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PROGRESS IN CLINICAL ENCAPSULATED ISLET XENOTRANSPLANTATION

David K.C. Cooper, MD, PhD, FRCS[1], **Shinichi Matsumoto, MD, PhD**[2], **Adrian Abalovich, PhD**[3], **Takeshi Itoh, PhD**[4], **Nizar I. Mourad, PhD**[5], **Pierre R Gianello, MD, PhD**[5], **Eckhard Wolf, DVM**[6], and **Emanuele Cozzi, MD, PhD**[7]

[1]Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA, USA

[2]Otsuka Pharmaceutical Factory, Tateiwa, Muya-cho, Naruto Tokushima, Japan

[3]Hospital Eva Peron de San Martin, Buenos Aires, Argentina

[4]Department of Regenerative Medicine and Transplantation, Faculty of Medicine, Fukuoka University, Fukuoka City, Fukuoka, Japan

[5]Laboratory of Surgery and Transplantation, Catholic University of Louvain, Brussels, Belgium

[6]Gene Center, LMU Munich and German Center for Diabetes Research (DZD), Munich, Germany

[7]Transplantation Immunology Unit, Padua University Hospital, and the Consortium for Research in Organ Transplantation (CORIT), Padua, Italy

Abstract

At the 2015 combined congress of the CTS, IPITA, and IXA, a symposium was held to discuss recent progress in pig islet xenotransplantation. The presentations focused on 5 major topics – (i) the results of 2 recent clinical trials of encapsulated pig islet transplantation, (ii) the inflammatory response to encapsulated pig islets, (iii) methods to improve the secretion of insulin by pig islets, (iv) genetic modifications to the islet-source pigs aimed to protect the islets from the primate immune and/or inflammatory responses, and (v) regulatory aspects of clinical pig islet xenotransplantation. Trials of microencapsulated porcine islet transplantation to treat unstable type 1 diabetic patients have been associated with encouraging preliminary results. Further advances to improve efficacy may include (i) transplantation into a site other than the peritoneal cavity, which might result in better access to blood, oxygen, and nutrients; (ii) the development of a more

Address for correspondence:- David K.C. Cooper MD, PhD, Thomas E. Starzl Transplantation Institute, Starzl Biomedical Science Tower, W1543, University of Pittsburgh, 200 Lothrop Street, Pittsburgh, PA 15261, USA, Te: 412-383-6961; Fax:412-624-1172, cooperdk@upmc.edu.

Authorship

David K. C. Cooper – coordinated and finalized the writing of the manuscript
Shinichi Matsumoto – carried out the clinical trial in New Zealand and supervised the trial in Argentina
Adrian Abalovich – carried out the clinical trial in Argentina with Dr. Matsumoto
Takeshi Itoh – carried out the studies on the inflammatory response
Nizar Mourad – carried out the studies on insulin secretion in pig islets with Dr. Gianello
Pierre R. Gianello – supervised the studies on insulin secretion in pig islets
Eckhard Wolf – contributed the section on genetic engineering of pigs for purposes of islet xenotransplantation
Emanuele Cozzi – contributed the section on regulatory aspects of clinical pig islet xenotransplantation

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biocompatible capsule and/or the minimization of a foreign body reaction; (iii) pig genetic modification to induce a greater secretion of insulin by the islets, and/or to reduce the immune response to islets released from damaged capsules; and (iv) reduction of the inflammatory response to the capsules/islets by improvements in the structure of the capsules and/or in genetic-engineering of the pigs and/or in some form of drug therapy.

Ethical and regulatory frameworks for islet xenotransplantation are already available in several countries, and there is now a wider international perception of the importance of developing an internationally-harmonized ethical and regulatory framework.

Introduction

Pancreatic islet allotransplantation is becoming increasingly successful, but is limited by the number of deceased organ donors who become available each year. Increasing attention is therefore being directed to other methods of treatment (reviewed in[1]). These approaches include the transplantation of pig islets.[2,3]

Free (nonencapulated) pig islets have maintained normoglycemia in streptozotocin (STZ)-induced diabetic, immunosuppressed nonhuman primates (NHPs) for periods >1 year[4–6] and encapsulated pig islets have maintained normoglycemia in nonimmunosuppressed NHPs for up to 6 months.[7] Encapsulated islets have the distinct theoretical advantage that exogenous immunosuppressive therapy may not be necessary, and therefore much effort is being made to explore this potential.

At the 2015 combined congress of the Cell Transplant Society (CTS), the International Pancreas and Islet Transplant Association (IPITA), and the International Xenotransplantation Association (IXA), a symposium was held at which recent progress in pig islet xenotransplantation was discussed. The presentations focused on 5 major topics – (i) the results of 2 recent clinical trials of encapsulated pig islet transplantation, (ii) the inflammatory response to encapsulated pig islets, (iii) methods to improve the secretion of insulin by pig islets, (iv) genetic modifications to the islet-source pigs aimed to protect the islets from the primate immune and/or inflammatory responses, and (v) regulatory aspects of clinical pig islet xenotransplantation.

