In vitro and in vivo expression of Galα–(1,3)Gal on porcine islet cells is age dependent

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Abstract

The expression of Galα–(1,3)Gal (αGal) on porcine islet cells remains controversial. Several groups have reported that porcine islet endocrine cells do not express αGal while we have shown in neonatal porcine islets (NPI) that β cells do express this antigen. We hypothesize that endocrine cells expressing αGal on NPI are less mature cells that may have originated from ductal cells and that expression of this antigen disappears as they develop into fully mature β cells. Thus, we further examined αGal expression on various porcine islet cell preparations and correlated this with the proportion of cytokeratin 7 (CK7)–positive ductal cells. In vitro and in vivo expression of αGal and CK7 was significantly (P<0.05) higher in less mature NPI cells compared with matured NPI and adult porcine islet cells while the reverse was observed in the proportion of β cells. Moreover, a significantly higher proportion of CK7–positive cells was detected in the Gal-expressing population compared with non-expressing cells. In contrast, a higher proportion of β cells was observed in the Gal-negative population compared with the Gal-positive population. These data showed a reduced expression of αGal and CK7 as porcine islet cells mature into β cells suggesting a possible role for αGal in the maturation of pancreatic endocrine β cells.


Introduction

Insulin independence in type 1 diabetes patients can now be achieved in a safe and reproducible manner when a sufficient islet cell mass (>9000 islets/kg body weight) is transplanted; however, this often requires more than one donor pancreas (Shapiro et al. 2000, Ryan et al. 2001). If islet transplantation is to become a widespread treatment for the majority of type 1 diabetes patients the supply of human pancreatic islet tissue will become a limitation. The shortage of available human allogeneic tissues for clinical transplantation has resulted in considering other sources of insulin-producing tissue. Porcine islets represent a practical alternative source of insulin-producing tissue since pigs are inexpensive, readily available, and they exhibit physiological and morphological characteristics comparable with humans. Adult porcine islets have been extensively studied; however, in most laboratories they are difficult to isolate or maintain in tissue culture. In contrast, we have previously developed a simple procedure for isolating abundant islets from neonatal pigs (Korbutt et al. 1996). Our group (Korbutt et al. 1996, Rayat et al. 2000) and others (Yoon et al. 1999, Trivedi et al. 2001) have shown that neonatal porcine islets (NPI) are capable of correcting diabetes in immunoincompetent mice. In addition, we have also shown that these cell aggregates are composed of approximately 40–45% endocrine cells as well as 50% ductal cells (Korbutt et al. 1996), suggesting the enormous growth potential of this tissue.

One of the main obstacles in porcine-to-human xenotransplantation is the occurrence of hyperacute rejection. The carbohydrate moiety Galα–(1,3)Gal (αGal) is highly expressed on the surface of porcine endothelial cells and is immunoreactive to human preformed natural antibodies. This reaction leads to activation of the recipient’s complement system and rapid destruction of the xenograft. The expression of αGal on specific porcine islet endocrine cells remains unclear. Some studies have shown that αGal is not expressed on adult or fetal porcine islet endocrine cells (Oriol et al. 1993, McKenzie et al. 1994, 1995a,b), while we have demonstrated that αGal is expressed in both insulin- and glucagon-producing neonatal porcine islet cells (Rayat et al. 1998). A recent study has shown that fetal porcine islet cells consistently express high levels of
αGal epitope, whereas most adult islet cells are void of this epitope (Bennet et al. 2000). The same group has also demonstrated that the strongest αGal antigen expression was detected in capillary endothelial cells, duct epithelial cells, and occasional macrophages in fetal porcine islet cell cluster preparations (Strokan et al. 2000) while mature endocrine–like cells were completely negative for the αGal antigen expression. However, some immature endocrine cells, scattered between the duct epithelial cells or inside the clusters, were weakly positive for αGal antigen. Based on our previous study (Rayat et al. 1998), we hypothesize that immature endocrine cells and some ductal cells present in NPI are the cells that we previously detected to express αGal antigen. To test this hypothesis, we further examined the presence of αGal in NPI and correlated this expression with cytokeratin 7 (CK7)–positive ductal cells in various porcine islet cell preparations. The preparations include (1) islet cells isolated and cultured from 3-day-old neonatal pigs (non-mature NPI), (2) microencapsulated NPI (in vitro matured) and (3) NPI grafted into non-diabetic SCID mice (in vivo matured NPI) as well as (4) adult porcine islets.

