated AO islets were implanted into autoimmune diabetic Bio Breeding/Organon (BB/O) rats ($n = 5$, allografts). Five isografts, five allografts, and three xenografts in AO recipients and five allografts in BB/O recipients resulted in normoglycemia. Two weeks after implantation, islets containing capsules were retrieved by peritoneal lavage, after which all animals that had become normoglycemic after transplantation returned to a state of hyperglycemia. Recovery rates of the capsules of these successful grafts, expressed as percentages of the initially implanted graft volume, varied from 72 % ± 7 % to 80 % ± 9 %. The associated pericapsular infiltrates (PCI) were similar in all groups and varied from 3.2 % ± 1.4 % to 8.3 % ± 2.6 %. Similar recovery rates and PCI were also found with empty capsules. However, the recovery rates of recipients with graft failures were lower and showed more PCI. Immunohistological staining of PCI showed no differences in the types of cells in the PCI on capsules with or without islets. We conclude that this early PCI is a capsule-induced foreign body reaction that is not influenced by MHC incompatibility or by the presence of autoimmune diabetes, and it should be avoided by improving the biocompatibility of the capsules.

Key words Islet transplantation, capsula · Alginate-polylysine, islet transplantation · Encapsulation, islet transplantation

Introduction

Alginate-polylysine microencapsulation of pancreatic islets has been shown to prolong graft function in experimental allogenic and xenogenic transplantation with normalization of blood glucose levels in chemically induced diabetic rats [1, 10, 19, 26] and mice [17, 22, 24], and in autoimmune diabetic rats [3, 7, 20, 30] and mice [24, 29]. The reported duration of normoglycemia varies considerably from a few days [1, 3] to almost 2 years [26]. Most of these studies agreed that cessation of graft function was associated with severe capsular overgrowth that had already been observed within 3–17 days after implantation [1, 3, 20, 24, 29, 30]. However, histological studies addressing the cause of this pericapsular infiltrate (PCI) are contradictory, suggesting either an immunological reaction to the encapsulated islet tissue [20, 24, 29] or a mere foreign body reaction to the capsule membrane [1, 3, 10, 22, 30].

The present study addresses the origin of the PCI by characterizing the PCI-producing cells on empty and on islet-containing capsules retrieved by peritoneal lavage. Technical imperfections of the capsule, such as bacterial contamination [3], incomplete alginate coating of the polylysine layer [2], or incomplete encapsulation of the islet tissue [33], may contribute to the PCI formation and, thereby, to the chance of early graft failure. This may bias the correct interpretation of the PCI formation eventually induced by the capsule per se. Therefore, we have used only islet-containing capsules derived from rats with well-functioning grafts for comparison to empty capsules in order to minimize the chance of possible technical imperfections contributing to the induction of the PCI under study.

The present study also investigates the role of MHC incompatibility in PCI formation by assessing islet-containing capsules retrieved from recipient rats with streptozotocin-induced diabetes after iso-, allo-, and xenotransplantation [20]. In addition, the influence of autoimmune diabetes was investigated by using spontaneously diabetic (BB/O) rats since it has been suggested that autoimmune diabetic rats are more prone to PCI formation than rats with chemically induced diabetes [2, 3].

Materials and methods

Design of the study

Empty capsules were implanted in streptozotocin-diabetic Albino Oxford (AO) and Lewis (Lew) rats and in autoimmune diabetic Bio Breeding/Organon (BB/O) rats. Encapsulated AO (isografts), Lew (allografts), and human (xenografts) islets were implanted in diabetic AO rats, and encapsulated AO islets were implanted in diabetic BB/O rats (allografts). Two weeks after implantation, microcapsules were recovered by peritoneal lavage. Nonfasting blood glucose levels were determined prior to transplantation, after transplantation, and following peritoneal lavage. In order to investigate whether the hyperglycemic state of the recipient animal enhanced the degree of capsular overgrowth, empty capsules were also implanted in normoglycemic AO, Lew, and BB/O rats.