Clinical trials of encapsulated pig islet transplantation

A nationally-regulated clinical trial of intra-peritoneal alginate-poly-L-ornithine-alginate (APA) encapsulated porcine islets in nonimmunosuppressed diabetic patients was recently undertaken in New Zealand, the first clinical trial of islet xenotransplantation under national regulatory oversight.[8] The study consisted of the transplantation of wild-type preweaned juvenile pig (aged 10–21 days)[9] islets by laparoscopy into the peritoneal cavity in 14 patients with unstable type 1 diabetes, without any immunosuppressive therapy.[8] The number of islets transplanted varied between 5,000IEq/kg and 20,000IEq/kg (5,000IEQ/kg n=4, 10,000IEQ/kg n=4, 15,000IEQ/kg n=4, 20,000IEQ/kg n=2). Outcome was monitored by the number of adverse events, the HbA1c, total daily insulin dose, and frequency of

unaware hypoglycemic episodes. The data were analyzed after follow-up of the patients for 52 weeks.

There were 4 adverse events, of which 3 may possibly have been related to the transplant procedure (discomfort, anxiety, and depressed mood). The number of 'unaware' hypoglycemic episodes experienced by the patients in all groups was reduced at 1 year after transplantation compared with pretransplantation, though the difference was not statistically significant, possibly because of the small number of patients studied. Since reductions in HbA1c and insulin doses were marginal, the efficacy of the transplants was assessed by calculating the transplant estimated function (TEF).[10] The TEF is calculated using the following formula:-

$$[\text{Daily insulin dose (IU)/kg body weight}+\text{HbA1c}/5.43(\text{pretransplant})]-[\text{Daily insulin dose (IU)/kg body weight}+\text{HbA1c}/5.43(\text{posttransplant})]$$

A TEF between 0.3 and 0.5 is considered to indicate partial graft function and a TEF >0.5 indicates full graft function. The overall average TEF was <0.3 in all groups, which suggested low graft function; however, 1 patient who received 5,000IEQ/kg showed a TEF of 0.58.

Tests for porcine endogenous retrovirus DNA and RNA were all negative. Parameters of the immune, eg, anti-Gal IgM and IgG antibody level, and inflammatory, eg, cytokine levels, responses have not yet been analyzed (as more patients have subsequently been added to the trial). These data will clearly be important to indicate the extent of protection of the islets provided by the capsules. It is possible that any breakage of capsules, thus exposing porcine islets, will result in an immune response.

Since the efficacy was not consistent and the transplantation of a high number of islets was not more effective than a low number, it was speculated that the transplantation of a large number of encapsulated islets might result in a relative deficiency in the supply of oxygen and nutrients to the islets. Therefore, it was decided that a smaller number of islets should be transplanted but, because of the lower numbers if islets per transplant, 2 transplants should be carried out in each patient.

A second clinical trial of encapsulated preweaned porcine islet xenotransplantation was therefore conducted in Argentina. This study was approved by the local ethical committee and the Buenos Aires region scientific committee of the Ministry of Health, and harmonized with Medsafe regulation of New Zealand. Patients received either 5,000IEQ/kg ×2 (n=4) or 10,000IEQ/kg ×2 (n=4), with the second transplant being carried out 3 months after the first. Sub-analysis of the efficacy data set demonstrated that when 10,000IEQ/kg were transplanted twice, the HbA1c was reduced to <7% and the average TEF was approximately 0.5. These improvements in the status of the patients have to date been maintained for more than 2 years. There has also been a significant ($p<0.05$) reduction in the number of unaware hypoglycemic episodes (Abalovich A and Matsumoto S, unpublished data)

Though encouraging, the results of these trials suggest that further improvements are required to improve the clinical outcome (discussed below).

Studies on the inflammatory response to microencapsulated pig islets

One of the major reasons for the lack of efficacy of microencapsulated pig islet transplantation is early nonfunction associated with an inflammatory reaction at the site of transplantation.[4,11–16] Previously, it has been demonstrated that high-mobility group box 1 (HMGB1), a damage-associated molecular pattern (DAMP) molecule,[17,18] is released from transplanted islets and triggers an inflammatory reaction leading to early graft loss of intrahepatic syngeneic islets in mice and autologous islets in humans.[19–23] Recent studies on early inflammation after the transplantation of microencapsulated porcine islets in mice are here summarized.

C57BL/6 mice were used as recipients, and APA-based microencapsulated porcine islets or APA-empty capsules were transplanted into the peritoneal cavity. The number of cells (including mononuclear cells) that accumulated around the capsules containing islets was significantly higher than that following the transplantation of empty capsules. Fluorescence-activated cell sorting (FACS) analysis revealed that tumor necrosis factor (TNF)-α-, interleukin (IL)-6-, interferon (IFN)-γ-, and/or IL-1β-positive macrophages, neutrophils, and dendritic cells infiltrated the peritoneal cavity after the placement of empty capsules or microencapsulated porcine islets. The cell characteristics were similar in both groups, but the number of accumulated cells was greater following microencapsulated islet transplantation.