We report here a direct correlation between αGal and CK7 expression on porcine islet cells both in vitro and in vivo. Our data show a reduction in the expression of αGal and CK7 as porcine islet cells mature into insulin-producing cells. This suggests a possible role for αGal in the maturation of pancreatic endocrine β cells and may explain in part why in some studies the expression of αGal on endocrine cells was not detected. We also demonstrate for the first time an in vitro method of increasing the proportion of β cells in NPI using alginate capsules and further culture of islets in medium supplemented with autologous serum. This may have implication(s) for the choice of NPI as alternative islets for clinical transplantation.

Materials and Methods

Preparation of non-mature NPI and adult porcine islets

As previously described (Korbutt et al. 1996), Landrace–Yorkshire neonatal pigs aged 1–3 days old (1·5–2·0 kg body weight) of either sex were anesthetized with halothane and subjected to laparotomy and exsanguination. The pancreases were removed, cut into small pieces and digested with 2·5 mg/ml collagenase (Sigma, St Louis, MO, USA). After filtration through a nylon screen (500 µm), non-mature spherical–shaped NPI were formed during 9 days of suspension culture in Ham’s F10 medium (Gibco, Burlington, Ontario, Canada) containing 10 mmol/l glucose, 50 µmol/l isobutylmethylxanthine (ICN Biomedicals, Montreal, Ontario, Canada), 0·5% bovine serum albumin (fraction V, radioimmunoassay grade; Sigma), 2 mmol/l L-glutamine, 10 mmol/l nicotinamide (BDH Biochemical, Poole, Dorset, UK), 100 U/ml penicillin, and 100 µg/ml streptomycin.

Adult porcine islets were isolated in Minneapolis from miniature swine Trolls (age 18–19 months) as previously described (Hering et al. 1995). The pancreases were intraductally distended with University of Wisconsin solution (Du Pont, Bad Homburg, Germany) containing 0·4% (w/v) collagenase (Serva, Heidelberg, Germany) and digested in a continuous digestion–filtration device at 32°C. Islets were purified using Ficoll–Na–diatrizoate density gradients on a Cobe 2991 cell separator (Cobe BCT, Inc., Lakewood, CO, USA) and then cultured at 37°C for 24 h. Following culture, islets were shipped to Edmonton, and maintained in culture for an additional 24–48 h prior to being assessed, as described below.

In vitro maturation of NPI

After 9 days of culture, non-mature NPI were microencapsulated in suspension in 1·0 ml 0·72% (w/v) highly purified alginate dissolved in Hank’s balanced salt solution (Rayat et al. 2000). Microcapsules (250–400 µm) were formed by passing the alginate/islet suspension through an electrostatic generator followed by collection in a 120 mmol/l CaCl₂ solution containing 10 mmol/l HEPES and 0·01% Tween 20 for 10 min to allow formation of capsules. The capsules were washed by gravity sedimentation in supplemented Ham’s F10 solution and cultured in the same medium with 10% autologous pig serum for an additional 7 days at 37°C. After culture, alginate capsules were dissolved by incubating the encapsulated islets in calcium-free medium for 15 min at 37°C. The in vitro matured islets were washed with calcium-free medium then dissociated into single cells as described below.

Preparation of single islet cell suspensions

Non-mature and in vitro matured NPI as well as adult porcine islets were dissociated into single cells by gentle agitation in calcium-free medium containing 15 µg/ml trypsin (Boehringer Mannheim, Laval, Quebec, Canada) and 4 µg/ml DNase (Boehringer Mannheim). The cell suspension was filtered through a 63 µm nylon screen to remove cell clumps and then centrifuged through Percoll (Sigma) of 1·040 g/ml density to eliminate dead cells and debris. Cells were then washed with phosphate-buffered saline and analyzed either by flow cytometry or placed on microscopic glass slides (FGR Steinmetz Inc., Surrey, British Columbia, Canada) which were then fixed in Bouin’s solution for 30 min, washed with 70% ethanol, and stored at 4°C prior to morphological assessment.

