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Xenotransplantation of pancreatic and kidney primordia – Where do we stand?

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Abstract

Lack of donor availability limits the number of human donor organs. The need for host immunosuppression complicates transplantation procedures. It is possible to 'grow' new pancreatic tissue or kidneys in situ via xenotransplantation of organ primordia from animal embryos (organogenesis of the endocrine pancreas or kidney). The developing organ attracts its blood supply from the host, enabling the transplantation of pancreas or kidney in 'cellular' form obviating humoral rejection. In the case of pancreas, selective development of endocrine tissue takes place post-transplantation. In the case of kidney, an anatomically-correct functional organ differentiates in situ. Glucose intolerance can be corrected in formerly diabetic rats and ameliorated in rhesus macaques on the basis of porcine insulin secreted in a glucose-dependent manner by beta cells originating from transplants. Primordia engraft and function after being stored in vitro prior to implantation. If obtained within a 'window' early during embryonic pancreas development, pig pancreatic primordia engraft in non immune suppressed diabetic rats or rhesus macaques. Engraftment of pig renal primordia transplanted directly into rats requires host immune suppression. However, embryonic rat kidneys into which human mesenchymal cells are incorporated into nephronic elements can be transplanted into non-immune suppressed rat hosts. Here we review recent findings germane to xenotransplantation of pancreatic or renal primordia as a novel organ replacement strategy.

Keywords

cell therapy; chronic kidney disease; diabetes mellitus; organogenesis; stem cell

Introduction

In 2004 [1-3] and again in 2005 [4] we reviewed for *Transplant Immunology* a body of literature originating from a number of laboratories around the world that established an experimental foundation for growing new organs in situ from transplanted embryonic organ primordia (organogenesis). The principles for organogenesis of endocrine pancreas or kidney were defined initially using rodent isotransplantation or allotransplantation models. While the use of human embryonic organs in human hosts has been contemplated [1,2-4,5] many laboratories have focused on the use of embryonic organs from the pig [4], a physiologically suitable donor for human pancreas or kidney replacement [6].

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Organogenesis of endocrine pancreas

It was established by 2005 [1-4] that rat or pig pancreatic primordia transplanted into mesentery undergo growth and differentiation and secrete insulin in a physiological manner into the portal venous system of hosts. Primordia transplanted in streptozotocin (STZ)-diabetic rats normalize host glucose tolerance. Exocrine tissue does not differentiate following transplantation of pancreatic primordia obtained early after organ formation from rat or pig embryos [on embryonic day 12.5 (E12.5) in rat or E28 in pig – just after the organ differentiates and prior to the time dorsal and ventral anlagen fuse]. Rather, islets differentiate in a connective tissue stroma following transplantation of rat pancreatic primordia into mesentery of rats or mice and individual alpha and beta cells engraft in mesentery post-transplantation of pig pancreatic primordia. If obtained sufficiently early (from E28 or E29, but not E35 embryos) pig pancreatic primordia engraft in non-immunosuppressed diabetic rats [4].

Subsequent to publication of our more recent *Transplant Immunology* review [4] Eventov-Friedman and co-workers implanted embryonic pig pancreatic tissues of different gestational ages beneath the kidney capsule of immunodeficient nonobese diabetic-severe combined immunodeficient (NOD-SCID) mice and immunocompetent diabetic mice that were immunosuppressed. Using NOD-SCID animals, they showed that pancreatic tissue obtained from E42 embryos exhibits reduced immunogenicity relative to that obtained from E56 embryos. Reduced immunogenicity was demonstrated by a lesser reduction in levels of circulating porcine insulin following immune reconstitution by infusion of human peripheral blood mononuclear cells (PBMC). In both models, it was possible to normalize level of glucose following pig pancreatic primordia transplantation [7]. As had been reported following transplantation of rat pancreatic primordia obtained relatively late during embryogenesis into kidneys of rats [4], limited differentiation of exocrine tissue was observed in mouse kidneys following implantation of E42 pig pancreas [6]. Subsequently, Brands et al transplanted human fetal pancreas into PBMC-reconstituted NOD-SCID mice and showed significantly decreased immunogenicity of tissue obtained during the first trimester of pregnancy relative to the second trimester. An analysis of immunoregulatory genes expressed in human fetal pancreas by microarray demonstrated the upregulation of several key immunoregulatory genes in second trimester tissue [5].