Thus, to determine the strength of the inflammatory response, inflammatory cytokines in the peritoneal cavity were measured by ELISA. The concentrations of inflammatory cytokines, including TNF-α and IL-6, in the peritoneal lavage fluid 7 days after microencapsulated porcine islet transplantation were significantly higher than those after empty capsule transplantation. In addition, the concentration of HMGB1 in the fluid was also significantly higher after microencapsulated islet transplantation. These findings suggested that damaged microencapsulated islets themselves release HMGB1 and induce further inflammation.

In order to confirm this observation, in vitro studies were performed. These revealed that empty capsules did not release HMGB1 into the culture medium. In contrast, damaged microencapsulated islets (induced by hypoxia - 1% O_2, 5% CO_2, and 94% N_2), released HMGB1 that could be detected outside of the capsules.

Next, to confirm whether HMGB1 itself activates mononuclear cells in the peritoneal cavity, FACS analysis was carried out. TNF-α-, IFN-γ-, and/or IL-6-positive macrophages were observed following intraperitoneal injection of HMGB1 itself. To complete the study, the efficacy of anti-inflammatory therapies on microencapsulated islet transplantation was investigated. Anti-TNF-α antibody treatment was associated with prolonged graft survival and improved glucose tolerance 30 days after transplantation.

Taken collectively, a proposed schematic of the early inflammatory reaction following microencapsulated islet transplantation is described Figure 1.

Modifications to the pigs aimed towards increasing islet insulin secretion

Most studies have investigated the issue of the primate immune response to the graft, or its prevention, and the great majority of genetically-engineered pigs have been designed in an attempt to protect their islets from the host immune reaction [5,24,25; reviewed in1]. Two often-overlooked aspects of porcine islets are (i) their poor secretory activity and (ii) their incapacity to respond to stimuli in a fashion similar to that seen with healthy islets from NHPs or humans.

A limited number of studies of pig islet function have been carried out, but data reported by Casu et al indicated that the demands on pig islets in their natural host are significantly less than after their transplantation into a primate, particularly if the new host is a monkey rather than a human.[26] Nondiabetic cynomolgus monkeys show lower levels of fasting and stimulated blood glucose, but higher levels of C-peptide and insulin than nondiabetic pigs (Table 1). The reported levels in humans lie between those of monkeys and pigs.[27] Graham, Schuurman, and their colleagues have confirmed these observations.[28–30]

Given the discrepancies in glucose metabolism between pig and monkey, it is perhaps surprising that several groups have demonstrated that pig islets can maintain a state of normoglycemia in STZ-induced diabetic monkeys for >6 months or even for 1 or 2 years (reviewed in[1]). Fortunately, genetic modification of the pig, even if this involves transgenesis with an insulin promoter, does not appear to reduce pig islet function further.[31,32]

As the ultimate goal of islet xenotransplantation is to restore controlled insulin secretion in the recipient, it seems crucial to ensure that the transplanted islets secrete enough insulin in response to stimuli to enable adequate control of glycemia. In the case of preclinical porcine islet xenotransplantation, this is often ensured by transplanting large numbers of islets.[5,24,33,34] However, this may likely increase the oxygen and nutrient demands of the graft, and possibly lead to decreased insulin secretion and accelerated graft failure. This may be particularly important when the islets are encapsulated where there may be a limited supply of nutrients.

Previous work from several groups of in vitro perifusion assays has shown that porcine islets do exhibit a biphasic pattern of glucose-stimulated insulin secretion.[35–38] However, after stimulation with 15mM glucose, isolated porcine islets secrete 6 to 3 times less insulin than human islets during the first and second phases, respectively; for perifused human islets the stimulation index is 13 during the first phase and 8 during the second phase, compared to 2 during both phases for porcine islets (Figure 2). This difference in the amplitude of islet secretory response cannot be explained by lower insulin content of pig islets (Table 2).

Unfortunately, our knowledge of porcine beta cell physiology remains limited since most beta cell physiologists have used the more easily-obtainable rodent islets in their studies. There exist, however, some data showing that glucose transport, phosphorylation, and utilization in pig islets are similar to those seen in rat islets.[39] Much less is known about stimulus-secretion coupling (relating to the pathways of activation of insulin production by beta cells that result mainly in ‘amplification’ of insulin production) in pig islets and, although there is general agreement that glucose-stimulated insulin secretion is significantly

increased in the presence of cAMP-increasing agents, eg, theophylline, forskolin, or IBMX,[37,40,41] there have been no studies to explore this or other amplifying pathways to improve pig islet secretory function.

