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

Authors

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Key words

- diabetes mellitus type 1
- xenotransplantation
- islet cells

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.

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Bibliography

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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.

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References

- 1 Ludwig B, Barthel A, Reichel A, Block NL, Ludwig S, Schally AV, Bornstein SR. Modulation of the pancreatic islet-stress axis as a novel potential therapeutic target in diabetes mellitus. *Vitam Horm* 2014; 95: 195–222
- 2 Achenbach P, Bonifacio E, Koczwara K, Ziegler AG. Natural history of type 1 diabetes. *Diabetes* 2005; 54 (Suppl 2): S25–S31
- 3 Meier JJ. Beta cell mass in diabetes: A realistic therapeutic target? *Diabetologia* 2008; 51: 703–713
- 4 Ludwig B, Reichel A, Kruppa A, Ludwig S, Steffen A, Weitz J, Bornstein SR. Islet transplantation at the Dresden Diabetes Center: five years' experience. *Horm Metab Res* 2015; 47: 4–8
- 5 Collaborative Islet Transplant Registry (CITR). www.citrregistry.org
- 6 Zimmerhackl LB, Junggraithmayr TC, Tibell A. Marginal donors in pediatric solid organ transplantation. *Pediatric transplantation* 2010; 14: 154–155
- 7 Morris MI, Fischer SA, Ison MG. Infections transmitted by transplantation. *Infect Dis Clin North Am* 2010; 24: 497–514
- 8 Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; 126: 663–676
- 9 Laflamme MA, Murry CE. Heart regeneration. *Nature* 2011; 473: 326–335
- 10 Weissman IL, Shizuru JA. The origins of the identification and isolation of hematopoietic stem cells, and their capability to induce donor-specific transplantation tolerance and treat autoimmune diseases. *Blood* 2008; 112: 3543–3553
- 11 Huang P, He Z, Ji S, Sun H, Xiang D, Liu C, Hu Y, Wang X, Hui L. Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. *Nature* 2011; 475: 386–389
- 12 Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernig M. Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* 2010; 463: 1035–1041
- 13 van der Windt DJ, Bottino R, Casu A, Campanile N, Smetanka C, He J, Murase N, Hara H, Ball S, Loveland BE, Ayares D, Lakkis FG, Cooper DKC, Trucco M. Long-term controlled normoglycemia in diabetic non-human primates after transplantation with hCD46 transgenic porcine islets. *Am J Transplant* 2009; 9: 2716–2726
- 14 Dufrane D, Goebbels RM, Gianello P. Alginate macroencapsulation of pig islets allows correction of streptozotocin-induced diabetes in primates up to 6 months without immunosuppression. *Transplantation* 2010; 90: 1054–1062
- 15 Cardona K, Korbitt GS, Milas Z, Lyon J, Cano J, Jiang W, Bello-Laborn H, Hacquoil B, Strobert E, Gangappa S, Weber CJ, Pearson TC, Rajotte RV, Larsen CP. Long-term survival of neonatal porcine islets in nonhuman primates by targeting costimulation pathways. *Nat Med* 2006; 12: 304–306

