Current Diabetes Reports (2021) 21: 3
https://doi.org/10.1007/s11892-020-01368-y

IMMUNOLOGY, TRANSPLANTATION, AND REGENERATIVE MEDICINE (L PIEMONTI AND V SORDI, SECTION EDITORS)

Novel Immunomodulatory Approaches for Porcine Islet Xenotransplantation

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Accepted: 19 November 2020 / Published online: 12 January 2021

Abstract

Purpose of Review Porcine islet xenotransplantation is a promising alternative to overcome the shortage of organ donors. For the successful application of islet xenotransplantation, robust immune/inflammatory responses against porcine islets should be thoroughly controlled. Over the last few decades, there have been numerous attempts to surmount xenogeneic immune barriers. In this review, we summarize the current progress in immunomodulatory therapy for the clinical application of porcine islet xenotransplantation.

Recent Findings Long-term graft survival of porcine islets was achieved by using anti-CD154 Ab-based regimens in a preclinical non-human primate (NHP) model. However, owing to a serious complication of thromboembolism in clinical trials, the development of an anti-CD154 Ab-sparing immunosuppressant procedure is required. The efficacy of new immunosuppressive practices that employ anti-CD40 Abs or other immunosuppressive reagents has been tested in a NHP model to realize their utility in porcine islet xenotransplantation.

Summary The recent progress in the development of immunomodulatory approaches, including the immunosuppressive regimen, which enables long-term graft survival in a pig-to-non-human primate islet xenotransplantation model, with their potential clinical applicability was reviewed.

Keywords Islet xenotransplantation · Porcine islet · Immunosuppressant · Immune rejection

Introduction

Type 1 diabetes (T1D) is a chronic autoimmune disease that involves the destruction of insulin-producing beta cells in the pancreas [1]. In 2000, the Edmonton group first demonstrated that seven T1D patients who underwent allogeneic islet transplantation became insulin-independent [2]. In clinical trials conducted thereafter, 25–50% of T1D patients who underwent islet transplantation stayed insulin-independent when followed up after 5 years and showed a significant

This article is part of the Topical Collection on *Immunology, Transplantation, and Regenerative Medicine*

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reduction in diabetes complications [3, 4]. Therefore, islet transplantation is regarded as an effective and a promising therapeutic option for T1D patients who suffer from frequent and severe hypoglycemia. However, a considerable gap exists between the number of patients who could benefit from such treatments and the number of available donors. Owing to the increase in T1D incidence, the number of patients who require islet transplantation is growing, but the rate of organ donation has remained constant [5]. Porcine islets have physiological and structural similarities to human islets, and porcine insulin differs from human insulin by only one amino acid residue [6]. Therefore, islet xenotransplantation from pig to human has been considered to be a good solution to overcome donor shortage by providing an unlimited source of islets.

However, immunological barriers to xenografts are greater than those to allografts [7]. For successful porcine islet xenotransplantation, species-based immunological barriers, such as instant blood-mediated inflammatory reactions (IBMIRs) and hyperacute rejection along with humoral and cellular immune responses, should be carefully controlled [8]. Over the last 20 years, efforts to find an effective immunomodulatory regimen for xenotransplantation have been underway, and considerable advances have been made in preclinical NHP studies.

Immune Responses Against Xenogeneic Porcine Islets

Transplantation across discordant species provokes a vigorous immune response to the host (Fig. 1). IBMIR is characterized by coagulation, activation of complements, secretion of chemokines that recruit immune cells, and release of pro-inflammatory cytokines that eventually leads to islet destruction [9]. Because islets are infused into the portal vein, transferred islets come into direct contact with blood, thereby inducing IBMIR [10]. Bennet et al. demonstrated that direct contact of adult pig islets with human blood in vitro or with cynomolgus monkey blood in vivo initiates IBMIR. As such, this progresses to a significant loss of islets owing to the activation of the complement, platelet, and intrinsic and extrinsic coagulation cascades followed by recruitment of monocytes and neutrophils [11]. Additionally, infused porcine islets induce the activation of innate immune cells by producing damage-associated molecular pattern (DAMP) molecules or inflammatory mediators [12]. In xenotransplantation, species-dependent differences in glycosylation patterns and in receptor–ligand interaction can induce the activation of a recipient's innate cells [13].