We demonstrated that glucose tolerance can be normalized following transplantation of E28 pig pancreatic primordia not only in adult streptozotocin (STZ)-diabetic rats, a model for type 1 diabetes in humans [8,9], but also in adult ZDF rats that manifest a type 2-like disease [10]. In addition, we showed that pig pancreatic primordia stored overnight prior to transplantation into STZ-diabetic rats, undergo engraftment and function that is comparable to primordia that are transplanted immediately following isolation [11]. Finally, we established that the requirement for exogenous insulin can be reduced in STZ-diabetic rhesus macaques following transplantation of pig pancreatic primordia [12]. As is the case for xenotransplantation from embryonic pig-into-adult rat [8-11], no host immunosuppression is required for engraftment of E28 pig pancreatic primordia following transplantation in the mesentery of diabetic rhesus macaques [12].

Our recent findings [8-12] and those of others [7] have advanced the concept that xenotransplantation of pig pancreatic primordia obtained early during development may be useful as a novel treatment for both types 1 and 2 diabetes. Here we will focus on data originating from our laboratory since 2005 [4] and provide a perspective on the future development of embryonic pancreas xenotransplantation.

Organogenesis of kidney

It had been delineated by 2005 that rat or pig renal primordia are 'preprogrammed' to differentiate into a kidney after transplantation into the mesentery of rodents with reduced functional renal mass (unilateral nephrectomy). For some congenic rat pairs (E15 PVG-RTI^c-into-adult PVG-RTI^{avl}), no host immunosuppression is required for allotransplantation across the rat major histocompatibility (RT1) locus. However, engraftment and survival of embryonic kidney xenografts (E15 rat-into-adult mouse; E28 pig-into-adult mouse; E 28 pig-into-adult rat) require that hosts be immunosuppressed. Newly 'grown' rat kidneys are capable of filtering blood, and urine is excreted following anastomosis between transplant and host rat ureters. Such transplants support life in otherwise anephric rat hosts. [3,4].

We had shown that E15 embryonic rat kidneys transplanted into adult mice attract a mouse vasculature [3,4]. Subsequently to 2005, we demonstrated that the predominant origin of endothelial cells post-transplantation of embryonic pig metanephroi into rats is host whereas mesangial cells originate predominantly from the donor [13]. Shown in Figure 1 are glomeruli from rat kidneys and pig kidneys and glomeruli within pig renal primordia transplanted into rats 8 weeks previously, stained with anti-rat endothelial antigen 1 (RECA-1) that is specific for rat endothelium, or anti CD31 that is specific for pig endothelium. The origin of the glomerular vasculature in transplants is rat (host). Non-glomerular renal vasculature is also of host origin [13].

Marshall et al [14] confirmed that prolongation of life in otherwise anephric rats can be achieved by transplanting a single renal primordium [15] and established that two transplanted primordia extend life longer than a single transplant. Campbell and co-workers showed that as is the case for reduction of the recipient's renal mass, pregnancy of host mice enhances the differentiation of transplanted embryonic kidneys [16].

While new observations delineated above [13,14,16] relating to transplantation of renal primordia are important, for purposes of this review we will concentrate on findings published since 2005 by Yokoo and co-workers [17,18] by Rosines et al [19] and by Kim et al [20,21]. Yolo and co-workers describe a novel method for renal organogenesis whereby human renal elements can be incorporated into rat kidneys and transplanted into non-immune suppressed rat hosts [17,18]. Rosines et al delineate a methodology for staged in vitro reconstitution and subsequent implantation of engineered rat kidney tissue using embryonic precursors [19]. Kim et al characterize the fate of isolated cells originating from rat fetal kidney after transplantation in the omentum or beneath the renal capsule of immunodeficient mouse [20], or rat [21] hosts.