- 16 Hering BJ, Wijkstrom M, Graham ML, Hardstedt M, Aasheim TC, Jie T, Ansiet JD, Nakano M, Cheng J, Li W, Moran K, Christians U, Finnegan C, Mills CD, Sutherland DE, Bansal-Pakala P, Murtaugh MP, Kirchof N, Schurman H-J. Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates. *Nat Med* 2006; 12: 301–303
- 17 Elliott RB. Towards xenotransplantation of pig islets in the clinic. *Curr Opin Organ Transplant* 2011; 16: 195–200
- 18 Elliott RB, Escobar L, Garkavenko O, Croxson MC, Schroeder BA, McGregor M, Ferguson G, Beckman N, Ferguson S. No evidence of infection with porcine endogenous retrovirus in recipients of encapsulated porcine islet xenografts. *Cell Transplant* 2000; 9: 895–901
- 19 Garkavenko O, Croxson MC, Irgang M, Karlas A, Denner J, Elliott RB. Monitoring for presence of potentially xenotic viruses in recipients of pig islet xenotransplantation. *J Clin Microbiol* 2004; 42: 5353–5356
- 20 Valdés-González RA, Dorantes LM, Garibay GN, Bracho-Blanchet E, Mendez AJ, Dávila-Pérez R, Elliott RB, Terán L, White DJ. Xenotransplantation of porcine neonatal islets of Langerhans and Sertoli cells: a 4-year study. *Eur J Endocrinol* 2005; 153: 419–427
- 21 Tuch BE, Keogh GW, Williams LJ, Wu W, Foster JL, Vaithilingam V, Philips R. Safety and viability of microencapsulated human islets transplanted into diabetic humans. *Diabetes Care* 2009; 32: 1887–1889
- 22 Basta G, Montanucci P, Luca G, Boselli C, Noya G, Barbaro B, Qi M, Kinzer KP, Oberholzer J, Calafiore R. Long-term metabolic and immunological follow-up of nonimmunosuppressed patients with type 1 diabetes treated with microencapsulated islet allografts: four cases. *Diabetes Care* 2011; 34: 2406–2409
- 23 Wynyard S, Nathu D, Garkavenko O, Denner J, Elliott R. Microbiological safety of the first clinical pig islet xenotransplantation trial in New Zealand. *Xenotransplantation* 2014; 21: 309–323
- 24 Elliott RB, Escobar L, Tan PL, Muzina M, Zwain S, Buchanan C. Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. *Xenotransplantation* 2007; 14: 157–161
- 25 Ekser B, Ezzelarab M, Hara H, van der Windt DJ, Wijkstrom M, Bottino R, Trucco M, Cooper DK. Clinical xenotransplantation: the next medical revolution? *Lancet* 2012; 379: 672–683
- 26 Cooper DK, Satyananda V, Ekser B, van der Windt DJ, Hara H, Ezzelarab MB, Schuurman HJ. Progress in pig-to-non-human primate transplantation models (1998–2013): a comprehensive review of the literature. *Xenotransplantation* 2014; 21: 397–419
- 27 Nagaraju S, Bottino R, Wijkstrom M, Trucco M, Cooper DK. Islet xenotransplantation: what is the optimal age of the islet-source pig? *Xenotransplantation* 2014, Aug 11 doi:10.1111/xen.12130 [Epub ahead of print]
- 28 Mohiuddin MM, Singh AK, Corcoran PC, Hoyt RF, Thomas ML 3rd, Lewis BG, Eckhaus M, Reimann KA, Klymiuk N, Wolf E, Ayares D, Horvath KA. One-year heterotopic cardiac xenograft survival in a pig to baboon model. *Am J Transplant* 2014; 14: 488–489
- 29 Cozzi E, Tallacchini M, Flanagan EB, Pierson RN 3rd, Sykes M, Vanderpool HY. The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes – chapter 1: Key ethical requirements and progress toward the definition of an international regulatory framework. *Xenotransplantation* 2009; 16: 203–214
- 30 Katholische Akademie Bayern. www.kath-akademie-bayern.de Zur Debatte, Heft 63/2014
- 31 Mueller NJ, Takeuchi Y, Mattiuzzo G, Scobie L. Microbial safety in xenotransplantation. *Curr Opin Organ Transplant* 2011; 16: 201–206
- 32 Scobie L, Takeuchi Y. Porcine endogenous retrovirus and other viruses in xenotransplantation. *Curr Opin Organ Transplant* 2009; 14: 175–179
- 33 Semaan M, Rotem A, Barkai U, Bornstein S, Denner J. Screening pigs for xenotransplantation: prevalence and expression of porcine endogenous retroviruses in Göttingen minipigs. *Xenotransplantation* 2013; 20: 148–156
- 34 Denner J, Tönjes RR. Infection barriers to successful xenotransplantation focusing on porcine endogenous retroviruses. *Clin Microbiol Rev* 2012; 25: 318–343
- 35 Denner J, Schuurman HJ, Patience C. The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes – chapter 5: Strategies to prevent transmission of porcine endogenous retroviruses. *Xenotransplantation* 2009; 16: 239–248
- 36 Denner J, Tönjes R, Takeuchi Y, Fishman J, Linda Scobie L. The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes – Chapter 5: Recipient monitoring and response plan for preventing disease transmission. *Xenotransplantation* in preparation
