Chapter 4

Immune Response Associated with Islet Xenotransplantation in Small and Large Animal Models

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Abstract

This chapter will review studies that examine the immune response to porcine neonatal pancreatic cell clusters (NPCC) in small and large animal models; specifically, the immune mechanisms that lead to the rejection of transplanted islet cells in mice, nonhuman primates, and humans will be discussed. In addition, current research on the *in vitro* and *in vivo* human immune responses to porcine NPCC is also included. Research into the immune responses that lead to islet cell death posttransplant allows for further understanding of how to better protect transplanted porcine NPCC in humans. Furthermore, this chapter will examine immune-related strategies that have shown to extend the life and/or function of porcine NPCC *in vitro* and *in vivo*, including techniques that work to modulate the immune system of the islet cell donor and/or the recipient. Finally, this chapter will identify future areas of research that have yet to be examined extensively in the literature, mostly pertaining to the human immune response to porcine NPCC in the clinical setting.

Keywords: complement and innate immunology, cell and tissue xenotransplantation, neonatal pancreatic cell clusters (NPCC)

1. Introduction

Clinical islet cell transplantation is currently considered as an alternative option for the treatment of unstable type 1 diabetes. Despite recent progress in the field, transplant recipients continue to experience a progressive loss of insulin independence for reasons that are not well understood [1]. In addition, the shortage of human islet cell donors and the necessity of

chronic immunosuppressant drugs are major barriers to the widespread application of islet cell transplantation in clinical practice.

Xenotransplantation addresses the shortage of available human donors in transplantation medicine. Porcine neonatal pancreatic cell clusters (NPCC) remain a strong option for islet cell xenotransplantation due to their relative ease of acquisition and low cost, as well as similarities in physiology between pig and human islet cells [2]. Similar to islet cell allotransplantation, the efficacy of transplanted porcine NPCC into animal models is limited by posttransplant cell damage likely resulting from acute host-mediated inflammatory and oxidative stress, as well as chronic immune cell-mediated responses.

This chapter will review studies that examine the immune response to porcine NPCC in small and large animal models; specifically, the immune mechanisms that lead to the rejection of transplanted islets in mice, nonhuman primates, and humans will be discussed. Research into the immune responses that lead to islet cell death posttransplant allows for further understanding of how to better protect transplanted porcine NPCC in humans. Furthermore, this chapter will examine immune-related strategies that have shown to extend the life and/or function of porcine NPCC both *in vitro* and *in vivo*, including techniques that work to modulate the immune system of the islet cell donor and/or the recipient. Lastly, this chapter will identify future areas of research that have yet to be examined extensively in the literature, mostly pertaining to the human immune response to porcine NPCC in preparation for the transplantation of porcine NPCC in patients with type 1 diabetes.

2. Background and history

Islet transplantation began in the 1970s, when Ballinger et al. demonstrated that diabetic rats could be made normoglycemic through injection of islet isografts into the portal vein [3]. Not long afterwards, the University of Minnesota performed successful autologous islet transplantations in patients that had undergone near-complete pancreatectomies [3]. From these experiments arose the goal of clinical islet transplantation as a viable treatment for type 1 diabetes.

However, the integration of islet transplantation into the clinical setting has seen several setbacks. Firstly, islet transplant recipients invariably return to a hyperglycemic state. Long-term follow-up of the earliest successful transplant recipients found that over 80% of these patients did not remain normoglycemic at the end of 2 years, even with adequate immunosuppression [3]. Further understanding of islet isolation protocols and the immune response to islet transplants has allowed for the 2-year failure rate to fall to 50% [1]; however, this remains a large barrier to the use of islet transplantation in a clinical setting. Secondly, as is true across the field of transplantation, there is a large shortage of donor tissue available. Xenotransplantation attempts to address this issue.