As with human allogeneic transplantation, the major immunological hurdle of xenotransplantation is adaptive immune responses, including T and B cells (Fig. 1). Although the exact role of B cells and antibody-secreting cells in graft rejection during xenotransplantation remains unclear, some studies conducted on NHPs reveal that the levels of anti-Gal IgG, anti-non-Gal, or anti-donor-specific antibodies were increased when xenografts were rejected [14, 15]. T cells are considered as primary effector cells of graft destruction. In a pig-to-NHP islet transplantation model, massive infiltration of $CD4^+$ and $CD8^+$ T cells was observed at rejected graft sites [16]. Our group conducted a flow cytometric analysis of rejected porcine islet grafts (liver), revealing that 60–80% of

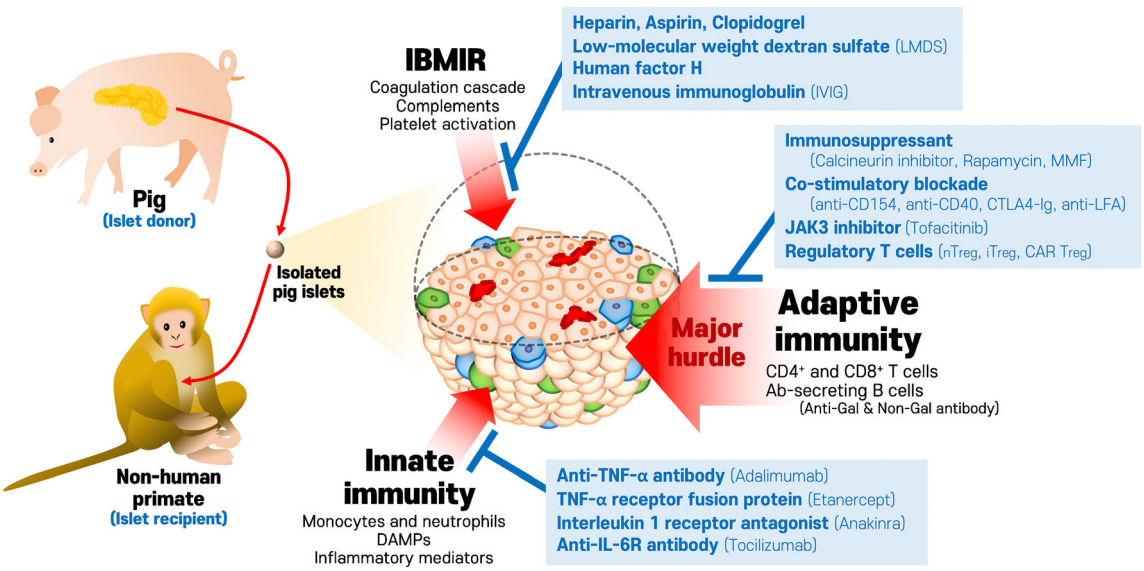

Fig. 1 Immunological hurdles and immunomodulatory approaches for porcine islet xenotransplantation. This diagram illustrates the immunological hurdles that occur to a graft throughout the transplantation process. After transplantation, the islet suffers instant blood-mediated inflammatory reaction (IBMIR), innate immune response, and adaptive immune response (red arrows). Subsequently, several therapeutic approaches have been proposed to protect transplanted islets from the hurdles (light blue boxes)

the infiltrating cells are T cells, and that most of them are memory type T cells (unpublished data). Peripheral blood RNA-seq analysis also suggested that T cell-signaling pathway was actually activated in rejecting monkeys [17]. Therefore, the suppression of T cell-mediated xeno-immune responses is key for successful xenotransplantation.