In vivo maturation of neonatal porcine islets

Non-mature NPI were transplanted under the left kidney capsule of non-diabetic C.B–17 SCID mice (C.B-Igh-1/p8 GbmstTac–Prkd+/Ly83+/p8 N7; Taconic Farms Inc., Germantown, NY, USA). Aliquots of 2000 NPI were aspirated into polyethylene tubing (PE-50), and spun to
Table 1 Cellular composition of non-mature, in vitro-matured neonatal porcine and adult pig islets. The numbers in parentheses represent the number of independent samples.

<table>
<thead>
<tr>
<th>Islets</th>
<th>αGal</th>
<th>Vimentin</th>
<th>CK7</th>
<th>Insulin</th>
<th>Glucagon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-mature NPI</td>
<td>19.7 ± 3.3a(14)</td>
<td>15.0 ± 3.9b(12)</td>
<td>28.0 ± 4.2c(14)</td>
<td>33.7 ± 3.8d(13)</td>
<td>19.5 ± 3.0(13)</td>
</tr>
<tr>
<td>In vitro-matured NPI</td>
<td>11.0 ± 2.8(7)</td>
<td>7.6 ± 1.3(7)</td>
<td>10.5 ± 1.8(9)</td>
<td>42.1 ± 3.1(9)</td>
<td>23.3 ± 1.4(9)</td>
</tr>
<tr>
<td>Adult pig</td>
<td>5.1 ± 1.3(6)</td>
<td>1.4 ± 0.5(4)</td>
<td>1.3 ± 0.5(6)</td>
<td>82.8 ± 1.2(6)</td>
<td>9.7 ± 3.2(6)</td>
</tr>
</tbody>
</table>

Table 2 Percentage of CK7- and insulin-positive cells in αGal-expressing and non-expressing NPI cells. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Cell population</th>
<th>n</th>
<th>CK7a</th>
<th>Insulinb</th>
<th>CK7/insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>αGal-positive</td>
<td>7</td>
<td>24.4 ± 10.4</td>
<td>11.7 ± 2.1</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>αGal-negative</td>
<td>9</td>
<td>1.8 ± 0.7</td>
<td>32.4 ± 3.8</td>
<td>1.4 ± 0.9</td>
</tr>
</tbody>
</table>

Histological analysis

Expression of αGal on single islet cells and paraffin graft sections (4 μm) was detected using biotinylated αGal-specific BS-1 isoelectin B4 from Bandeiraea simplicifolia (1:25 dilution; Sigma; see Rayat et al. 1998). CK7 expression was detected by treating the paraffin graft sections with 0.1 M citrate buffer. The sections were then heated for 1.5 min using a domestic microwave. Single islet cell preparations were exposed to citrate buffer and heated for 30 s. CK7 was detected using a monoclonal mouse anti-human CK7 antibody (1:200 dilution; Dako Diagnostics Canada Inc., Mississauga, Ontario, Canada) and biotinylated anti-mouse IgG secondary antibody (1:200 dilution; Vector Laboratories, Burlingame, CA, USA). Monoclonal mouse anti-porcine vimentin (1:1000; Dako Diagnostics) was used as a marker for mesenchymal and/or rapidly dividing cells.

Form a pellet. The pellet of islets was gently placed under the kidney capsule with the aid of a micromanipulator syringe. Once the tubing was removed, the capsulotomy was sealed with a disposable high-temperature cautery pen (Aaron Medical Industries, St Petersburg, FL, USA). Graft-bearing kidneys were harvested on days 1, 7, 30, 50, and more than 200 days post-transplantation. Tissues were fixed in Bouin’s solution and then stained with appropriate antibodies as described in Materials and Methods.