Indeed, insulin granule exocytosis triggered by glucose metabolism, and the ensuing rise in cytosolic calcium concentration, is further regulated by 2 major amplifying pathways[42,43] - (i) a cAMP-dependent pathway, activated physiologically by binding of glucagon-like peptide-1 (GLP-1) to its G-protein-coupled receptor on beta cells and mimicked by cAMP-increasing drugs, leading to activation of protein kinase A, and (ii) a cholinergic pathway, activated by binding of acetylcholine or cholecystokinin to a type 3 muscarinic receptor (M3R) and mimicked by carbachols or partially by direct protein kinase C ligands, eg, phorbol esters. Both of these pathways increase the number of readily-releasable insulin granules in beta cells[44] and result in a greater secretory response to glucose stimulation.

Figure 3 illustrates the effects of transgenic activation of protein kinase A and protein kinase C on pig islet insulin secretion during static incubation assays by means of adenoviral transduction to express glucagon-like peptide-1 and activated muscarinic receptor type 3 in islet beta cells. In control experiments, the stimulation index was 3.3 for neonatal islets and 3.0 for adult islets. Higher stimulation indices in neonatal islets can be explained by their lower basal secretion compared to adult islets. This is probably due to cell damage sustained by adult islets during isolation, whereas neonatal islets are subjected to a less aggressive isolation and purification process and have the opportunity to recover in culture for 8–10 days before being used in insulin secretion assays. Islets coexpressing glucagon-like peptide-1 and activated muscarinic receptor type 3 showed significantly improved insulin secretion, with a stimulation index of 7.3 and 5.5 in neonatal and adult islets, respectively.

Inducing these changes permanently in porcine beta cells by means of genetic engineering might be a novel and helpful approach to increase insulin secretion from isolated pig islets, bringing their secretory function closer to that of human islets and rendering them more efficient in controlling host glycemia in both preclinical and clinical trials without the need to transplant extremely high numbers of islets. In summary, this approach might enable transplanted pig islets to secrete higher amounts of insulin that might more readily maintain normoglycemia in primates.

Other potential genetic modifications of islet-source pigs

Remarkable progress has been made in restoring glucose control by transplanting free wild-type porcine islets into diabetic NHPs, though this has largely been achieved through blockade of the CD40/CD154 costimulation pathway with an anti-CD154mAb.[6] At present, anti-CD154mAbs are not clinically applicable because they have been demonstrated to be thrombogenic in both patients and NHPs.[45–47] If encapsulated pig islets are to be transplanted, it will be important to demonstrate that this can be successfully achieved in the absence of (or minimal) exogenous immunosuppressive therapy. This may possibly be achieved by the genetic engineering of the islet-source pigs.

There is evidence from organ and artery patch transplant studies that certain genetic manipulations of the source pig may allow less potent immunosuppressive therapy to be successful in preventing graft failure. For example, anti-CD40mAb alone or in combination with belatacept successfully prolongs genetically-engineered pig heart, kidney, or artery patch grafts for periods of several months to >2 years.[48–53] The absence of galactose-α1,3-galactose (αGal) expression and/or the expression of a human complement-regulatory protein reduces the T cell response to a pig graft as well as protecting against the humoral response.[54,55] There is every prospect, therefore, that judicious genetic engineering of the islet source pigs, eg, α1,3-galactosyltransferase gene-knockout (GTKO) pigs expressing 1 or more human complement- and coagulation-regulatory proteins, will result in a successful outcome without the need for anti-CD154mAb therapy [reviewed in[56]], and may even allow the transplantation of encapsulated islets without exogenous immunosuppressive therapy.

Intraportal xenotransplantation of free wild-type porcine islets incurs considerable loss of cells due to the instant blood-mediated inflammatory reaction (IBMIR), a result of activation of complement, platelets, and the coagulation cascade [reviewed in[4]]. Islets from GTKO pigs (lacking expression of αGal) and/or expressing 1 or more human complement-regulatory proteins (eg, hCD46, hCD55), have been tested in NHPs. GTKO only modestly improved the outcome of intraportally-transplanted adult pig islets,[25] presumably because αGal expression on wild-type islets steadily reduces as the pig matures.[57] Expression of hCD46, however, supported long-term survival, although it had no effect on islet loss in the early posttransplant period.[24] GTKO was shown to be advantageous for engraftment and survival of neonatal islet-like cell clusters (NICCs), which express high levels of αGal, in STZ-induced diabetic rhesus monkeys receiving immunosuppressive therapy.[25] In combination with transgenic expression of hCD55 and hCD59, GTKO NICCs attenuated IBMIR after intraportal transplantation into nondiabetic baboons.[58]

To date, transgenic strategies to specifically overcome T cell-mediated islet xenograft destruction, such as beta cell-specific expression of the costimulation blockade agent, LEA29Y,[59] still require validation in a NHP model. It is worth mentioning that transplantation of LEA29Y-expressing islet cells into marmoset monkeys was not associated with the transmission of porcine microorganisms.[60] Although the 3 subtypes of porcine endogenous retrovirus were present in all islet-source pigs, and cytomegalovirus in some, no transmission to the recipient monkey was documented. The screening program also included porcine lymphotropic herpes virus and hepatitis E virus, but neither was detected in the donor or recipient.