At peritoneal lavage, the volume of the recovered microcapsules was measured and compared to the initial volume of implanted microcapsules. The degree of capsular overgrowth was quantified by determining the percentage of capsules with PCI in histological sections of the recovered microcapsule specimens. The PCI was histologically characterized by routine staining, as well as by immunohistochemical staining with monoclonal antibodies directed against different cell types.

Animals and islet isolation

We used male rats of the highly inbred AO strain ($RT1^u$; 280–350 g, bred at our own animal facilities), the Lew strain ($RT1^l$; 300–350 g, Harlan CPB, Zeist, The Netherlands), and the BB/O strain ($RT1^u$; breeding colony derived from Organon, Oss, The Netherlands). Diabetes in AO and Lew rats was induced by intravenous injection of streptozotocin (75 mg/kg, Zanosar). The animals were considered diabetic if the nonfasting blood glucose levels, determined under light ether anesthesia, remained over 20 mM for at least 2 weeks. Normoglycemic BB/O rats were used as graft recipients at 6 weeks of age, i.e., before the onset of hyperglycemia. Diabetic BB/O rats were used within 1–2 weeks of developing hyperglycemia, which occurred usually at 3–4 months of age. These animals were maintained on daily insulin therapy (Novo Lente, 2.0 U/day subcutaneously), which was discontinued 2–3 days prior to transplantation.

Rat donor islets were isolated with a two-stage collagenase incubation [31]. Briefly, the donor pancreas was distended by ductal infusion of 10 ml Krebs' Ringer (KRH) solution (buffered with 25 mM HEPES, pH 7.4, containing 10 % bovine serum albumin). The excised pancreas was cut into small pieces that were incubated successively for 10 min at 37 °C with 1.2 and 0.7 mg/ml collagenase (Sigma type XI, 1730 U/mg, Sigma Chemical, St. Louis, Mo., USA). The islets were purified by means of a five-layer discontinuous dextran gradient (MW 60–90 kD, Sigma) in KRH [12]. Subsequently, 1000–1500 islets per donor pancreas were hand-picked with a total islet volume of 3.0–4.0 μl/pancreas, calculated from the islet diameters of a 5 % sample [9].

Human islets were isolated from cadveric donor pancreases within 12 h after removal from the donor [28]. The pancreas was removed after preliminary in situ perfusion with UW solution and stored at 4 °C until isolation. Islets were isolated from the body and tail of the pancreas. Collagenase was dissolved in KRH and perfused through a cannula that was inserted into the duct of the gland. The pancreas was digested at 37 °C for 12–18 min and then dissociated by teasing and trituration. The islets were purified by means of a four-layer discontinuous dextran gradient in KRH, after which pure islets were hand-picked and quantified identically to rat islet preparations.

Islet encapsulation

A 2.5 % (w/v) Na-alginate solution (Alginate Keltone LV, lot 23814A, Kelco, San Diego, Calif., USA; dry-sterilized with ethylene oxide) with 2000 freshly hand-picked islets per milliliter was converted into islet-containing microdroplets [32]. Alginate-polylysine-alginate (APA) encapsulation was further performed as previously described [11]. Each islet transplant consisted of 10.0–15.0 μl islet tissue, which amply equals one equivalent of the endocrine volume of a normal rat pancreas [8]. The microcapsules had diameters of 700–850 μm. Total settled graft volumes varied between 1.5 and 2.5 ml per islet graft, determined with a 10-ml measure cylinder, with an accuracy of ± 0.1 ml. Similar volumes of empty microcapsules with comparable capsule diameters were also implanted. Both islet isolation and encapsulation procedures were carried out under sterile conditions with sterilized media.