Pancreatic Primordia for ZDF (Type 2) Diabetes

The ZDF rat is an inbred strain derived from a colony of Zucker fatty rats. ZDF and Zucker fatty animals have an autosomal recessive mutation in the gene (fa) that encodes the leptin receptor [22]. Homozygous Zucker fatty rats (fa/fa) manifest hyperphagia, obesity and severe insulin resistance, but remain normoglycemic. ZDF homozygous males (fa/fa) become hyperglycemic starting after 6 weeks of age and thereafter spontaneously develop overt diabetes [23]. Homozygous females become overtly diabetic beginning at age 6-8 weeks if maintained on a diabetogenic high fat diet [22,23]. In ZDF males and females, hyperglycemia occurs concomitant with markedly elevated levels of circulating insulin and failure of insulin secretion in response to a glucose challenge, mimicking the pathophysiology of human type 2 diabetes mellitus [23]. Diet restriction (15g/day standard rat chow) permits the use of ZDF rats as breeders, but does not reverse glucose intolerance or insulin resistance [23]. Once developed, diabetes in ZDF males & females is irreversible [22,23].

To define the utility for transplantation of pig pancreatic primordia in an animal model of human type 2 diabetes, we implanted E28 primordia into the mesentery of diabetic ZDF rats. In combination with a standard diet, transplantation of E28 pig pancreatic primordia normalizes glucose tolerance in diabetic ZDF males and females and ameliorates (ZDF diabetic females) or eliminates (ZDF diabetic males) insulin resistance in formerly diabetic rats [10]. Porcine insulin is detectable in plasma of formerly diabetic ZDF rats that received pig pancreatic primordia transplants. Levels peak at 15 minutes after an oral glucose load [10]. To localize porcine insulin-producing cells following implantation of pig pancreatic primordia into rats, in-situ hybridization was performed using a porcine proinsulin-specific antisense probe [10]. Cells expressing porcine proinsulin mRNA that stain with use of the antisense probe are present in liver, mesenteric lymph nodes and pancreas at 40 weeks post-transplantation [10].

Shown in Figure 2 are cells within germinal centers (arrows) and medullary sinuses (arrowheads) of mesenteric lymph nodes that stain with the antisense probe (A, & C red-brown stain), but not with the sense probe (B & D). No staining is observed in pancreas if an excess of unlabeled antisense probe is added to labeled antisense probe [10].

To confirm that transcripts identified in transplanted ZDF rat tissues by in-situ hybridization are for porcine proinsulin, RT-PCR was performed using primers designed to amplify a porcine proinsulin RNA sequence different from the one recognized by the anti-sense probe. A band is amplified from RNA originating from transplanted ZDF rat liver that corresponds to a transcript present in adult pig pancreas [10]. Sequencing confirms that transcripts are for porcine proinsulin [10]. A first phase insulin release characteristic of beta cells results within 1 minute of glucose addition to media in which mesenteric lymph nodes from transplanted ZDF rats are incubated in vitro [10].

Preservation of pig pancreatic primordia in vitro

It had been established by 2005 that rat renal primordia can be stored in vitro prior to successful allotransplantation [3]. To determine whether transplanted pig pancreatic primordia engraft, differentiate, and function in rat hosts after preservation in vitro, we implanted pig pancreatic primordia into STZ-diabetic rats either directly or after 24 hours of suspension in a University of Wisconsin-based growth-factor containing 'preservation' solution [11]. We demonstrated engraftment in mesentery and mesenteric lymph nodes and normalization of glucose levels in STZ-diabetic rat hosts following transplantation of preserved E28 pig pancreatic primordia comparable to glucose normalization after transplantation of non-preserved E28 pancreatic primordia [11].