- 37 Yamada K, Yazawa K, Shimizu A, Iwanaga T, Hisashi Y, Nuhn M, O'Malley P, Nobori S, Vagefi PA, Patience C, Fishman J, Cooper DK, Hawley RJ, Greenstein J, Schuurman HJ, Awwad M, Sykes M, Sachs DH. Marked prolongation of porcine renal xenograft survival in baboons through the use of alpha1,3-galactosyltransferase gene-knockout donors and the cotransplantation of vascularized thymic tissue. *Nat Med* 2005; 11: 32–34
- 38 Kuwaki K, Tseng YL, Dor FJ, Shimizu A, Houser SL, Sanderson TM, Lancos CJ, Prabhasarath DD, Cheng J, Moran K, Hisashi Y, Mueller N, Yamada K, Greenstein JL, Hawley RJ, Patience C, Awwad M, Fishman JA, Robson SC, Schuurman HJ, Sachs DH, Cooper DK. Heart transplantation in baboons using alpha1,3-galactosyltransferase gene-knockout pigs as donors: initial experience. *Nat Med* 2005; 11: 29–31
- 39 Ekser B, Kumar G, Veroux M, Cooper DK. Therapeutic issues in the treatment of vascularized xenotransplants using gal-knockout donors in nonhuman primates. *Curr Opin Organ Transplant* 2011; 16: 222–230
- 40 Mohiuddin MM, Singh AK, Corcoran PC, Hoyt RF, Thomas ML 3rd, Ayares D, Horvath KA. Genetically engineered pigs and target-specific immunomodulation provide significant graft survival and hope for clinical cardiac xenotransplantation. *J Thorac Cardiovasc Surg* 2014; 148: 1106–1113
- 41 Petersen B, Ramackers W, Lucas-Hahn A, Lemme E, Hassel P, Queisser AL, Herrmann D, Barg-Kues B, Carnwath JW, Klose J, Tiede A, Friedrich L, Baars W, Schwinzer R, Winkler M, Niemann H. Transgenic expression of human heme oxygenase-1 in pigs confers resistance against xenograft rejection during ex vivo perfusion of porcine kidneys. *Xenotransplantation* 2011; 18: 355–368
- 42 Oropeza M, Petersen B, Carnwath JW, Lucas-Hahn A, Lemme E, Hassel P, Herrmann D, Barg-Kues B, Holler S, Queisser AL, Schwinzer R, Hinkel R, Kupatt C, Niemann H. Transgenic expression of the human A20 gene in cloned pigs provides protection against apoptotic and inflammatory stimuli. *Xenotransplantation* 2009; 16: 522–534
- 43 Petersen B, Ramackers W, Tiede A, Lucas-Hahn A, Herrmann D, Barg-Kues B, Schuettler W, Friedrich L, Schwinzer R, Winkler M, Niemann H. Pigs transgenic for human thrombomodulin have elevated production of activated protein C. *Xenotransplantation* 2009; 16: 486–495
- 44 Ezzelarab M, Garcia B, Azimzadeh A, Sun H, Lin CC, Hara H, Kelishadi S, Zhang T, Lin YJ, Tai HC, Long C, Wagner R, Thacker J, Murase N, McCurry K, Barth RN, Ayares D, Pierson RN 3rd, Cooper DK. The innate immune response and activation of coagulation in alpha1,3-galactosyltransferase gene-knockout xenograft recipients. *Transplantation* 2009; 87: 805–812
- 45 Poirier N, Azimzadeh AM, Zhang T, Dilek N, Mary C, Nguyen B, Tillou X, Wu G, Reneaudin K, Hervouet J, Martinet B, Coulon F, Allain-Launay E, Karam G, Souillou JP, Pierson RN 3rd, Blanche G, Vanhove B. Inducing CTLA-4-dependent immune regulation by selective CD28 blockade promotes regulatory T cells in organ transplantation. *Sci Transl Med* 2010; 2: 17ra10
- 46 Klymiuk N, van Buerck L, Bähr A, Offers M, Kessler B, Wuensch A, Kurome M, Thormann M, Lochner K, Nagashima H, Herbach N, Wanke R, Seissler J, Wolf E. Xenografted islet cell clusters from INSLEA29Y transgenic pigs rescue diabetes and prevent immune rejection in humanized mice. *Diabetes* 2012; 61: 1527–1532
- 47 Del Rio ML, Seebach JD, Fernandez-Renedo C, Rodriguez-Barbosa JL. ITIM-dependent negative signaling pathways for the control of cell-mediated xenogeneic immune responses. *Xenotransplantation* 2013; 20: 397–406
- 48 Ding Q, Lu L, Zhou X, Zhou Y, Chou KY. Human PD-L1-overexpressing porcine vascular endothelial cells induce functionally suppressive human CD4+ CD25hiFoxp3+ Treg cells. *J Leukoc Biol* 2011; 90: 77–86
- 49 Plege-Fleck A, Lieke T, Römermann D, Düvel H, Hundrieser J, Buermann A, Kraus L, Klempnauer J, Schwinzer R. Pig to rat cell transplantation: reduced cellular and antibody responses to xenografts overexpressing PD-L1. *Xenotransplantation* 2014; Jul 9 doi:10.1111/xen.12121 [Epub ahead of print]
- 50 Waldmann H, Adams E, Fairchild P, Cobbald S. Regulation and privilege in transplantation tolerance. *J Clin Immunol* 2008; 28: 716–725
- 51 Muller YD, Golshayan D, Ehrichou D, Wekerle T, Seebach JD, Buhler LH. T regulatory cells in xenotransplantation. *Xenotransplantation* 2009; 16: 121–128
- 52 Inverardi L, Pardi R. Early events in cell-mediated recognition of vascularized xenografts: cooperative interactions between selected lymphocyte subsets and natural antibodies. *Immunol Rev* 1994; 141: 71–93
- 53 Schneider MK, Seebach JD. Current cellular innate immune hurdles in pig-to-primate xenotransplantation. *Curr Opin Organ Transplant* 2008; 13: 171–177