Implantation and removal of implants

Either empty capsules or islet-containing microcapsules were implanted immediately after encapsulation. The capsules were suspended in 4.0 ml RPMI culture medium (with 100 U/ml penicillin G and 100 μg/ml streptomycin) and subsequently injected with a 10-gauge cannula into the peritoneal cavity of ether anesthetized rats. Blood was taken from the tail vein and the glucose concentration was determined with glucose test tapes (Reflolux, Boehringer, Mannheim, Germany). Two weeks after implantation, the capsules were removed by peritoneal lavage. This was performed by infusion of 10 ml prewarmed (37 °C) Krebs' Ringer solution through a small midline incision, after which the capsules were flushed out of the abdomen. This procedure was repeated three times. The final vol-

ume of the retrieved microcapsules was determined with the aid of a 10-ml measure cylinder and expressed as a percentage of the initial volume of implanted capsules. The abdomen was closed with a two-layer suture.

Histological evaluation

Specimens of the recovered microcapsules were fixed in 4 % paraformaldehyde, buffered with 0.1 M phosphate, and processed for glycol methacrylate embedding [13]. Embedded capsules were sectioned at 2 μm and stained with Romanowsky-Giemsa stain [15]. The degree of capsular overgrowth was expressed as the number of capsules with PCI per 100 recovered microcapsules. A part of the capsules was fixed in Bouin's solution for paraffin processing and stained with aldehyde-fuchsin. Statistically significant differences between the percentages of capsules with PCI of various groups were calculated using the unpaired Student's *t*-test. Values are given as mean ± SE. The level of significance was chosen at P, less than 0.05.

At the time of peritoneal lavage, pancreas biopsies were also taken in order to determine whether native beta cells were present (BB/O rats) or whether beta-cell regeneration had occurred (streptozotocin-diabetic animals). Aldehyde-fuchsin-positive beta cells in native islets were counted and expressed as a percentage of the total number of beta cells per islet in healthy control animals [10].

Immunohistochemistry

Microcapsules, retrieved by peritoneal lavage, were put into 1-ml plastic reservoirs and frozen in liquid freon. After removal from the reservoirs, 6-μm sections were cut on a cryostat microtome and dried overnight for immunohistochemical staining [16]. Briefly, sections were fixed in acetone containing 0.5 % H_2O_2 to inactivate endogeneous peroxidase and incubated with monoclonal antibodies ED1 and ED2 against monocytes and macrophages and subset macrophages [6], HIS-40 against IgM-bearing B lymphocytes [5], MCR OX-19 against $CD5^+$ T lymphocytes [4], HIS-48 against neutrophilic granulocytes [14], 1F119 against dendritic cells [21], and Asialo-GM1 directed to natural killer (NK) cells [23]. Specimens were rinsed three times in phosphate-buffered saline (PBS) and subsequently incubated with the appropriate horseradish peroxidase-conjugated second-step reagents, supplemented with 1 % volume normal rat serum to prevent nonspecific binding. After rinsing, peroxidase activity was demonstrated by applying 3,3′-diaminobenzidine tetra HCl (Sigma, St. Louis, Mo., USA) containing hydrogen peroxide. The specimens were counterstained with hematoxylin eosin. In control sections, PBS was used instead of the first stage monoclonal antibody.

Results

Blood glucose levels

Implantation of empty microcapsules into either normoglycemic or hyperglycemic recipients had no influence on nonfasting blood glucose levels. Streptozotocin-diabetic AO recipients of encapsulated isografts (AO islets, Fig. 1 A) or encapsulated allografts (Lew islets, Fig. 1 B) became normoglycemic in five out of six cases.