Shown in Figure 3 is in-situ hybridization of tissue sections from mesenteric lymph nodes (A-F) from formerly STZ-diabetic Lewis rats into which E28 pig pancreatic primordia had been transplanted 1 year previously. Arrows delineate medullary sinus (A & B). Cells expressing porcine proinsulin mRNA that stain with use of an antisense probe (red-brown stain) are delineated by the arrow (A) and arrowheads (C & E). B, D & F are sections stained using a sense probe. A non-staining cell in F is shown (arrowhead) with morphology similar to a stained cell in E (arrowhead).

Pig to non-human primate xenotransplantation of pancreatic primordia

We transplanted E28 pig pancreatic primordia in the mesentery of STZ-diabetic rhesus macaques [12]. Long-term engraftment of pig beta cells within liver, pancreas and mesenteric lymph nodes post-transplantation of E28 pig pancreatic primordia into STZ-diabetic rhesus macaques was demonstrated by five complementary techniques: 1) electron microscopy; 2) positive immune-histochemistry for insulin; 3) positive RT-PCR and 4) in-situ hybridization for porcine proinsulin mRNA; and 5) porcine insulin detected in plasma using sequential

affinity chromatography, HPLC and mass spectrometry. Insulin requirements were reduced in one macaque followed over 22 months post-transplantation. Of potential importance for application of this transplantation technology to treatment of diabetes in humans and confirmatory of our previous findings in Lewis and ZDF rats, no host immunosuppression is required [12].

Shown in Figure 4 are sections of mesenteric lymph node from a diabetic rhesus macaque that had been transplanted 407 days previously with E28 pig pancreatic primordia. In situ hybridization was performed using pig proinsulin antisense (A, C) or sense (B, D) probes. Cells within medullary sinuses stain (red-brown stain) with the antisense, (A, C) but not the sense (B, D) probe.

Models for host tolerance of transplanted pig pancreatic primordia

Our ability to transplant pig pancreatic primordia obtained on E28 without immunosuppression into rats or rhesus macaques may reflect the reduced immunogenicity of pig pancreatic primordia obtained on E42 relative to E56 reported by Eventov-Friedman et al [7], and the reduced immunogenicity of human pancreatic primordia obtained during the first trimester of pregnancy relative to the second trimester, each demonstrated using humanized NOD-SCID mice models [5]. However, E35 pig pancreatic primordia do not engraft in non-immunosuppressed rats hosts [9] suggesting that an alternative explanation applies for transplants obtained within the E28-E29 time window. One such explanation is host tolerance on the basis of chimerism (Figures 2-4) as proposed by Abraham et al. to explain successful xenotransplantation of human pancreatic islet-derived progenitor cells in multiple tissues of non-immunosuppressed immunocompetent mice [24].

In contrast to islet formation that occurs within connective tissue stroma following transplantation of E12.5 rat pancreatic primordia into diabetic rats or mice [1] or within stroma containing some exocrine elements following transplantation of E42 pig pancreatic primordia [7] or first-trimester human pancreatic primordia [5] into mouse kidneys, individual endocrine cells engraft in tissues following transplantation of E28 pig pancreatic primordia into mesentery of diabetic rats [8-11] or STZ-diabetic rhesus macaques [12]. During normal pancreatic organogenesis, individual endocrine cells first migrate away from primitive ducts prior to coalescing into islets. Migration and coalescence are guided by cell adhesion molecules [25]. The failure of individual pig endocrine cells to coalesce into islets post-transplantation of E28 pig pancreatic primordia into rats or rhesus macaques likely results from the absence of adhesion molecules in the rat or macaque mesenteric interstitium that are recognized by pig endocrine cells. In contrast, rat endocrine cells recognize mouse adhesion molecules, so islets are formed following transplantation of E12.5 rat embryonic primordia into mesentery of rats or mice [1,4]. Similarly, it is possible that exocrine components differentiating following transplantation of E42 pig pancreatic primordia [7] or first-trimester human pancreatic primordia [5] beneath the renal capsule of mice provide sufficient cell adhesion substrate so as to permit the formation of islets within the nonendocrine tissue [5,7].

