

Xenotransplantation of Porcine Islet Cells as a Potential Option for the Treatment of Type 1 Diabetes in the Future

Authors

B. Reichart¹, H. Niemann², T. Chavakis^{3, 4, 5, 6}, J. Denner⁷, E. Jaeckel⁸, B. Ludwig^{3, 4, 9}, G. Marckmann¹⁰, A. Schnieke¹¹, R. Schwitzer¹², J. Seissler¹³, R. R. Tönjes¹⁴, N. Klymiuk¹⁵, E. Wolf¹⁵, S. R. Bornstein^{3, 4, 9, 16}

Affiliations

Affiliation addresses are listed at the end of the article

Key words

- diabetes mellitus type 1
- xenotransplantation
- islet cells

received 23.09.2014

accepted 20.10.2014

Bibliography

DOI <http://dx.doi.org/10.1055/s-0034-1395518>
Published online:
December 15, 2014
Horm Metab Res 2015;
47: 31–35
© Georg Thieme Verlag KG
Stuttgart · New York
ISSN 0018-5043

Correspondence

Prof. Dr. med. S. R. Bornstein
Medizinische Klinik und
Poliklinik III
Technische Universität Dresden
Universitätsklinikum Carl
Gustav Carus
Fetscherstrasse 74
01307 Dresden
Tel.: +49/351/458 5955
Fax: +49/351/458 6398
Stefan.Bornstein@uniklinikum-
dresden.de

Abstract

Solid organ and cell transplantation, including pancreatic islets constitute the treatment of choice for chronic terminal diseases. However, the clinical use of allogeneic transplantation is limited by the growing shortage of human organs. This has prompted us to initiate a unique multi-center and multi-team effort to promote translational research in xenotransplantation to bring xenotransplantation to the clinical setting. Supported by the German Research Foundation, an interdisciplinary group of surgeons, internal medicine doctors, diabetologists, material sciences experts, immunologists, cell biologists, virologists, veterinarians, and geneticists have established a collaborative research center (CRC) focusing on the biology of xenogeneic cell, tissue, and organ transplantation. A major strength of this consortium is the inclusion of members of the regulatory bodies, including the Paul-Ehrlich

Institute (PEI), infection specialists from the Robert Koch Institute and PEI, veterinarians from the German Primate Center, and representatives of influential ethical and religious institutions. A major goal of this consortium is to promote islet xenotransplantation, based on the extensive expertise and experience of the existing clinical islet transplantation program. Besides comprehensive approaches to understand and prevent inflammation-mediated islet xenotransplant dysfunction [immediate blood-mediated inflammatory reaction (IBMIR)], we also take advantage of the availability of and experience with islet macroencapsulation, with the goal to improve graft survival and function. This consortium harbors a unique group of scientists with complementary expertise under a cohesive program aiming at developing new therapeutic approaches for islet replacement and solid organ xenotransplantation.

Introduction

Type 1 diabetes and novel therapeutic strategies

The prevalence of type 1 diabetes in Western Europe and North America is about 0.5% of the population, with an increasing trend, and now affecting approximately 2 million subjects [1]. Type 1 diabetes pathogenesis involves a complex autoimmune reaction leading to complete destruction of the insulin-producing beta-cells of the pancreas. Consequently, the disease requires life-long substitution with insulin [2]. Despite insulin therapy, the serious and potentially life-threatening complications of the disease make it imperative to develop a curative therapeutic approach for the treatment of type 1 diabetes. In this context, preventive strategies aiming at the protection of islets, for example, by blocking inflammation, as well as approaches to induce

the regeneration of islet cell mass and function have been proposed [3]. Moreover, beta cell replacement therapies have been developed, including the transplantation of either the complete pancreas or isolated islets. Restoration of the islet cell mass by allogeneic islet transplantation represents a therapeutic option especially in type 1 diabetes patients with a very labile metabolic situation [4,5]. This technique has been successfully established in routine clinical practice at few specialized centers. Although allotransplantation of whole pancreas or islets appears to be promising for the treatment of type 1 diabetes, there are still major limitations, mainly resulting from shortage of donor supply and the relatively high number of islet cells required for a single patient, as well as complications associated with islet transplantation, such as infections [6,7].

To combat the disease, autologous stem-cell based therapies, for example, by reprogramming differentiated somatic cells into pluripotent cells [8] or by controlled differentiation of pluripotent cells [9,10] have been proposed. This would not only help overcome the limited resources of differentiated cells but also avoid the potential complications associated with the otherwise required immunosuppression for allogeneic transplants. However, such approaches may bear the risk of the tumorigenic potential associated with pluripotent stem cells [11,12]. Therefore, stem cell-based attempts may not compensate for the prevalent shortage of donor organs in the near future.