While the idea of xenotransplantation dates back to the sixteenth century, it was not until the 1980s that a better understanding of immunosuppression allowed for clinical islet xenotransplantation to be attempted with any success [4]. From 1990 to 1993, Groth et al.

performed islet xenotransplants with NPCC into 10 type 1 diabetic patients [5]. Though all 10 patients remained insulin dependent, 4 patients secreted small amounts of insulin up to 400 days posttransplant. In 2002, at the XIXth International Congress of the Transplantation Society, Valdes-Gonzalaez et al. reported 12 transplants of NPCC into children with type-1 diabetes [6]. At 1-year posttransplant, five of the patients who received transplants required less insulin, and one patient was entirely insulin-independent. Most recently, Matsumoto et al. demonstrated that transplantation of encapsulated NPCC into the peritoneal cavity of patients with type 1 diabetes was able to maintain normoglycemia in these patients for over 600 days posttransplant without immunosuppression [7]. These experiments demonstrate that transplantation of NPCC could have a place in the clinical treatment of type 1 diabetes.

While xenotransplantation comes with its own set of immune-related complications, scientists still believe that islet xenografts are a good alternative to islet allografts. Because they are much less vascular, islet transplants are less immunogenic than full organ transplants, and so do not present the same challenges that a heart or kidney xenotransplantation would present. In addition, NPCC are relatively low cost and easily acquired, and would therefore solve the problem of islet donor shortage. Unfortunately, the problem of islet transplant recipients' inevitable return to a hyperglycemic state is also a problem in xenotransplantation.

As is seen in islet allotransplantation, immunosuppression techniques increase the lifespan of islet xenografts *in vivo* [8–12], demonstrating that islet xenograft failure has an immune-dependent mechanism. Scientists continue to attempt to elicit this mechanism, as therapies targeted at controlling this immune response will allow for longer islet transplantation survival.

3. Ideal age of porcine islet donors

3.1. Adult porcine islets

Successful autograft, allograft, and xenograft transplantation has been done using adult porcine islets. There are several advantages to obtaining islets from older pigs. Firstly, larger numbers of islets can be obtained from a single adult pig pancreas. Secondly, these mature islets, when isolated, are individually larger in size (**Figure 1**) and the potential for insulin secretion is greater [13]. This has been demonstrated in several studies, which have shown that the return to normoglycemia is faster post transplantation in experiments with mice and nonhuman primates [12, 14, 15]. Lastly, adult porcine islets express certain immunogenic antigens, such as galactose alpha 1,3-galactose (alpha Gal) to a lesser extent than neonatal [16] or fetal porcine islets [13].

Unfortunately, adult porcine islets are delicate. They are more susceptible to ischemic injury and so are difficult to keep viable in culture [17, 18]. Also, the quality of islets obtained from adult pigs varies greatly depending on the exact age and breed of the donor pig [13, 19]. Lastly, although adult porcine islets express certain antigens to a lesser extent as stated above, it is thought that islets isolated from adult pigs are overall more immunogenic than islets from neonatal or fetal pigs, increasing the need for immunosuppressive drug regimens [2, 16].