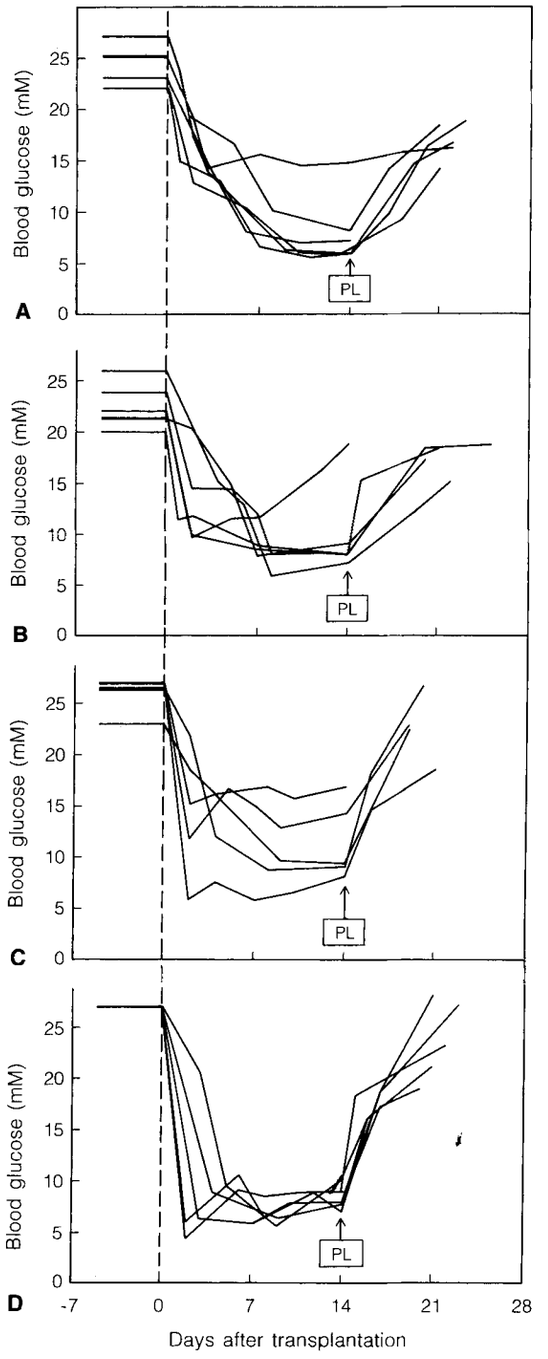

Fig. 1 A–D Effect of intraperitoneal implantation of microencapsulated islets and subsequent peritoneal lavage on nonfasting blood glucose levels: **A** isogenic transplantation, AO rats, $n = 6$. One animal died due to peritoneal lavage; **B** allogenic transplantation, Lew islets, AO recipients, $n = 6$. One animal died due to peritoneal lavage; **C** xenogenic transplantation, human islets, AO recipients, $n = 5$; **D** allogenic transplantation, AO islets, autoimmune diabetic BB/O recipients, $n = 5$

Table 1 Recovery rates and percentages of capsules with pericapsular infiltrate (PCI) at 2 weeks after implantation in streptozotocin (AO and Lew) and autoimmune (BB/O) diabetic rats

Implant	Donor	Recipient	*n*	% Recovery	% PCI
Empty capsules	–	AO	9	82 ± 1	10.3 ± 5.0
	–	Lew	5	85 ± 2	6.4 ± 1.6
	–	BB/O	4	82 ± 4	4.0 ± 1.0
Isografts	AO	AO	5[a]	75 ± 4	3.2 ± 1.4
			1[b]	28	50
Allografts	Lew	AO	5[a]	72 ± 7	5.5 ± 1.5
			1[b]	25	45
	AO	BB/O	5[a]	80 ± 9	8.0 ± 2.1
Xenografts	Human	AO	3[a]	80 ± 8	8.3 ± 2.6
			2[b]	60, 83	16, 17

[a] Successful islet grafts with blood glucose levels ≤ 10.0 mM at the time of graft removal
[b] Graft failures with blood glucose levels > 10.0 mM at the time of graft removal

Table 2 Recovery rates and percentages of capsules with pericapsular infiltrate (PCI) at 2 weeks after implantation in healthy (normoglycemic) recipients