Histological analysis

Expression of αGal on single islet cells and paraffin graft sections (4 μm) was detected using biotinylated αGal-specific BS-1 isoelectin B4 from Bandeiraea simplicifolia (1:25 dilution; Sigma; see Rayat et al. 1998). CK7 expression was detected by treating the paraffin graft sections with 0.1 M citrate buffer. The sections were then heated for 1.5 min using a domestic microwave. Single islet cell preparations were exposed to citrate buffer and heated for 30 s. CK7 was detected using a monoclonal mouse anti-human CK7 antibody (1:200 dilution; Dako Diagnostics Canada Inc., Mississauga, Ontario, Canada) and biotinylated anti-mouse IgG secondary antibody (1:200 dilution; Vector Laboratories, Burlingame, CA, USA). Monoclonal mouse anti-porcine vimentin (1:1000; Dako Diagnostics) was used as a marker for mesenchymal and/or rapidly dividing cells.

The presence of pancreatic hormones was determined using guinea pig anti-porcine insulin antibody (1:1000 dilution; Dako Diagnostics) and a rabbit anti-porcine glucagon antibody (1:1000 dilution; Dako Diagnostics). Secondary antibodies were biotinylated goat anti-guinea pig IgG (1:200; Vector Laboratories) and goat anti-rabbit IgG (1:200 dilution; Vector Laboratories). The avidin–biotin complex/horseradish peroxidase (ABC/HP; Vector Laboratories) method with 3,3-diaminobenzidinetetrahydrochloride (DAB; BioGenex, San Ramon, CA, USA) was used to visualize the positive reaction (brown color).

For double staining, paraffin sections were stained as described above with either biotinylated αGal-specific lectin or mouse anti-human CK7 antibody for 30 min followed by 20 min with the appropriate biotinylated secondary antibody. The ABC/HP complex using DAB as chromagen was used to detect cells expressing αGal or CK7. The same tissue sections were subsequently stained with guinea pig anti-porcine insulin antibody and biotin-conjugated goat anti-guinea pig secondary antibody. The avidin–biotin complex/alkaline phosphatase (Vector Laboratories) method and Fast Red as chromagen (Vector Laboratories) were used to obtain a red reaction.

Fluorescence-activated cell sorter (FACS) analysis

To further characterize the expression of αGal on non-mature NPI, single islet cell suspensions were stained with fluorescein isothiocyanate (FITC)-conjugated BS-1
Age-dependent expression of αGal on porcine islet cells
isolectin B4 (1:50 dilution; Sigma) for 1 h on ice, washed three times, and analyzed by flow cytometry (EPICS Elite ESP flow cytometer; Coulter, Hialeah, FL, USA). Cells expressing or not expressing αGal were collected and subsequently stained for the co-expression of CK7 and/or insulin as described above. Positive controls for this experiment included porcine aortic endothelial cells stained with FITC–conjugated IB4 lectin using the same protocol for islet cells.

**Statistical analysis**

Results are expressed as means ± S.E.M. of at least four independent observations. Significant differences between experimental groups were tested using ANOVA and unpaired t-test. A P value of 0·05 was considered to be statistically significant.

**Results**

**Cellular composition of neonatal and adult porcine islets**

The cellular composition of non-mature and in vitro matured NPI as well as adult porcine islets was assessed with respect to the proportion of cells expressing insulin, glucagon, αGal, CK7 and vimentin (Table 1). Immunostaining for the expression of αGal revealed a significantly (P<0·05) higher proportion of αGal-expressing cells in non-mature NPI (19·7 ± 3·3%) compared with those islets that were further matured in vitro (11·0 ± 2·8%). Moreover, this expression was further reduced in adult porcine islets (5·1 ± 1·3%). A similar pattern of expression was observed for CK7 and vimentin. The percentage of CK7- and vimentin-positive cells was significantly higher in non-mature NPI than in both in vitro matured NPI and adult pig islets. Non-mature NPI consisted of 28·0 ± 4·2% CK7-positive cells compared with 10·5 ± 1·8 and 1·3 ± 0·5% for in vitro matured NPI and adult pig islets respectively. Vimentin-positive cells in non-mature NPI was approximately twofold (15·0 ± 3·9 vs 7·6 ± 1·3%) and tenfold (15·0 ± 3·9 vs 1·4 ± 0·5%) higher than that observed in both in vitro matured NPI and adult pig islets respectively. In contrast to the reduction in αGal, vimentin and CK7 expression, a significant increase (P<0·001) in the proportion of insulin-positive cells in highly matured islet cell preparations was observed. In particular, non-mature NPI contained 33·7 ± 3·8% insulin-positive cells compared with 42·1 ± 3·1 and 82·8 ± 1·2% in both in vitro matured NPI and adult porcine islets respectively.