Immunomodulatory Drugs to Control Immune Responses Against Porcine Islets

To prevent early graft loss caused by IBMIR, a combination of anti-coagulant, anti-platelet, and anti-complement agents has been used (e.g., heparin, aspirin, clopidogrel, low molecular weight dextran sulfate, human factor H, intravenous immunoglobulin). Although investigators have focused their efforts toward IBMIR reduction, more than half of the islets transplanted via the portal vein are immediately lost after the transplantation. Fortunately, it has been proven that if a sufficient number of islet cells survive, normoglycemia can be achieved in the NHP model [8]. This result suggests that IBMIR can be partly compensated by a substantial number of pig islets.

Also, blockades of pro-inflammatory cytokines such as TNF-α neutralizing Ab (adalimumab), interleukin-1 receptor antagonist (anakinra), IL-6 antagonist (tocilizumab) have been used in pig to NHP xenotransplantation since increased pro-inflammatory cytokines produced by innate immune cells significantly contribute to the graft rejection [18, 19].

To control the B cell immune response, B cell depleting anti-CD20 Abs (namely, rituximab) have been used in kidney, heart, and embryonic pancreatic cell transplantations and have shown beneficial effects in an NHP model [20, 21]. Because anti-CD20 Abs cannot target bone marrow-resident plasma cells that do not express CD20, it has been recommended that agents that inhibit B cell activation factors or survival factors should be used (e.g., belimumab, tabalumab) for further controlling the humoral immune response [22]. In our experiences from the implementation of CD154–CD40 axis blockade strategies, we did not observe anti-donor antibody response before graft rejection, suggesting that CD154–CD40 axis blockage also regulates the B cell immune response.

Suppression of effector T cell response is the most critical factor for long-term graft survival. T cell depleting polyclonal antibodies, such as anti-thymocyte globulins (ATGs), have been used since the mid-1970s, after which began the era of biological immunosuppressant development [23]. Subsequently, more refined monoclonal antibodies against CD3 (muromonab, OKT3) were developed to selectively deplete T cells [24]. In the 1980–1990s, a significant advancement in the transplantation field was achieved by the introduction of calcineurin inhibitors (CNI; cyclosporine and tacrolimus) [24]. The CNIs inhibit calcineurin from the dephosphorylating nuclear factor of activated T cells (NFAT) and prevent translocation of NFAT into the nucleus, thereby markedly reducing acute graft rejection [25]. More recently, immunomodulatory drug developments have focused on the identification of targets that control T cell activation and proliferation, such as co-stimulation blockade (e.g., anti-CD154, anti-CD40, anti-CD28). Blockage of co-stimulatory receptors and their ligands is an attractive strategy to prevent acute graft rejection as well as to maintain immunosuppression because signaling mediated by accessory molecules or co-stimulatory molecules is indispensable for fine-tuning T cell activation [26].

So far, immunomodulatory regimens used in pig-to-NHP xenotransplantation consist of co-stimulation blockades, cell activation blockades, cell-depleting Abs, and anti-proliferation agents (anti-CD154, belatacept, abatacept, anti-thymocyte globulin, tacrolimus, MMF, and sirolimus, etc.) (Fig. 1) [27, 28]. Although the remarkable advancements made in the field of immunomodulatory drugs for controlling T cell immune response have been encouraging, immunomodulatory regimen for islet xenotransplantation in clinical settings still needs to be improved.

Efficacy of Anti-CD154 Ab in Porcine Islet Xenotransplantation

CD154, also called CD40L, is a member of tumor necrosis factor (TNF) superfamily and is predominantly expressed on activated T cells. The interaction between CD40, mainly expressed on antigen-presenting cells (APCs), and CD40L controls the overall cellular process of immune cells such as activation, proliferation, and differentiation [29]. Numerous NHP studies have demonstrated that blocking of CD154 effectively prevented graft rejection. Based on the results from murine models, anti-CD154 Abs were found to reduce the proliferation and differentiation of allo-specific T cells by promoting the generation of tolerogenic plasmacytoid DC [30, 31]. Moreover, anti-CD154 Abs induce the conversion of $CD4^+$ T cells into $Foxp3^+$ regulatory T cells [32].