Implant	Recipient	*n*	% Recovery	% PCI
Empty capsules	AO	9	81 ± 4	10.5 ± 1.1
	Lew	5	76 ± 6	13.2 ± 2.4
	BB/O	4	64 ± 8	10.6 ± 1.2

Three out of five diabetic AO recipients of encapsulated human islets (Fig. 1 C) and all five spontaneously diabetic BB/O recipients of encapsulated AO islets (Fig. 1 D) became normoglycemic. All diabetic recipients that had become normoglycemic after implantation of iso, allo or xenografts returned to a state of hyperglycemia within 1 week after peritoneal lavage.

Recovery of capsules and degree of pericapsular infiltration

Through the small midline incision for peritoneal lavage, large numbers of freely floating capsules were seen in the peritoneal cavity. Recovery rates and percentages of capsules with PCI retrieved from animals that were hyperglycemic at the time of implantation are shown in Table 1. Similar and not statistically significantly differing percentages of recovery were obtained from recipients of empty capsules and from recipients of successful iso, allo and xenografts. Also, the associated degrees of PCI were found not to be significantly different, ranging from 4.0 % ± 1.0 % to 10.3 % ± 5.0 % in the three groups with empty capsules and from 3.2 % ± 1.4 % to 8.3 % ± 2.6 % in the four groups with functioning islet grafts. Neither the percentages of PCI on empty and islet-containing microcapsules nor the percentages of PCI on capsules retrieved from different recipient rat strains reached statistical significance in any comparison between any pair of experimental groups. From diabetic recipients that had not become normoglycemic after iso, allo and xenotransplantation (graft failures), we recovered 25 %, 28 %, 60 %, and 83 % of the initially implanted capsules, with 45 %, 50 %, 16 %, and 17 % capsules with PCI, respectively. This indicates that higher percentages of PCI are associated with lower recovery rates.

When empty capsules were implanted into normoglycemic recipients (Table 2), observations similar to those about the hyperglycemic recipients were made. Most capsules were free of capsular overgrowth and no significant differences in PCIs were found between the rat strains tested. Hyperglycemia in recipient rats, as such, does not enhance capsular overgrowth since the percentages of PCI on empty capsules retrieved from hyperglycemic recipients were not higher than those on empty capsules retrieved from normoglycemic recipients.

Histology of retrieved microcapsules

Routine staining showed that the majority of recovered capsules had intact alginate-polylysine membranes and that capsules retrieved from recipients with well-functioning islet grafts contained many viable aldehyde-fuchsin-positive islets. Capsules with a PCI were severely infiltrated by fibroblasts, fibrocytes, foreign body giant cells, and collagen deposition, irrespective of whether these capsules had been recovered from empty capsules

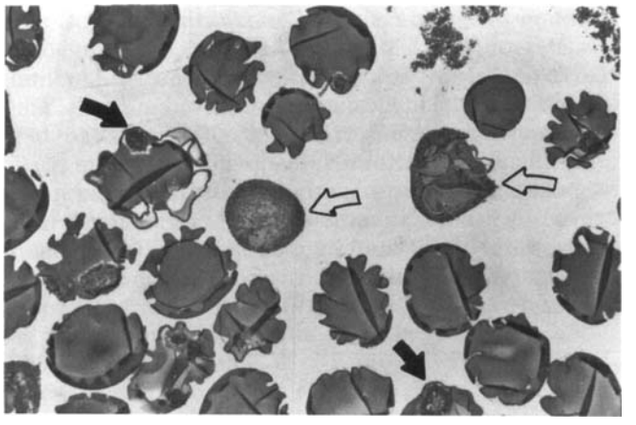

Fig. 2 Glucol methacrylate embedded histological section of encapsulated human islets recovered from a streptozotocin diabetic AO rat at 2 weeks after implantation. *Open arrows* show remnants of capsules with severe PCI; *closed arrows* show encapsulated islets without PCI. Note some islets with central necrosis in capsules without PCI. Capsule surface deformation was due to fixation and embedding artifacts (Romanowsky-Giemsa stain, × 50)