- 54 Li S, Waer M, Billiau AD. Xenotransplantation: role of natural immunity. *Transplant Immunol* 2009; 21: 70–74
- 55 Weiss EH, Lilienfeld BG, Muller S, Muller E, Herbach N, Kessler B, Wanke R, Schwinzer R, Seebach JD, Wolf E, Brem G. HLA-E/human beta2-microglobulin transgenic pigs: protection against xenogeneic human anti-pig natural killer cell cytotoxicity. *Transplantation* 2009; 87: 35–43
- 56 Marigliano M, Bertera S, Grupillo M, Trucco M, Bottino R. Pig-to-non-human primates pancreatic islet xenotransplantation: an overview. *Curr Diab Rep* 2011; 11: 402–412
- 57 Goto M, Tjernberg J, Dufrane D, Elgue G, Brandhorst D, Ekdahl KN, Brandhorst H, Wennberg L, Kurokawa Y, Satomi S, Lambris JD, Gianello P, Korsgren O, Nilsson B. Dissecting the instant blood-mediated inflammatory reaction in islet xenotransplantation. *Xenotransplantation* 2008; 15: 225–234
- 58 Vaithilingam V, Tuch BE. Islet transplantation and encapsulation: an update on recent developments. *Rev Diabetic Studies* 2011; 8: 51–67
- 59 Ludwig B, Zimerman B, Steffen A, Yavriants K, Azarov D, Reichel A, Vardi P, German T, Shabtay N, Rotem A, Evron Y, Neufeld T, Mimon S, Ludwig S, Brendel MD, Bornstein SR, Barkai U. A novel device for islet transplantation providing immune protection and oxygen supply. *Horm Metab Res* 2010; 42: 918–922
- 60 Ludwig B, Rotem A, Schmid J, Weir GC, Colton CK, Brendel MD, Neufeld T, Block NL, Yavriants K, Steffen A, Ludwig S, Chavakis T, Reichel A, Azarov D, Zimmermann B, Maimon S, Balyura M, Rozenshtein T, Shabtay N, Vardi P, Bloch K, de Vos P, Schally AV, Bornstein SR, Barkai U. Improvement of islet function in a bioartificial pancreas by enhanced oxygen supply and growth hormone releasing hormone agonist. *Proc Natl Acad Sci USA* 2012; 109: 5022–5027
- 61 Barkai U, Weir GC, Colton CK, Ludwig B, Bornstein SR, Brendel MD, Neufeld T, Bremer C, Leon A, Evron Y, Yavriants K, Azarov D, Zimmermann B, Maimon S, Shabtay N, Balyura M, Rozenshtein T, Vardi P, Bloch K, de Vos P, Rotem A. Enhanced oxygen supply improves islet viability in a new bioartificial pancreas. *Cell Transplant* 2013; 22: 1463–1476
- 62 Neufeld T, Ludwig B, Barkai U, Weir GC, Colton CK, Evron Y, Balyura M, Yavriants K, Zimmermann B, Azarov D, Maimon S, Shabtay N, Rozenshtein T, Lorber D, Steffen A, Willenz U, Bloch K, Vardi P, Taube R, de Vos P, Lewis EC, Bornstein SR, Rotem A. The efficacy of an immunoisolating membrane system for islet xenotransplantation in minipigs. *PLoS One* 2013; 8: e70150
- 63 Ludwig B, Reichel A, Steffen A, Zimerman B, Schally AV, Block NL, Colton CK, Ludwig S, Kersting S, Bonifacio E, Solimena M, Gendler Z, Rotem A, Barkai U, Bornstein SR. Transplantation of human islets without immunosuppression. *Proc Natl Acad Sci USA* 2013; 110: 19054–19058