Neural cell adhesion molecule (NCAM) is an important regulator of endocrine cell aggregation during islet development [26]. Crnic et al. showed that loss of NCAM function causes the formation of lymph node metastasis in a transgenic model of pancreatic beta cell carcinogenesis [rat insulin promoter1- Tumor antigen 2 (Rip1Tag2)]. Metastatic spread was facilitated by up-regulated pancreatic lymphangiogenesis possibly induced by disaggregation of endocrine cells [27]. Failure of endocrine cells to aggregate following transplantation of E28 pig pancreatic primordia in the mesentery of rats or rhesus macaques may induce a comparable lymphangiogenesis, and trafficking of differentiating, but non-malignant pig endocrine cells beta cells to regional (mesenteric) lymph nodes. Tolerance in the setting of the chimerism that

results (Figures 2-4) may occur on the basis of ‘T-cell paralysis’ [28] from host exposure to antigen plus swine leukocyte antigen (SLA) II on pig beta cells [29-30] in the absence of second costimulatory signal [31], or there may be another explanation. Whatever the basis for tolerance may be, it is likely that establishment of chimerism (pig endocrine cells in mesenteric lymph nodes) [8-12] requires lymphangiogenesis that occurs following transplantation of primordia in mesentery under conditions that endocrine cells do not aggregate into islets. A similar intratumoral lymphangiogenesis induced by disaggregated pancreatic endocrine cells is proposed to facilitate their lymphatic invasion and targeting to mesenteric lymph nodes [32, 33].

The intestinal epithelium is exposed constantly to foreign material. Food antigens and commensal bacteria constitute the bulk of the antigenic load and the ‘default’ reaction of the immune system confronted with them leads to systemic unresponsiveness. This phenomenon is designated oral tolerance [34] and represents a key feature of intestinal immunity [35]. Antigen transport via afferent lymphatics into the draining mesenteric lymph nodes is obligatory for oral tolerance induction [35]. Oral tolerance cannot be induced in chemokine (C-C motif) receptor 7 (CCR-7)- deficient mice that display impaired migration of dendritic cells from the intestine to mesenteric lymph nodes, suggesting that immunologically relevant antigen is transported in a cell-bound fashion [35]. Transplantation of E28 pig pancreatic primordia in the mesentery could initiate a chain of events that results in a type of ‘oral tolerance’ for implants.

Novel methods for renal organogenesis

Yokoo et al injected human mesenchymal stem cells (hMSC) labeled with LacZ into E9.5 mouse embryos or E11.5 rat embryos at the site of early renal organogenesis, and subjected the whole embryos to culture. After 48 hours of whole culture, metanephroi were dissected from whole embryos and cultured in vitro for 6 days. It was found that hMSC-derived LacZ-labeled cells contribute to renal structures in organ-cultured metanephroi [17]. Subsequently, the investigators implanted LacZ- labeled hMSC that had been transfected with glial cell line-derived neurotrophic factor into the nephrogenic site of E11.5 rat embryos. Following 48 hours of whole embryo culture, metanephroi containing hMSC were dissected out and transplanted into the omentum of uninephrectomized rats. No immunosuppression was required for engraftment. Transplants enlarged over 2 weeks in non-immunosuppressed rats, became vascularized by host vessels and contained hMSC-derived LacZ-positive cells that were morphologically identical to resident renal cells. These findings suggest that self-organs from autologous MSC can be generated using inherent developmental and angiogenic systems [18].