In 2006, two path-breaking groups published studies on long-term porcine islet graft survival in a diabetic NHP model. Hering et al. demonstrated WT porcine adult islet survival for > 187 days after treatment with anti-IL-2R, anti-CD154, FTY720, and rapamycin [16]. The Cardona et al. group showed WT neonatal porcine survival more than 260 days by treatment with anti-IL-2R, anti-CD154, CTLA-4 Ig, and rapamycin [33]. Following this achievement, other groups published successes of long-term porcine islet graft survival in NHPs. By using islets from transgenic pigs expressing human CD46, van der Wint et al. accomplished long-term graft survival in NHPs treated with ATG, anti-CD154, and MMF [34]. Moreover, Thompson et al. observed Gal-deficient islet

graft survival for 249 days when the recipient NHPs were treated with anti-LFA, anti-CD154, CTLA-4 Ig, and MMF [35]. Bottino et al. presented data on prolonged survival of transgenic pig islet graft for 365 days after treatment with ATG, anti-CD154, and MMF [36].

Finally, in 2015, Shin et al. demonstrated the consistent long-term WT porcine graft survival for over 180 days in 4 out of 5 Rhesus monkeys and the longest survival reaching 965 days by treating with an immunosuppressant regimen composed of ATG, CVF, anti-TNF, anti-CD154, rapamycin, and regulatory T cells [37, 38]. Until now, all groups who achieved long-term graft survival used anti-CD154 antibody (Table 1). Hence, it seems undeniable that targeting CD154 is efficacious in attaining long-term graft survival by overcoming xenogeneic immunological hurdles. However, these preclinical studies were not implemented in clinic settings because clinical trials of anti-CD154 Ab treatment were unfortunately terminated owing to serious prothrombotic events. Thus, investigators need to pay attention to another aspect: CD40 blockade.

Immunosuppressive Regimens Based on Anti-CD40 Abs

Thompson et al. first published that combined anti-IL-2R, anti-CD40 Abs (Chi220), CTLA-4 Ig, and rapamycin treatment prolonged porcine islet graft survival in NHPs to a maximum of 203 days (with a mean of 90.8 days) (Table 1) [39]. However, Chi220 was not suitable as a maintenance drug in transplantation because of significant peripheral B cell depletion in Chi220 treatment recipients.

Thus, our group tested the efficacy of another clone of non-depleting anti-CD40 Abs (2C10R4) with or without the addition of belatacept (CTLA-4 Ig) or tacrolimus because 2C10R4 could prolong the survival of porcine kidney and heart xenotransplantation in NHPs [20, 41]. Unfortunately, in porcine islet xenotransplantation setting, the maximum graft survival period in the NHP group treated with anti-CD40 Abs alone was 41 days (Table 1) [40••]. Anti-CD40 Abs (2C10R4) did not prolong islet survival further when combined with belatacept. Although 2C10R4 in combination with tacrolimus prolonged islet survival, it was not as effective as anti-CD154 Abs (only one of the five monkeys treated with tacrolimus (CNI) plus anti-CD40 Abs maintained normoglycemia for more than 6 months). As it has been shown that higher dose of anti-CD40 Abs (2C10R4) is required for the successful prolongation of porcine heart in NHP [41], dose escalation study would be required to confirm the efficacy of 2C10R4-based regimen.

Currently, it is reported that new fully humanized anti-CD40 Abs (ASKP1240) are undergoing phase II clinical trials and have effectively prolonged islet allograft survival in the NHP model [42]. Novartis also has announced that new fully humanized anti-CD40 Abs (CFZ255) have prolonged the graft survival in kidney-transplanted patients with a lesser number of side effects as opposed to a CNI-based regimen. Thus, in addition to the development of new antibodies that could effectively prevent porcine islet rejection without serious pan-depletion of immune cells, efficacies of the abovementioned anti-CD40 Abs (ASKP1240, CFZ255) need to be tested in pig-to-NHP xenotransplantation models.