Other genetic modifications of source pigs with potential beneficial effects include the removal of the additional 2 known xenoantigens, (I) N-glycolylneuraminc acid, ie, knockout of the cytidine monophosphate-N-acetylneuraminic acid hydroxylase gene *CMAH*[61] and (ii) the Sd(a) antigen, ie, knockout of the β1,4N-acetylgalactosaminyltransferase gene (*β4GALNT2*)[62] (reviewed in[63]). Human antibody binding to pig cells not expressing these 3 antigens is greatly reduced.[64,65] Alternatively, or in addition, transgenic expression of inhibitors of coagulation and/or inflammation, eg, tissue factor pathway inhibitor, CD39, human thrombomodulin, and human endothelial protein C receptor,[5,32] and of cytoprotective molecules like heme oxygenase-1 and A20 [reviewed in[66]] are likely to be beneficial.

Regulatory frameworks pertinent to clinical islet xenotransplantation

The clinical trial of microencapsulated pig islet transplantation in New Zealand is to date the only 1 conducted under full national regulatory oversight.[8] The wide scale application of clinical islet xenotransplantation will require an ethically appropriate environment and the existence of regulatory frameworks in any country in which a trial is planned. What progress has been made in this respect?

The IXA has previously published 2 important documents that remain key references with regard to the fundamental ethical and regulatory requirements necessary to enable the initiation of xenotransplantation procedures in diabetic patients.[67,68] One of these has recently been updated.[3] As far as the regulatory aspects, the effort by the USA to closely and effectively regulate xenotransplantation is remarkable. Indeed, through the involvement of experts from the US Food and Drug Administration (FDA) and other executive agencies, the USA has developed a comprehensive regulatory paradigm that includes a set of laws and regulations, a centralized authority with regulatory oversight, and a set of guidance documents.[69–72] Such a regulatory system includes regulations on source animals, the specifications for xenotransplantation products, and the clinical issues that need to be addressed for the use of xenotransplantation products in humans specifically with regard to islet xenotransplantation.[73] The US regulations have served as a model for the development of regulatory frameworks in other parts of the world.

In Europe, several directives have been issued over the years by the European Parliament to regulate xenotransplantation within the European Union (EU). Such directives address the safety procedures to deal with genetically-modified organisms (GMOs), the standardization of good practice for clinical trials, and define procedures for gene therapy and somatic cell therapy using xenogeneic cells.[68,74,75] In addition, the EU regulation on Advanced Therapy Medicinal Products (ATMP)[76] establishes the specific rules concerning the authorization, supervision, and pharmaco-vigilance of ATMP (namely, products relating to gene therapy, somatic cell therapy, and tissue engineering), which are intended to be placed on the market in the EU. The European Medicines Agency has also issued a Guideline that addresses the scientific requirements for xenogeneic cell-based medicinal products for human use.[77]

Among the nonEU Member States, Switzerland has released a specific ordinance that is more stringent than existing guidelines elsewhere in the world.[78] In Japan, the Ministry of Health, Labour and Welfare, the Japan Society for Transplantation, and the Japanese Society for Xenotransplantation are currently working jointly to update the *'Public Health Guidelines on Infectious Disease Issues in Xenotransplantation'*. In addition, to enable marketing approval of xenogeneic products, the Act on the Safety of Regenerative Medicine and the Amended Pharmaceutical Affairs Act, namely the Pharmaceutical and Medical Devices Law, have been approved.[79,80]

Likewise, many other countries, including Canada, China, and Australia, appear to have developed, or are developing, the necessary set of national regulations. In South Korea, with the help of the Xenotransplantation Research Center (a multi-year program for preclinical and clinical xenotransplantation research supported by the Korean government since 2004),

in 2012 the Ministry of Health and Welfare prepared a draft Act on Xenotransplantation that closely resembles the US framework. A bill on promotion and regulation of advanced regenerative medicinal products has been submitted by a member of National Assembly to safely expedite clinical application of these products, eg, stem cells and tissue-engineered products. Xenogeneic cell products were included in the bill and, if it passes, clinical trials of porcine islet transplantation could be initiated.

It is highly relevant that the IXA has previously encouraged the successful initiation of clinical xenotransplantation trials in the context of a well-coordinated international effort under the expert guidance of the World Health Organization (WHO). Indeed, following the 2004 World Health Assembly Resolution WHA57.18, which urged member states to "allow xenotransplantation only when effective national regulatory control and surveillance mechanisms overseen by National Health Authorities are in place", the WHO has coorganized 2 global consultations to enable harmonization of xenotransplantation procedures at a global level.[81,82]

Regrettably, however, several cases of unregulated xenotransplantation have been reported since the 2009 IXA Consensus Statement. National Regulatory Authorities are strongly encouraged to identify and prohibit such unregulated practices.