Table 3 Immunohistochemical staining of pericapsular infiltrates on empty capsules and islet-containing alginate-polylysine microcapsules retrieved 2 weeks after implantation (+++ many positive cells, ++ some positive cells, + a single positive cell, – no positive cells)

Monoclonal antibody	Specificity	Empty capsules[a]	Islet containing capsules[b]
ED1	Macrophages	+++	+++
ED2	Subset macrophages	++	++
HIS40	IgM (B lymphocytes)	–	–
HIS48	Granulocytes	+	+
MCR OX-19	CD5 (T lymphocytes)	–	–
1F119	Dendritic cells	–	–
Asialo GM1	NK cells	–	–

[a] Empty capsule specimens retrieved from AO and BB/O recipients
[b] Islets containing capsules retrieved from AO rats with iso-, allo- and xenografts, and from BB/O rats with allografts

or islet-containing implants. Most of these capsules were broken, often with minimal remnants of the alginate-polylysine capsule (Fig. 2). Table 3 shows the outcome of the immunohistochemical staining of PCIs. There were many ED1$^+$ and ED2$^+$ macrophages (Fig. 3a, b), some HIS-48+ granulocytes, but no B or T lymphocytes (Fig. 3c, d), dendritic cells, or NK cells in the PCIs. No differences in the PCIs of empty capsules and islet-containing capsules were found no matter whether they were retrieved from normoglycemic or hyperglycemic rats. Also, PCIs on islet-containing capsules retrieved after iso, allo or xenotransplantation were similar, just as the cellular composition of the PCIs on encapsulated allografts retrieved from either streptozotocin diabetic or autoimmune diabetic rats.

Histology of the pancreas of the recipient

Native pancreases from streptozotocin-treated animals showed some aldehyde-fuchsin-positive beta cells. This, however, was never more than 5 % of the number of beta cells of healthy controls. Native pancreases from BB/O rats with microencapsulated allografts contained no aldehyde-fuchsin-positive beta cells. Most of these islets were severely infiltrated by mononuclear cells, demonstrating active insulitis at the time of grafting.