JAK3 Inhibitor as a Candidate Drug for Porcine Islet Xenotransplantation

Although CNIs, including cyclosporine and tacrolimus, revolutionized the field of transplantation by preventing acute rejection, CNIs have several drawbacks in terms of islet transplantation.

Table 1 Summary of immunomodulatory protocol based on anti-CD154 or anti-CD40

	Donor	Immunomodulatory regimen	Maximum graft survival	Reference
Anti-CD154	WT (adult)	Anti-IL-2R + anti-CD154 + FTY720 + Rapamycin	> 187	[16]
	WT (neonatal)	Anti-IL-2R + anti-CD154 + CTLA-4 Ig + Rapamycin	> 260	[33]
	hCD46 (adult)	ATG + anti-CD154 +MMF	396	[34]
	GTKO (neonatal)	Anti-LFA-1 + anti-CD154 + CTLA-4 Ig + MMF	249	[35]
	Muti-Tg (neonatal)	ATG + anti-CD154 + MMF	365	[36]
	WT (adult)	ATG + CVF + anti-TNF + anti-CD154 + Rapamycin + T_{reg}	> 603	[37]
Anti-CD40	WT (neonatal)	Anti-IL-2R + anti-CD40 (Chi220) + CTLA-4 Ig + Rapamycin	> 203	[39]
	WT (adult)	ATG + CVF + anti-TNF + anti-CD40 (2C10R4) + Tacrolimus	> 320	[40••]

First, CNIs are cytotoxic to pancreatic ß cells [43]. Calcineurin is also expressed in ß cells and plays a role in proliferation, differentiation, and insulin secretion. Thus, CNIs induce ß cell death as well as inhibit ß cell proliferation and differentiation; they are also associated with post-transplant ß cell failure. In addition to this, CNIs exert nephrotoxic effects. Long-term exposure to CNIs could cause an irreversible damage to the renal structure and its function by inducing morphological changes (e.g., arteriolar hyalinosis, interstitial fibrosis, and/or tubular atrophy) [44].

Second, CNIs impair the activation and generation of regulatory T cells (T_{reg}). Because the Foxp3 promoter and enhancer regions contain NFAT-binding sites, the inhibition of NFAT causes a decrease in Foxp3 mRNA expression [45]. Indeed, cyclosporine-treated T_{reg} decreases Foxp3 expression by reducing suppressor activity, and tacrolimus increases the transformation of T_{reg} into conventional T cells [46, 47]. Therefore, the development of a new drug that effectively inhibits T cell activation while being innocuous to ß cells and T_{reg} is necessary.

Janus kinases (JAKs) are cytoplasmic tyrosine kinases that participate in signaling of the members of the family of cytokine receptors, which share a common gamma chain. There are four mammalian JAKs: JAK1, 2, 3, and tyrosine kinase 2 [48]. Binding of cytokines to receptors delivers an activation signal to JAK, which phosphorylates the receptor and creates the docking sites for signal transducers and activators of transcription (STATs) [48]. Because JAK/STAT signaling plays a pivotal role in lymphocyte activation, proliferation, and differentiation, JAK inhibitors show potential as immunomodulatory drugs [49]. Moreover, unlike other JAKs, *JAK3* is predominantly expressed in *hematopoietic cells*. Thus, a specific JAK3 inhibitor may have selective inhibitory effects on immune cells [50]. Another merit of the JAK3 inhibitor is that it is a T_{reg} friendly immunomodulatory drug. Sewgobind et al. documented that the JAK3 inhibitor preserves the suppressive function of T_{reg} while inhibiting effector T cell function [51]. Because of these properties, JAK3 inhibitors are considered suitable as immunomodulatory drugs for transplantation.

Indeed, in a murine cardiac transplantation model, tofacitinib (JAK3 inhibitor) increased graft survival in a dose-dependent manner [52]. Furthermore, we recently published data revealing that tofacitinib-based IS protocol significantly prolongs graft survival with median survival days (MSD) of 86.5 days in an NHP allo-islet transplantation model [53•]. Notably, using the tofacitinib-based immunosuppression regimen, we achieved consistent porcine islet graft survival in three of seven NHPs for at least 6 months with a significantly reduced need for exogenous insulin (unpublished data). Thus, the development of new regimens based on tofacitinib could be a promising immunomodulatory therapy for porcine islet xenotransplantation.