Rosines et al described a stepwise, in vitro method of engineering rat kidney-like tissue capable of being implanted in adult rats. The investigators devised a modular approach that sequentially induces an epithelial tubule (the Wolffian duct) to undergo in vitro budding, followed by branching of a single isolated bud and its recombination with metanephric blastema. Transplanted recombined tissue develops glomeruli that are vascularized by the host and tubular structures that are functionally capable of organic ion transport [19]. The approach includes several points where tissue can be propagated. The data show how functional kidney tissue can assemble in three dimensions by means of independent modules interacting in vitro.

Kim et al isolated fetal kidney cells from rats by mincing embryonic kidneys, digesting them using collagenase/dispase and filtering the digested tissue through a nylon mesh. Polyglycolic acid scaffolds were used as three dimensional matrices to transplant cells into the omentum of immunodeficient mice. Cells were transplanted into the renal subcapsular region and cortex using fibrin gel as a matrix. Three weeks post-transplantation of cells originating from E17.5

embryos, histological analyses of grafts revealed the formation of renal structures including tubules and glomeruli in host kidneys and omentum. More glomeruli and tubules with more mature structures were observed in host kidneys than omentum when cells originating from E14.5 embryos were transplanted. However, the formation of nonrenal tissue such as bone and cartilage was also observed. In contrast cells from E20.5 embryos showed little differentiation into renal structures [20]. In another study, these investigators transplanted renal cells originating from E17.5 rat embryos beneath the capsule of remnant kidneys in non-immunosuppressed Sprague Dawley rats that underwent 1 5/6th nephrectomy. An increased number of glomeruli was observed 10 weeks later in rats that received embryonic cell transplants relative to the number observed following transplantation of renal cells originating from adult kidneys, and rats with embryonic transplants had relatively better renal function [21]. The studies of Kim et al represent the first in vivo structural reconstitution of renal tissue by transplanted isolated fetal kidney cells. The findings suggest such implants might be useful for partial augmentation of damaged kidney structures [20,21].

Summary and Conclusions

As reviewed previously for *Transplant Immunology* [1-4], transplantation of embryonic organ primordia to replace the function of diseased organs offers theoretical advantages relative to transplantation of either pluripotent ES cells, or of fully differentiated (adult) organs: 1) Unlike ES cells, organ primordia differentiate along defined organ-committed lines. There is no requirement to steer differentiation and no risk of teratoma formation. In the case of embryonic pancreas, the glucose sensing and insulin releasing functions of beta cells that differentiate from primordia are functionally linked. In the case of embryonic kidney, a three-dimensional, anatomically correct and functional organ differentiates in situ; 2) The growth potential of cells within embryonic organs is enhanced relative to those in terminally-differentiated organs; 3) The cellular immune response to transplanted primordia obtained early during embryogenesis is attenuated relative to that directed against adult organs; 4) Early organ primordia are avascular. The ability of cellular primordia to attract a host vasculature renders them less susceptible to humoral rejection than are adult organs with donor blood vessels transplanted across a discordant xenogeneic barrier; and 5) Organ primordia differentiate selectively. In the case of embryonic pancreas, exocrine pancreatic tissue does not differentiate following transplantation, obviating complications that can result from exocrine components such as the enzymatic autodigestion of host tissues.

The finding that it is possible to transplant pig pancreatic primordia to non-human primates without an immunosuppression requirement [12] is very important because it establishes the potential for transplantation in humans without the complications of immunosuppressive agents. Primordia engraft and secrete porcine insulin in the primate circulation. Exogenous insulin requirements are reduced. However, glucose tolerance has yet to be normalized in any transplanted primate. Until reproducible normalization is demonstrated, and the long-term safety of pancreatic primordia transplantation is established in non-human primates, it is premature to apply this technology in humans.