Comment

In summary, trials of microencapsulated porcine islet transplantation to treat unstable type 1 diabetic patients have been carried out with encouraging preliminary results. Further advances will hopefully make this approach a treatment of choice for patients with this condition. What, however, can be done to improve the efficacy of encapsulated islet xenotransplantation further?

i. A different site (rather than the peritoneal cavity) might result in better access to blood, and thus to oxygen and nutrients. The implant site could possibly be improved by placing the encapsulated islets within the omentum or implanting them into a prevascularized subcutaneous site, either alone or in a device.

ii. A more biocompatible capsule and/or the minimization of a foreign body reaction are also research targets. Biocompatibility of the capsules might be improved by making them a larger size (1.5mm)[83–85] and/or by avoiding the use of poly-L-ornithine, which is the likely cause of any inflammatory response. A more rapid metabolic response, ie, normalization of glycemia, may be achieved by reducing the conformational size of the capsules, thus minimizing the distance between the islets and nearby blood vessels.[86] Any foreign body reaction could be suppressed by the administration of anti-inflammatory and/or anti-cytokine drugs.

iii. The inflammatory response to the capsules and islets might be reduced by improvements in the structure of the capsules and/or in genetic-

engineering of the pigs and/or in some form of drug therapy. A greater understanding of the response might provide therapeutic targets.

iv. Genetic modifications to the pigs may induce a greater secretion of insulin by the islets.

v. The immune response to islets that are released from damaged capsules may be reduced by use of islets from genetically-engineered pigs. Because of the high costs and ethical constraints of NHP studies, there is a need for a hierarchical screening pipeline of (a) in vitro assays, (b) improved 'humanized' mouse models,[87] and (c) large diabetic animal models.[88]

Ethical and regulatory frameworks for islet xenotransplantation are already available in several countries, and there is now a wider international perception of the importance of developing an internationally-harmonized ethical and regulatory framework.

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Abbreviations

APA	alginate-poly-L-ornithine-alginate
FDA	US Food and Drug Administration
HMGB1	high-mobility group box 1
IBMIR	instant blood-mediated inflammatory reaction
IXA	International Xenotransplantation Association
αGal	galactose-α1,3-galactose
GTKO	α-1,3-galactosyltransferase gene-knockout
NHP	nonhuman primate
STZ	streptozotocin
TEF	transplant estimated function

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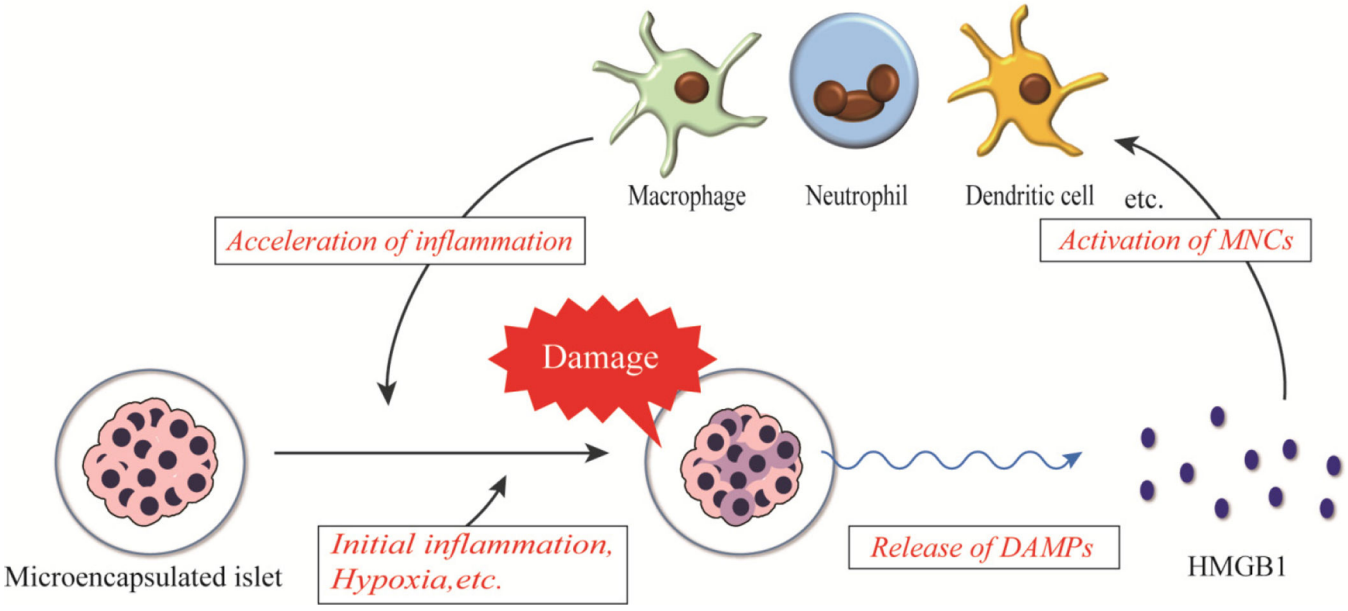

Figure 1. Proposed schema of the early inflammatory reaction following the transplantation of microencapsulated pig islets into the peritoneal cavity of the mouse

Transplanted microencapsulated islets are damaged by hypoxia and/or inadequate nutrients as well as by the inflammatory reaction occurring against the microcapsules themselves. Damaged islets then release HMGB1, which stimulates the accumulation of mononuclear cells and induces further inflammation, leading to graft loss. Blockade of these inflammatory pathways improves the outcome of microencapsulated pig islet transplantation in a mouse model.