**Expression of CK7 and insulin in αGal-expressing and non-expressing NPI cells**

Following FACS analysis of dissociated non-mature NPI cells that had been stained with FITC–conjugated IB4-lectin, Gal-expressing and non-expressing cell populations were collected and the proportion of CK7- and insulin-positive cells in these two populations was determined by immunocytochemical staining (Table 2). The αGal-expressing population was comprised of a significantly (P<0·003) higher proportion of CK7-positive cells (24·4 ± 10·4%) than that observed in the αGal-negative cell fraction (1·8 ± 0·7%). In contrast, the αGal non-expressing cell population consisted of a significantly (P<0·005) higher proportion of insulin-positive β cells than those present in the αGal-positive cell fraction (32·4 ± 3·8 vs 11·7 ± 2·1% respectively). There was no significant difference in the number of cells staining double positive for both CK7 and insulin in either the αGal-expressing or non-expressing populations (0·7 ± 0·2 vs 1·4 ± 0·9% respectively). These data suggested that a proportion of less mature endocrine β cells can express the αGal xenoadenogen while the majority of mature endocrine β cells lose the expression of this antigen.

**In vivo expression of αGal and CK7**

To examine the fate of αGal and CK7 expression in vivo, non-mature NPI were transplanted and allowed to mature under the kidney capsule of non-diabetic C.B-17 SCID mice. On day 1 post-transplantation, a high proportion of αGal-positive cells were observed in the graft (Fig. 1A and B). A strong reaction of IB4 lectin with ductal epithelium and some centrally located islet β cells was noted. A similar intensity of positive immunostaining reaction for αGal was also observed on day 7 (data not shown). However, by day 30 post-transplantation (Fig. 1C and D) a reduction in positive staining for αGal was observed and this continued to decrease by day 50 post-transplantation (Fig. 1E and F). At these times, αGal-expressing cells were mostly concentrated on luminal structures and some cells stained for both αGal and insulin were still present. More than 200 days post-transplantation, the αGal-positive cells were almost undetectable in the grafts while numerous clusters of β cells stained positive for insulin comprised the majority of the graft (Fig. 1G and H).

![Figure 1](http://www.endocrinology.org/)

**Figure 1** αGal and insulin staining on NPI xenografts following transplantation into C.B-17 SCID mice. Grafts were harvested on days 1 (A and B), 30 (C and D), 50 (E and F) and more than 200 days (G and H) post-transplantation. αGal-positive cells are shown as brown structures whereas insulin-positive cells are stained red. αGal expression was highly expressed on day 1 then decreased by day 30 and day 50 and was subsequently non-detectable more than 200 days post-transplantation. Scale bars represent 100 μm for A, C, E and G and 50 μm for B, D, F and H.
To determine whether an association exists between αGal with less mature islet cells in vivo, islet xenografts were stained with anti-CK7 and anti-insulin antibodies. Similar to that observed in αGal expression, a strong reaction of anti-CK7 antibody to ductal epithelium was detected in less mature islet preparations (Fig. 2). On day 1 post-transplantation, the ductal epithelia were present mostly around the β cells that were arranged as small cell clusters closely attached to the ductal epithelium (Fig. 2A and B). Some clusters contained one or two β cells and some were devoid of β cells (clear round structures). On day 7 post-transplantation, the epithelial duct cells began to form more luminal structures that contained cells positive for insulin and CK7 as observed in Fig. 1. On day 30 post-transplantation, the CK7-positive ductal cells were largely arranged as luminal structures consisting of a higher proportion of insulin-positive cells budding off from the CK7-positive ducts (Fig. 2C and D). At this time, the majority of islet clusters containing β cells were present closely adjacent to the ductal epithelium. Few CK7-positive ducts could be identified on day 50 post-transplantation and these ducts were mostly located in the center of the graft (Fig. 2E and F). More than 200 days post-transplantation, no CK7-positive ductal cells were detected and the grafts consisted mainly of insulin-positive cells (Fig. 2D and H). These data showed that pig β cells were derived from ducts present in the islet preparations that also express αGal xenoreagent.