Renal function of developed primordia post-transplantation from embryonic-into-adult rat is low and prolongation of life in otherwise anephric hosts is too short to permit the gathering of meaningful data regarding filtration, tubular or endocrine function [3,4]. This is probably explained by the fact that rat primordia do not grow to the size of native kidneys after allo or isograft transplantation [14,15]. However, the kidneys that do develop are unstructurally normal [3,4] and nephron number is approximately 30% of that in native kidneys [36]. The phenotype of such kidneys is reminiscent of that observed early in rat models of oligomeganephronic congenital hypoplasia [37,38].

The finding that pig primordia transplanted in rats grow to a size larger than native rat kidneys in rat hosts [9] suggests that the hypoplasia may be more characteristic of transplanted rat renal primordia than of pig renal primordia. Hypoplasia can result from excessive cell death in metanephric blastema [36]. Rapidly-dividing blastema cells in renal primordia could be placed at risk during the time of relative hypoperfusion that occurs between dissection from donor embryos and revascularization in situ. It may be that pig renal primordia, cells in which divide more slowly over a longer gestation period, are at reduced risk for apoptosis relative to rat primordia during the time of relative hypoperfusion. Experiments currently in progress will determine whether transplantation of pig kidneys in rats or larger animals will prolong the recipient's life long-term. If so, embryonic pig kidneys may prove a suitable source for replacement of human renal function.

Alternatively, it may be that the utility for transplanted renal primordia will prove to be as a scaffold to support the development and differentiation into kidney tissue of transplanted human cells such as hMSCs [17,18], or that a more modular approach to renal organogenesis [19-21] will enable its use as a renal replacement therapy.

If and when one or another iteration of the technology for organogenesis of endocrine pancreas or kidney can be safely and effectively used in humans, it will provide in essence, an unlimited supply of donor organs. This will result in a paradigm shift in how the world thinks about organ replacement: 1) there will be no need to transport organs across long distances; 2) transplantation can be done electively at a convenient time; 3) transplantation can be offered to high-risk individuals and can be repeated as needed; and 4) transplantation can be offered to patients currently not candidates including type 2 diabetics [10].

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Figure 1.

Photomicrographs of stained sections of: A,B) rat kidney; or C,D) pig kidney and E,F) a pig renal primordium from an E28 embryo 8 weeks post-transplantation into rat mesentery, stained with rat-specific RECA-1 (A,B,E) or pig-specific CD31 (C,D,F). Glomerular capillaries in transplants stain positive for RECA-1 specific for rat endothelium (E) and negative for CD31 specific for pig endothelium (F). Magnifications are shown for A-D (in A) and E & F in E. Reproduced with permission [13].

Figure 2.

In situ hybridization was performed using pig proinsulin antisense or sense probes on tissue originating from diabetic ZDF rats into which pig pancreatic primordia had been transplanted 40 weeks previously: A., C). Mesenteric lymph node stained using antisense probe; B, D) Mesenteric lymph node stained using sense probe. Arrow delineates germinal centers in A & B; Arrowhead delineates medullary sinus in A & B. Arrowheads delineate cells that stain positive for porcine proinsulin mRNA in C. Magnifications are shown for A & B (B) and for C & D (D). Reproduced with permission [10].

Figure 3.

A-F) Sections of mesenteric lymph nodes from a formerly diabetic STZ-rat into which preserved E28 pig pancreatic primordia had been transplanted 1 year previously: In situ hybridization was performed using pig proinsulin antisense (A,C,E) or sense (B,D,F) probes. Arrow delineates medullary sinus in A and B. Arrowhead designates cells that stain positive for porcine proinsulin mRNA (C, E) and a negative-staining cell with similar morphology (F). Magnifications are shown in B for A & B; in D for C & D; and in F for E-F. Reproduced with permission [11].

Figure 4.

In situ hybridization was performed using pig proinsulin antisense (A, C) or sense probes (B, D) on sections of mesenteric lymph node originating from a STZ-diabetic rhesus macaque 407 days post-transplantation of E28 pig pancreatic primordia. Magnifications are shown for A & B (B) and C & D (D). Reproduced with permission [12].