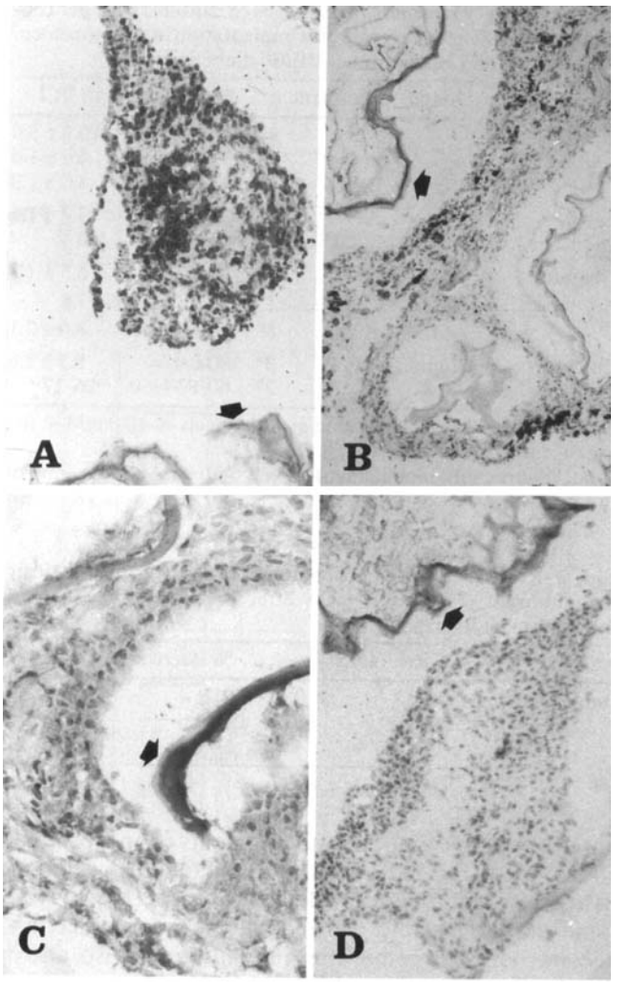

Fig. 3 A–D Immunohistochemically stained specimens of capsules with PCI: **A** PCI on isograft retrieved from a streptozotocin diabetic AO rat, stained with ED1 mAb (macrophages): many positive cells; **B** PCI on allograft retrieved from autoimmune diabetic BB/O rat, stained with ED2 mAb (macrophages): many positive cells; **C** PCI on allograft retrieved from autoimmune diabetic BB/O rat, stained with HIS40 mAb (B lymphocytes): no positive cells; **D** PCI on allograft retrieved from autoimmune diabetic BB/O rat, stained with OX19 mAb (T lymphocytes): no positive cells. Counterstained with H&E, × 200. Capsule distorsion (→) was due to freezing artifacts

Discussion

The main issue addressed by this study was the question of whether PCI on alginate-polylysine microencapsulated islets is induced by the capsule membrane itself or by the presence of islet tissue within the capsule. Capsules were retrieved at a fixed time after implantation in the peritoneal cavity in order to allow for a sound comparison of the degrees of PCI on capsules implanted under different conditions. This period was chosen to be 2 weeks since we wanted to investigate the role of intact and technically adequate capsules in the process of PCI formation and to exclude the contribution of possible technical imperfections of the capsules. Since technical imperfections, as such, are known to be associated with a pericapsular reaction [2, 3, 33], it is likely that a severe PCI will develop more rapidly in the presence than in the absence of technical imperfections. A severe PCI interferes with graft function and is usually observed in a

much shorter time after implantation than 2 weeks [1, 3, 20, 24]. Thus, by choosing a 2-week period and by restricting our comparisons to the capsules retrieved from well-functioning grafts, we maximized our chances of studying technically adequate capsules only and minimized our chances of including technically imperfect capsules in our studies.

Our study indicates that capsular overgrowth is a typical foreign body reaction since the PCIs on recovered microcapsules contained fibroblasts, fibrocytes, giant cells, and collagen deposition, together with $ED1^+$ and $ED2^+$ macrophages and a solitary granulocyte [25]. Thus, the capsule membrane itself, rather than the islet tissue within the capsule, seems to be responsible for inducing the PCI. This corroborates previous observations of others [3, 22, 30] and is further substantiated by our finding that not only the cellular composition but also the degree of PCI is similar on empty capsules and islet-containing microcapsules. Furthermore, the absence of B and T lymphocytes and NK cells in immunohistochemically stained sections of PCI suggests that antibody and/or cellularly mediated graft rejection is unlikely to be involved in PCI formation. The absence of these cells in the PCI on capsules retrieved from the BB/O rats suggests that, in these animals, the PCI is not of autoimmune origin since it has been demonstrated that T lymphocytes and NK cells play a major role in autoimmune beta cell destruction in biobreeding rats [18, 27]. As regards the immunocompetence of the diabetic biobreeding rat recipients, we found many mononuclear lymphocytes in islets of native pancreases, which suggests active insulitis at the time of grafting.

Like others [20], we have expressed the degree of overgrowth as the percentage of recovered capsules with PCI. Unlike others, however, we have also assessed the recovery rate of capsules from each animal. Our findings indicate that severe overgrowth is associated with low recovery rates. This may explain the finding of Mazaheri et al. [20, 30] that a longer graft survival time was associated with less PCI. They mention only an overall recovery rate of approximately 50 % in a wide variety of experiments. It may well be that in this particular set of experiments the recovery rate was much lower. If the number of capsules recovered was low, then the percentage of recovered capsules with PCI could easily also have been quite low, while a large number of capsules had not been recovered as a consequence of overgrowth and subsequent firm adhesion within the abdominal cavity. This reasoning is quite plausible since graft failure is consistently associated with severe capsular overgrowth, as described in several reports [1, 3, 24, 29], including our previous [10] and present studies.