Xenotransplantation

As an alternative therapeutic approach, xenogeneic islet transplantation has been experimentally performed in preclinical diabetic animal models with promising results. It may therefore represent a promising approach to overcome donor organ shortage [13–17]. First clinical trials have evaluated the efficacy and microbiological safety [18–23]. In one trial, live encapsulated porcine islet cells were found 9.5 years after transplantation [24].

The unique advantage of xenotransplantation is the potentially unlimited availability of donor organs on demand. The ideal donor xenotransplant should have a similar size and physiology as well as a similar anatomic location compared to the human organ. These criteria are largely fulfilled by the pig (*Sus scrofa*) and in addition, this species has a relatively short generation time of about 4 months, and is highly fertile. Furthermore, genetic engineering is well established in the pig, thus providing an opportunity to genetically modify and optimize the donor xenotransplant for better matching with the human host. Considerable progress has been made to produce genetically modified pig organs for nonhuman primate xenotransplantation [25–28].

However, the social and ethical acceptance of animals as donors of xenotransplants has to be clarified [29,30]. Additionally, serious safety concerns have been raised by the possibility of transmitting infectious pathogens from the donor species to the human patient, although preventive actions like housing and breeding of donor animals under specific pathogen-free (SPF) conditions and screening of the donor prior to transplantation are expected to minimize infectious risks [31–33]. Wynyard et al. [23] reported the case of 14 patients from New Zealand with severe unaware hypoglycemia, who were treated with microencapsulated porcine islets. Up to 52 post-transplant weeks, no transmission of either PERV or other porcine microorganisms was detected by sensitive PCR and immunological methods. However, in addition to the risk posed by PERV [34], other viruses also need to be considered, which are difficult to eliminate even under SPF production of pigs, for example, hepatitis E virus and herpes viruses [31]. In order to implement the highest possible safety standards, the International Xenotransplantation Association (IXA) has established guidelines for xenotransplantation [35,36].

Strategies to accomplish immunological tolerance of pig xenotransplants

Rejection of porcine xenotransplants by the human immune system is mediated via a series of humoral and cellular mechanisms. The hyperacute rejection (HAR) results from pre-formed antibodies directed against the α 1,3-galactosyl-galactose (α Gal) epitopes on porcine endothelial cells. The generation of immune complexes induces a fulminant activation of the host comple-

ment system with rapid disruption of the graft endothelial system and irreversible organ damage within a few hours. Interestingly, xenotransplantation experiments with grafts from genetically engineered pigs lacking functional α 1,3-galactosyltransferase (*GGTA1*, also termed as α 1,3 GT) genes, thereby being devoid of the α Gal-epitope, have yielded promising results with regard to enhanced graft survival and function in baboons [37–39].

The porcine xenotransplants are challenged by the acute humoral xenotransplant rejection (AHXR; also known as acute vascular rejection or delayed xenotransplant rejection), which is primarily induced by the existence of pre-formed antibodies (presumably directed against the porcine endothelial Neu5Gc-epitope). Strategies to prevent the humoral xenotransplant rejection are based on the production of transgenic animals lacking the immunologically relevant epitopes or expressing human complement regulatory proteins (e.g., CD46, CD55, CD59) on endothelial cells [40] or other human immunological proteins involved in endothelial activation such as heme oxygenase 1 (HO-1) [41] or tumor necrosis factor induced human protein A20 [42]. Furthermore, expression of human antithrombotic or anticoagulant genes such as tissue factor pathway inhibitor (TFPI), endothelial protein C receptor (EPCR) or thrombomodulin (TM) may also enhance xenotransplant survival [43].

Since islet preparations usually contain only little or no endothelial cells and vascular structures, cellular graft reactions (CXR) play the dominant role in islet xenotransplant rejection. Blocking T-cell activation by antigen presenting cells (APC) via interfering with the co-stimulatory systems CD40-CD40L and/or CD80/86-CD28 could be utilized to reduce CXR [15,16,40,44,45]. After xenotransplantation under the kidney capsule, transgenic porcine islet cell clusters expressing the T cell costimulation blocking molecule LEA29Y normalized blood glucose levels of diabetic immuno-deficient mice and were, in contrast to wild-type porcine islets, protected against rejection by human immune cells [46]. T cell activation is regulated not only by co-stimulatory but also by co-inhibitory receptor-ligand interactions. Thus, enhancing inhibitory signals, for example, by transgenic expression of respective ligands on porcine cells and tissues, is an attractive new concept to diminish human anti-pig cellular immune responses [47]. The observation that immune responses to pig cells overexpressing the human inhibitory ligand PD-L1 (CD274) are particularly weak in vitro and in vivo supports the relevance of this approach [48,49]. Combining blockade of co-stimulatory signaling pathways (e.g., by CTLA-4. Ig/LEA29Y) with an enhancement of inhibitory signals by targeting the PD-1/PD-L1 pathway should be highly effective in controlling cell-mediated rejection of xenotransplants. Another feasible approach to inhibit T-cell mediated graft rejection appears to be the induction of regulatory T-cells (Tregs) [50], although the relevance of Tregs in xeno graft rejection requires further evaluation [51]. Also, natural killer (NK) cells, macrophages and neutrophils are critical components of the cellular response in xenotransplant rejection [52–54]. For example, expression of HLA-E, an inhibitor of NK cell activation has been found to protect porcine cells from destruction by primate NK cells [55]. Other promising targets to protect xenotransplants (porcine Islets) from host-directed lysis are associated with the so-called immediate blood-mediated inflammatory reaction (IBMIR) [56,57] and the resulting activation of complement factors and coagulation [57].