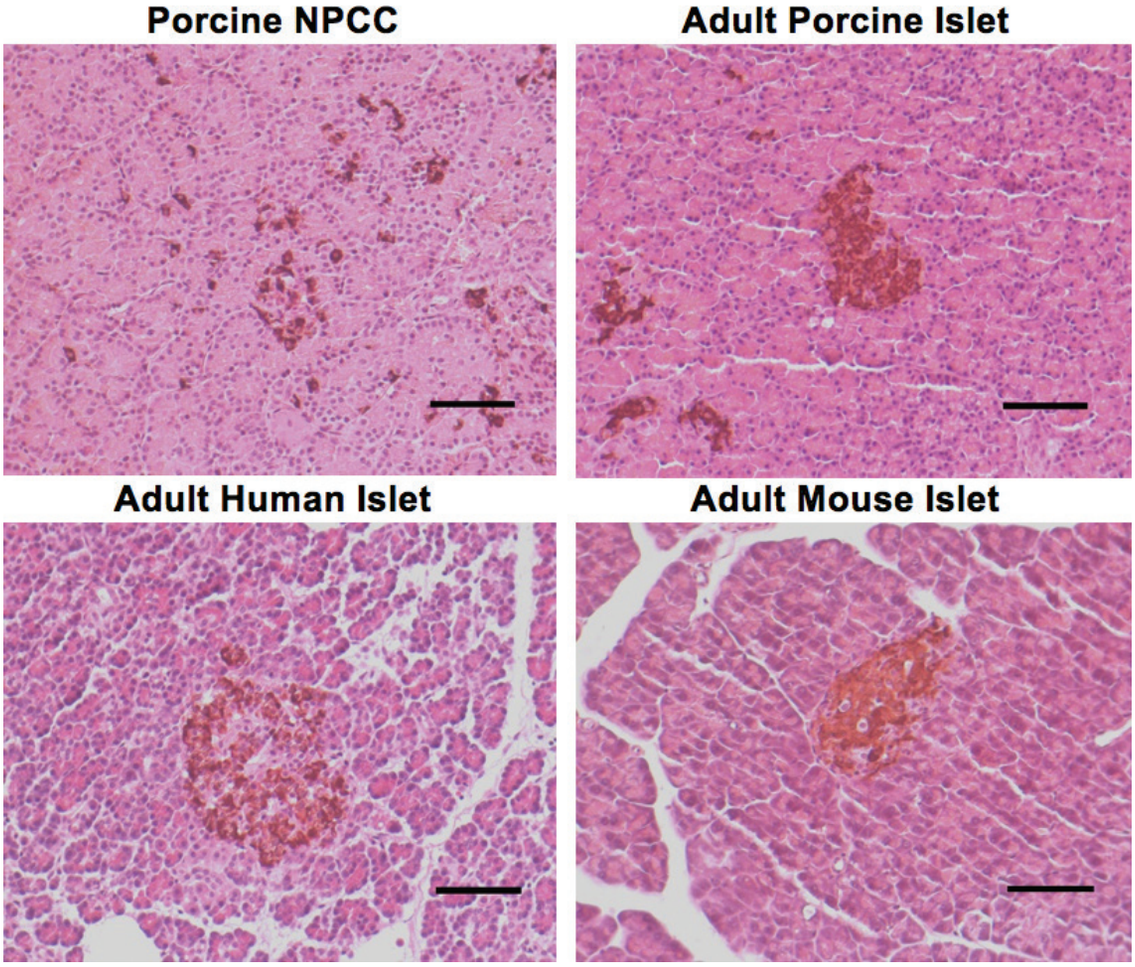

Figure 1. Islets in neonatal pig, adult pig, and adult human, adult mouse pancreas. Islets are depicted as brown structures representing insulin-positive beta cells in the islets, which are surrounded with exocrine tissue. Pancreas sections were counterstained with Harris' hematoxylin and eosin. Scale bar represents 100 μm.

3.2. Porcine neonatal pancreatic cell clusters

Porcine NPCC have also been used to successfully reverse diabetes in small [8, 10, 20] and large animal models [9, 21]. It is widely believed that neonatal pigs are the best source of islets for xenotransplantation, for several reasons. Firstly, the pancreas of a neonatal pig is less fibrous and the islets are easier to isolate than those of an adult pig. Porcine NPCC also maintain growth capacity after isolation, and may continue to grow even after transplantation [13]. They also appear to be less susceptible to ischemic injury after isolation and keep better in culture [17, 18].

Disadvantages of using porcine NPCC include an increased time to return islet recipients to normoglycemia due to their immature nature compared to adult islets (**Figure 1**). NPCC also have an increased presence of antigens on their surface (i.e., alpha Gal), and require a high number of donor pigs for a single transplantation [2, 13, 18].

3.3. Porcine fetal pancreatic cell clusters (FPCC)

Porcine FPCC have many of the same advantages that porcine NPCC have, including the resilience of the cells to ischemic injury and the ability to mature and maintain growth capacity after isolation [13].

However, fetal islets have shown to secrete very small amounts of insulin in response to glucose stimulation and can take up to months to achieve normoglycemia, even in small animal models. Much like porcine NPCC, many donor pigs are required for a single transplantation [13]. Neither large animal islet allotransplantation nor nonhuman primate xenotransplantation has been successfully achieved with porcine FPCC.