**Discussion**

NPI constitute an attractive source of xenogeneic insulin-producing tissue for clinical transplantation (Korbut et al. 1996, Yoon et al. 1999, Rayat et al. 2000, Trivedi et al. 2001); however, several aspects of the model need further investigation before this tissue can be considered for treating patients with type 1 diabetes. In particular, the susceptibility of porcine pancreatic endocrine cells to immunologic destruction remains an active field of research in xenotransplantation. We have previously shown that the xenoreactive αGal xenoreagent is expressed on NPI cells and its expression is not restricted to non-endocrine cells (Rayat et al. 1998). Furthermore, our previous study also demonstrated that the proportion of β cells staining positive for αGal residues was greater in freshly isolated (less mature) preparations than those allowed to mature further during a 9-day culture period (Rayat et al. 1998). We therefore hypothesized that less mature porcine islet β cells express αGal antigen and, once fully matured into islet endocrine cells, αGal expression disappears.

In the present study, we have demonstrated using both in vitro and in vivo models of islet maturation that αGal expression on NPI cells significantly decreases with maturation of the cells. We have found that αGal is highly expressed in less mature NPI cells and that its expression decreases once the cells differentiate into fully mature islet β cells. This reduction in αGal expression occurred when non-mature 9-day-cultured NPI were allowed to mature in vitro by microencapsulation followed by an additional 7 days culture with serum or when they were transplanted under the kidney capsule of non-diabetic C.B-17 SCID mice. In non-mature NPI, αGal was expressed on 20% of the islet cells then, following further in vitro maturation, the proportion of αGal-expressing cells decreased to 11% and further reduction (5%) was observed in fully developed adult porcine islets. Interestingly, the proportion of endocrine cells but not β cells was comparable between non-mature NPI and in vitro matured NPI but not with adult pig islets. We found that the proportion of glucagon-secreting cells as well as polypeptide (8.7 and 10.4% respectively, data not shown) or somatostatin-secreting cells (8.2 and 8.0% respectively, data not shown) were significantly higher in these two preparations compared with adult porcine islets (4.5% for polypeptide and 4.0% for somatostatin). It is possible that these cells might have undergone apoptosis during maturation to maintain the homeostasis in the pancreas as more β cells are formed. The observation that the proportion of vimentin- and CK7-positive ductal cells decreased while the proportion of β cells increased during the in vitro maturation process supports the concept that our in vitro system of NPI cell maturation is a valid model of porcine islet maturation. For example, during this culture period it is plausible that CK7-positive cells differentiate into hormone-expressing cells and thereby suggests a mechanism for the change in relative proportions of these cells.

To further characterize the phenotype of αGal-expressing cells present in non-mature NPI, we used FACS analysis to isolate αGal-expressing cells from those that do not. Our data showed that ~25% of the αGal-positive population co-expressed CK7 and only ~12% were insulin positive. On the other hand, the αGal-negative population consisted of <2% of the cells co-expressing CK7 and ~33% were insulin positive. It is possible that αGal is expressed in a subpopulation of less mature NPI cells that are potentially precursors of

**Figure 2** CK7 and insulin staining on NPI xenografts from C.B-17 SCID mice. On day 1 (A and B) both CK-7 (brown stain) and insulin-positive cells (red stain) are scattered within the graft. Some cells stained positive for both CK7 and insulin can be detected and some islets are devoid of staining. On day 30 (C and D) and day 50 (E and F) a high proportion of insulin-positive cells appeared to be budding off from aggregates of CK7-positive ductal cells. More than 200 days (G and H) post-transplantation these CK7-positive cells were no longer detected as β cells positive for insulin comprised the majority of the xenograft. Scale bars represent 100 μm for A, C, E and G and 50 μm for B, D, F and H.
hormone-secreting cells or transitional cytodifferented forms of insulin/glucagon cells that eventually lose αGal expression upon becoming fully differentiated into a mature form. It is generally believed that during fetal and neonatal development, islet neogenesis occurs from precursor cells that bud off from pancreatic ductules (Bouwens et al. 1994). Since the present study has demonstrated that 24% of the αGal-expressing cells present in the non-mature NPI expressed the ductal cell marker CK7, it is possible that these cells are islet endocrine cell precursors.