The relation between capsular overgrowth and graft failure is complex. At 2 weeks after implantation, there is a mild degree of PCI on both empty capsules and islet-containing capsules. It stands to reason that this overgrowth will gradually increase, resulting in graft failure after several weeks or months [10], and that modifications in the composition of the capsule may reduce or even prevent this reaction [24, 33]. In a few cases, however, not mild but severe overgrowth and graft failure are apparent within 2 weeks. Although inadequacies of the enzymatic isolation procedure in these particular cases could explain the graft failures as such, they cannot be held responsible for the severe overgrowth since we have frequently observed encapsulated islets with central cell necrosis in intact capsules without PCI. Furthermore, it is unlikely that severe overgrowth was induced by MHC incompatibility since severe overgrowth was observed in both isogenic and allogenic strain combinations and, to a lesser extent, in the xenogenic strain combination. Thus, the most appropriate explanation seems to be that early graft failure with severe capsular overgrowth is the consequence of technical imperfections of the capsule. Such imperfections may include imperfect coating of the polylysine with alginate [2] or an incidentical lack of spherical smoothness of the capsule surface [32, 33]. These capsular imperfections are more likely to occur when microcapsules are produced with islet tissue within the capsule since we observed only mild – never severe – capsular overgrowth on empty microcapsules. Moreover, this may also explain the observation of others who found more capsular overgrowth on islet-containing capsules than on empty microcapsules [2, 3, 24, 29, 30]. Incomplete encapsulation and, thereby, incomplete immunoisolation of the islet cannot be excluded [33], although we found no immunohistochemical evidence of an immune reaction in our early failures.

As regards the degree of PCI, our results indicate that this is not dependent on the histocompatibility between donor and recipient since no differences were observed after isotransplantation or allotransplantation, and since the degree of PCI on the three successful xenografts was similar. Also, our results suggest that the degree of capsular overgrowth is not enhanced by the presence of autoimmune diabetes in biobreeding rat recipients. This observation seems to differ from that of Cole et al. [3], who reported more capsular overgrowth on capsules retrieved from biobreeding rats than on capsules retrieved from streptozotocin-diabetic rats. Similar observations were made by Weber et al. [29] in autoimmune and streptozotocin-diabetic mice. In both studies, however, the periods after implantation were considerably shorter in autoimmune than in streptozotocin-diabetic recipients, and the recovery rates and degree of PCI were quantified in neither of the studies. Ricker et al. [24] also reported different degrees of PCIs in streptozotocin- and autoimmune diabetic mice, but after similar periods after implantation. Their results cannot be interpreted in detail since they have not presented capsule recovery rates and since several of their experimental groups did not include more than one or two animals. The observed

overgrowth, as such, was probably related to the chemical composition of the capsule since the same study reported that the use of another poly-l-lysine preparation markedly reduced overgrowth in autoimmune diabetic recipients.

In summary, both the histological composition and the degree of capsular overgrowth were similar in all experimental groups when assessed on microcapsules derived from recipients with well-functioning grafts at 2 weeks after implantation. This indicates that this early PCI on microencapsulated islets is a capsule-induced foreign body reaction that is not influenced by MHC incompatibility or by the presence of autoimmune diabetes. The degree of this foreign body reaction may be expected to increase with time after implantation, eventually resulting in loss of graft function. The results of our study therefore suggest that further studies should address the avoidance of this foreign body reaction by improving the biocompatibility of the capsules, *i. e.*, by improving the chemical composition and/or purity of the applied alginates [34], in combination with flawless results of the mechanical encapsulation method.

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