In conclusion, experimental xenograft transplantation from genetically modified pigs or pharmacologic modification of HAR, AHXR, CXR, and IBMIR appear to be promising strategies to improve graft survival.

Macroencapsulation for xenogeneic transplantation

The requirement of chronic immunosuppression to prevent graft rejection bearing several risks for the host is a major limitation for beta cell replacement. In order to overcome these obstacles, micro- and macro-encapsulation appear to be reasonable strategies to separate the graft from the host immune system and prevent rejection [58]. For example, Dufrane et al. [14] described the successful xenotransplantation of alginate-encapsulated porcine islets into a streptozotocin-induced diabetic animal model. In this setting, glycemic control could be accomplished over several months in animals transplanted with encapsulated islets without immunosuppression, while nonencapsulated islets were rejected within a week. Furthermore, Ludwig et al. [59,60] developed a subcutaneously implantable macrochamber system containing alginate-immobilized islets that are protected from the host immune system by a permeable Teflon membrane. In addition, this device contains a refillable oxygen chamber system to provide the islets with oxygen. This system has been optimized and subcutaneous implantation of the macro-chamber system containing immobilized rat islets in diabetic mini-pigs resulted in persistent glycemic control over 3 months without immunosuppression, demonstrating a sustained xenotransplant function [61,62]. In addition, this macro-chamber system has been successfully used for the transplantation of human islets without immunosuppression in a pilot-study conducted in a patient with type-1-diabetes [63]. These data indicate that macroencapsulation of islets may be a promising approach to prevent graft rejection in allo- and xenogeneic beta cell replacement therapies.

Summary

Many specific hurdles and obstacles related to the field of xenotransplantation have been identified and this knowledge has paved the way for the development of a variety of encouraging concepts utilizing porcine xenotransplants for beta cell replacement. Considering the enormous recent progress in our understanding of immunological mechanisms, the transfer of genetic engineering techniques into routine practice and the advances in material sciences, medical engineering and technology, xenotransplantation of pancreas or islets appears to be a promising approach for the treatment of type 1 diabetes.

Conflict of Interest

The authors declare that they have no conflicts of interest in the authorship or publication of this contribution.

Affiliations

- ¹ Institute for Surgical Research at the Walter-Brendel-Centre for Experimental Medicine, Ludwig-Maximilians-University, Munich, Germany
- ² Friedrich-Loeffler-Institute Mariensee, Federal Research Institute for Animal Health, Neustadt, Germany

- ³ Department of Medicine III, University Hospital Carl Gustav Carus, Dresden, Germany
- ⁴ Centre for Diabetes Research, Paul Langerhans Institute Dresden, Dresden, Germany
- ⁵ Department of Clinical Pathobiochemistry, Technische Universität Dresden, Dresden, Germany
- ⁶ Institute for Clinical Chemistry and Laboratory Medicine, Technische Universität Dresden, Dresden, Germany
- ⁷ Robert-Koch-Institute, Berlin, Germany
- ⁸ Medical School of Hannover, Department Gastroenterology, Hepatology, Endocrinology, Diabetology, Hannover, Germany
- ⁹ Center for Regenerative Therapies Dresden, Technische Universität Dresden, Dresden, Germany
- ¹⁰ Institute for Ethics, History and Theory of Medicine, Ludwig-Maximilians-University, Munich, Germany
- ¹¹ Chair of Livestock Biotechnology, Technical University of Munich, Freising, Germany
- ¹² Transplant Laboratory, Clinic for General-, Visceral-, and Transplantation Surgery, Hannover Medical School, Hannover, Germany
- ¹³ Medizinische Klinik und Poliklinik IV, Diabetes Zentrum, Ludwig-Maximilians-University, Munich, Germany
- ¹⁴ Paul-Ehrlich-Institute, Federal Institute for Vaccines and Biomedicines, Langen, Germany
- ¹⁵ Gene Center, Molecular Animal Breeding and Biotechnology, Ludwig-Maximilians-University, Munich, Germany
- ¹⁶ Department of Endocrinology and Diabetes, King's College, London, England

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