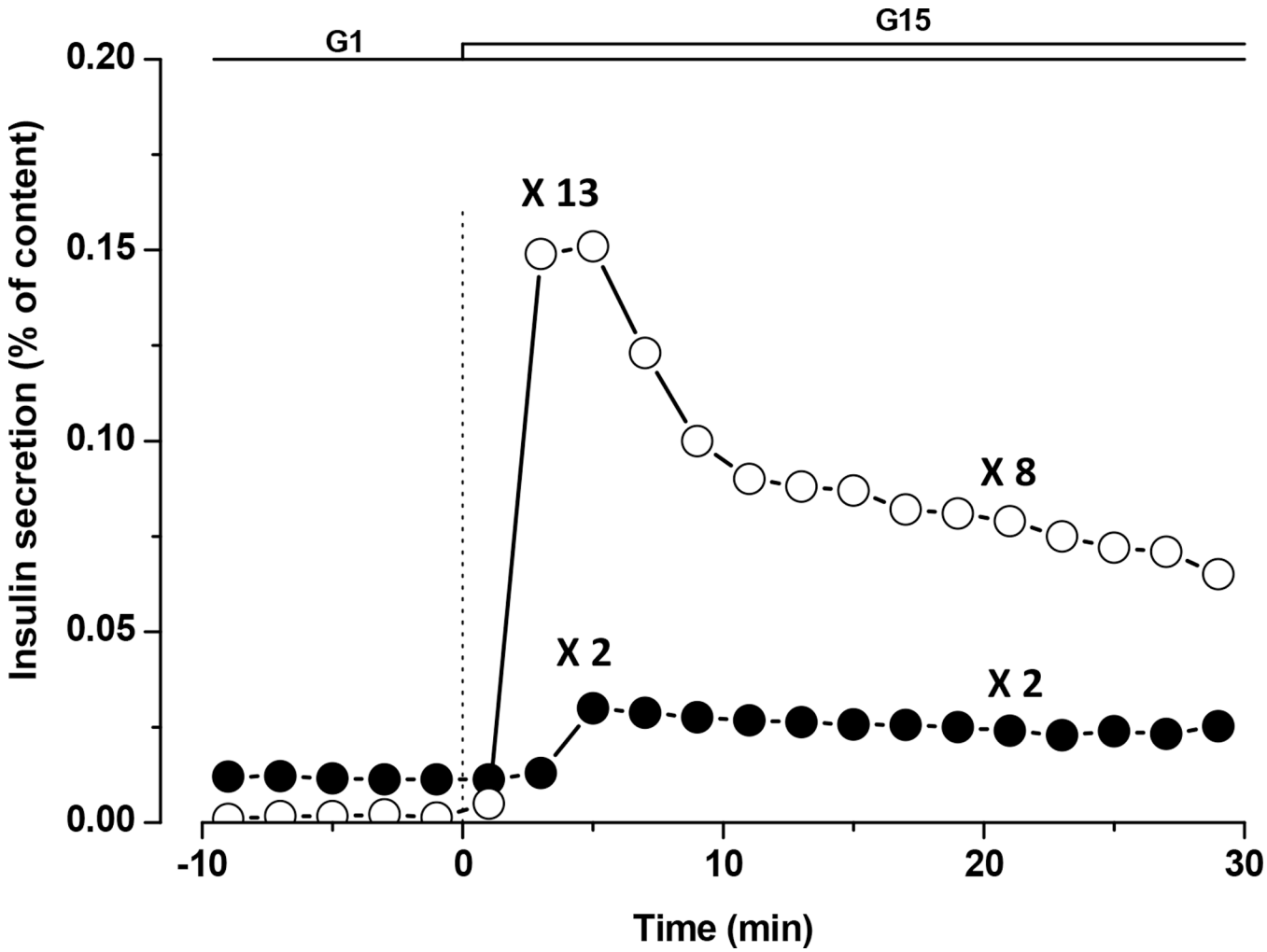

Figure 2. Comparison of glucose-induced insulin secretion from perifused pig (●) and human (○) islets

Batches of 200–600 islets were perifused in Krebs medium containing 1 mM glucose (G1) then 15 mM glucose (G15) as indicated at the top of the figure. Insulin secretion was then measured in the effluent fractions. Numbers along the curves indicate stimulation index during first [2–8 min] and second [10–30 min] phases. Values are means ± SEM for n=3–4 from 4 different preparations.