Therapeutic Application of T_{reg} in Porcine Islets Xenotransplantation

T_{regs} are defined as small subsets of T cells which constitute 5–10% of T cells in healthy humans, non-human primates, and mice [54]. T_{regs} have been regarded as a potential therapeutic tool for the prevention of graft rejection because they are essential for the induction and maintenance of tolerance [54]. The efficacy of T_{regs} to prevent organ rejection have been tested in MHC-mismatched hematopoietic transplantation and graft-versus-host disease models [55, 56]. In a human clinical setting, adoptive transfer of ex vivo-expanded $CD4^+CD25^+CD127^-$ T_{regs} effectively suppressed graft-versus-host disease [57]. Furthermore, therapeutic potential of adoptively transferred $CD4^+$ T_{regs} has been tested in rodent xenotransplantation models. Infusion of ex vivo-expanded polyclonal human $CD4^+$ T_{regs} prevents the rejection of transplanted porcine islets in humanized mice [58]. Our group injected ex vivo-expanded T_{reg} cells into porcine islet-transplanted rhesus monkeys under treatment with an anti-CD154 Ab-based regimen [37]. Although the number of experiments was not large enough for conducting statistical analysis, it seems that graft survival in T_{reg}-infused groups was increased. However, T_{regs} alone were not sufficient to achieve long-term graft survival. In the pig-to-NHP islet xenotransplantation performed by our group, ex vivo-expanded $CD4^+CD25^+CD127^-$ T_{regs} were infused at early time points (6–20 days post transplantation). T_{regs}-infused recipient monkeys showed better glycemic control, and engraft porcine islets were protected by a maintenance regimen composed of CD40–CD154 blockade + sirolimus. However, engrafted porcine islets were completely rejected by activated T cells after cessation of immunosuppressant administration [38]. This result indicates that infused $CD4^+$ T_{regs} could not induce tolerance against porcine islets. However, despite unsatisfactory results, it is still an undeniable fact that T_{regs} are an attractive alternative to conventional immunosuppressive drugs. Therefore, further studies are required to define T_{reg} types and optimize infusion doses, frequency, and intervals for the success of porcine islet xenotransplantation.

Conclusions

Porcine islet xenotransplantation has great potential to cure severe type 1 diabetes by providing unlimited sources for insulin production. Despite its necessity, the application of islet xenotransplantation in clinical settings seemed insurmountable owing to the strong immune responses against xeno islets along with safety concerns. However, remarkable progress over the last 20 years in the development of immunomodulatory drugs has paved the way to clinical islet xenotransplantation. Technically, we are close to establishing a

clinically applicable immunomodulatory regimen by employing JAK3 inhibitors. However, in our experience, JAK3 inhibitor-based regimen did not appear to as effective as an anti-CD154-based regimen, suggesting that further fine-tuning of the regimen is still required. Another aspect to consider for overcoming the xenogeneic immunological barrier could be the testing of new CD40 clones (ASKP1240, CFZ255). Furthermore, we suggest that cell-based therapy, including adoptive regulatory T cell infusion or Chimeric Antigen Receptor T_{reg} cells (CAR T_{reg}), could be promising for overcoming the xenogeneic immune barrier. We believe that successful porcine islet xenotransplantation is an attainable goal and expect that clinical trials of porcine islet xenotransplantation will be performed in the near future under legitimate regulations.

Authors' Contributions C-G. Park is the corresponding author for the manuscript. S-H. Hong, H-J Kim and C-G. Park contributed to writing, and S-J. Kang contributed to the literature search. C-G. Park is responsible for the final approval of the manuscript.

Funding C.G Park is supported by a grant from the Korea Healthcare Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (Project No. HI13C0954) and also supported by a grant from National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIT) (Grant No. 2019R1A2C2085287).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

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