To examine further the association of αGal expression with maturation of porcine islet cells into β cells in vivo, non-mature NPI were transplanted under the kidney capsule of non-diabetic C.B17-SCID mice and then subsequently examined for the expression of αGal and insulin. Grafts in these mice matured with time after transplantation as assessed by the significant increase in relative proportion of β cells. In contrast, αGal-positive cells were most prominent on days 1 and 7 post-transplant and were no longer detected after 50 days. This pattern of αGal expression further suggests that αGal is highly expressed in immature developing porcine islet tissue and that its expression is dependent upon the maturity of the tissue. It has been shown that, during development, specific antigens are expressed at early developmental stages only to disappear as the cells mature (Kukowska-Latallo et al. 1990). Since our data have demonstrated that expression of αGal is age dependent, this possibly suggests that its expression may play a role in the development of porcine islets. Exactly what this role is remains to be determined. Furthermore, the function(s) of many oligosaccharides present on glycolipids and glycoproteins is not known because of the complicated pathways that have evolved to synthesize them. It has, however, been suggested (Kukowska-Latallo et al. 1990) that oligosaccharides expressed on cell-surface proteins provide a protective cellular coating that allows changes in cellular shape and motility. These cellular properties are expected to play a more significant role in less mature/developing islet precursor cells in the native porcine pancreas. For example, during islet β-cell neogenesis, newly formed β cells are believed to bud from ductules and initially appear as extra-islet endocrine cells until they completely develop into distinct intact islet structures (Wang et al. 1995, Bouwens & Pipeleers 1998, Strokan et al. 2000). Similarly, we have shown, in the neonatal porcine pancreas, that the islet endocrine cells are not arranged as islets but rather randomly scattered as single cells or small clusters of cells frequently arranged alongside or within the duct lining (Korbut et al. 1996). Taken together, αGal and possibly other oligosaccharides may play a role in the migration of endocrine precursor cells from the ductules that ultimately leads to islet formation at a later developmental stage.

In our initial publication describing the isolation of NPI cell aggregates (Korbut et al. 1996), we demonstrated that, following transplantation of non-mature NPI under the kidney capsule of diabetic nude mice, the graft β-cell mass increased 20- to 30-fold. More recently, Trivedi et al. (2001) demonstrated that this increase in β-cell mass is attributable to proliferation of existing β cells and differentiation of ductal cells into β cells. In the present study, we have shown that non-mature NPI contain a high proportion of precursor CK7-positive ductal cells and that, following implantation into C.B-17 SCID mice, the proportion of CK7-positive cells decreased while the majority of the islet cells stained positive for insulin. Thus, the present data are in agreement with other studies (Strokan et al. 2000, Trivedi et al. 2001), thereby providing additional support for the concept that pancreatic ductal cells exhibit the inherent ability to differentiate into insulin-producing β cells. Moreover, the presence of more CK7-positive cells in non-mature NPI compared with in vivo matured NPI and adult pig islets indicates the existence of islet endocrine precursors in non-mature NPI, thereby denoting that this tissue constitutes an attractive model to study islet cell neogenesis.

It is conceivable that less mature NPI cells that express αGal may be more susceptible to humoral-induced xenograft rejection than fully matured islet endocrine cells that have lower αGal expression. It has, however, been previously demonstrated (McKenzie et al. 1995) that expression of αGal in adult porcine islets can be increased under a variety of tissue culture conditions including varying proportions of CO₂ with oxygen. Therefore, if αGal expression in adult porcine islets can also be increased post-transplantation, possibly by immunological activation, then NPI and adult pig islets may be equally susceptible to antibody-mediated hyperacute rejection.

We conclude from this study that expression of αGal on NPI cells is highly associated with the age of the tissue and that by day 200 post-transplantation its expression is no longer detectable. Furthermore, by using in vitro and in vivo models of NPI maturation, the present study demonstrates that, as the islet tissue develops into mature β cells, the proportion of CK7-positive cells decreases while the relative number of insulin-positive cells increases. Taken together, these results suggest that αGal-expressing cells are less mature NPI cells.

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