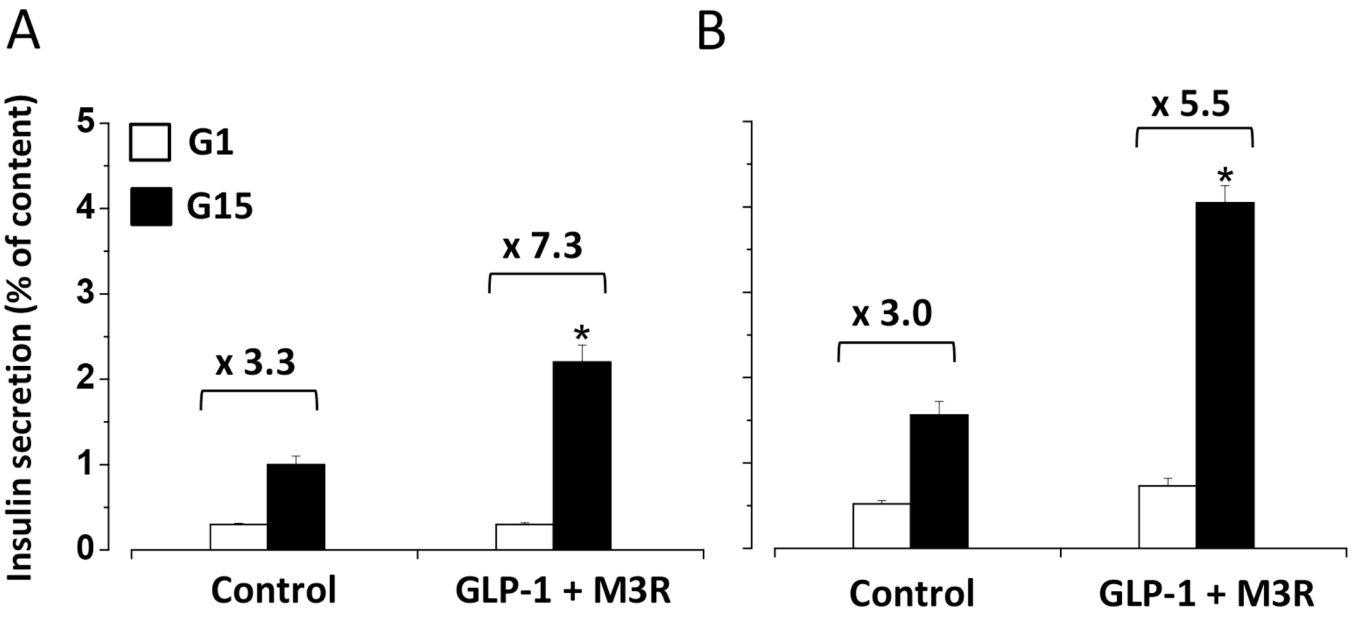

Figure 3. Insulin secretion from isolated pig islets

Neonatal **(A)** and adult **(B)** isolated islets were exposed to 200 MOI viral expression vector carrying sequences coding for glucagon-like peptide-1 and activated muscarinic receptor type 3 for 48 hours. Batches of 200 islets were incubated for 2 hours in 1 ml Krebs medium containing 1 mM glucose (G1) or 15 mM glucose (G15). Insulin secretion was measured in the incubation media and expressed as a percentage of total insulin content of each batch of islets. Numbers above pairs of columns indicate G15/G1 stimulation index (* $p<0.05$). Values are means ± SEM for n=38–46 from 10 different neonatal preparations and n=40–50 from 11 different adult preparations.

Table 1

Fasting blood glucose, C-peptide, insulin, and glucagon levels in monkeys (*Macaca fascicularis*), pigs, and humans *

	Cynomolgus monkeys	Pigs	Humans
Blood glucose (mmol·L^{-1})	2.2 – 4.1 (3.2±0.1)	4.0 – 5.2 (4.8±0.2)	3.9 – 5.6
C-peptide (nmol·L^{-1})	0.47 – 3.14 (1.39±0.09)	0.11 – 0.32 (0.16±0.04)	0.17 – 0.66
Insulin (pmol·L^{-1})	15 – 201 (109±11)	7 – 12 (9±1)	34 – 138
Glucagon (pmol·L^{-1})	18.7 – 179.4 (54.3±6.9)	11.3 – 13.8 (12.5±1.0)	5.7 – 28.7

* Reproduced with permission from[26].

Data are presented as minimum and maximum (mean±SE in parentheses). Human data are obtained from the literature and were measured in venous plasma[27,89]. Monkey C-peptide, insulin, and glucagon levels are significantly higher than the equivalent levels in the pig ($p<0.001$, $p=0.021$, and $p<0.001$, respectively).

Table 2

Comparison of insulin content in adult pig, neonatal pig and human islets *

	Adult pig	Neonatal pig	Human
Insulin content (μU/IEQ)	616 ± 113	175 ± 59	725 ± 362

* Islet insulin was extracted in acid-ethanol by ultrasound sonification. Values are means ± SD for n=4–6 from 15 different preparations.