4. Immune response in mouse

Many studies examining the postporcine NPCC transplantation immune response have been performed in rodent models. Mice are often used as a mammalian model organism, due to relative ease of acquisition, short gestation period, and well-studied and sequenced genome. C57BL/6 mice in particular are the most widely used rodent in laboratory experiments, and have been used extensively in the study of postislet transplantation environment.

4.1. Hyperacute rejection

If the serum of the transplant recipient has natural preformed antibodies with specificity for antigens on the transplanted tissue, a process known as hyperacute rejection can occur. This can lead to an antibody-mediated destruction of the transplant that begins immediately after transplantation. The presence of preformed natural antibodies against the transplant can occur from prior exposure to either the antigen present on the transplant or an antigen similar enough that it can be recognized by the same antibody. This becomes especially important in xenotransplantation, as different species express antigens that can be recognized by preformed natural antibodies commonly made by the human immune system.

The main difference in hyperacute rejection of pig-to-rodent transplants is that these species share a similarity that pigs and nonhuman primates and pigs and humans do not. Pigs and rodents, along with most other mammals, synthesize the enzyme alpha 1,3-galactosyltransferase and produce alpha Gal, and so do not produce anti-Gal antibodies [22]. Some nonhuman primates and humans lack this enzyme, and therefore do produce anti-alpha Gal antibodies. Hyperacute rejection occurs in pig-to-nonhuman primates and pig-to-human xenotransplants when preformed natural antibodies in the recipient, largely anti-alpha Gal antibodies, recognize alpha Gal present within the transplanted tissue. Anti-alpha Gal antibody-mediated rejection does not occur in the mouse model. Because of this difference and the resulting lack of clinical application, hyperacute rejection in mouse models is not a main focus of xenotransplantation research.

4.2. Instant blood-mediated inflammatory reaction (IBMIR)

Islet transplantation triggers an inflammatory reaction when transplanted intravascularly in mice. This reaction involves activation of the coagulation cascade and complement pathways, which damages the transplanted islets. These events are known to occur in all transplants, including autologous transplants, but it has been shown that IBMIR occurs on a larger scale in xenotransplantation [23].

However, IBMIR in the pig-to-mouse model has been poorly researched. This is because islet transplantation in mice is often not done by injecting the porcine NPCC into the portal vein and is instead done through injecting the porcine NPCC into the peritoneum (for encapsulated porcine NPCC) or under the kidney capsule. This does not allow for an IBMIR response that would be comparable to nonhuman primate or human islet transplantation models.

A study that did attempt transplantation into the portal vein of mice showed that the inflammatory mediators in the posttransplant environment are largely acute-phase cytokines, such as TNFα and IFN-γ [24]. It has been shown that blockade of these inflammatory pathways, such as by using an anti-TNFα antibody, improved the survival of the islet transplant and improved glucose tolerance *in vivo* [24].

4.3. Adaptive immune response

T cell-mediated rejection is key in the rejection of porcine NPCC xenografts by mouse recipients [25]. There are two pathways of antigen recognition by T cells that are important in transplantation: the direct pathway and the indirect pathway. The direct pathway occurs when T cells recognize an antigen that is presented on the surface of a donor antigen-presenting cell (APC). The indirect pathway occurs when the T cells recognize an antigen that is presented on the surface of a host APC. Activation of either of these pathways can lead to subsequent activation of T cells and destruction of a transplant [18].

The indirect pathway of T cell activation becomes increasingly dominant as the evolutionary disparity between the transplant donor and recipient increases; likewise, the direct pathway of T cell activation is dominant in the rejection of allotransplants. As expected, in the pig-to-mouse xenotransplant, the indirect pathway is responsible for T cell-mediated rejection [25, 26]. It has been demonstrated that CD4+ T cell activation is essential for porcine NPCC xenograft rejection to occur, whereas CD8+ T cells are only minimally involved [25].

5. Immune response in nonhuman primate

Nonhuman primates (NHP), specifically old world monkeys, constitute the only research animal in which the occurrence of transplant rejection and the efficacy of immunosuppression can be observed in the presence of a human-like complicated and redundant immune system. As such, numerous studies of pig-to-NHP islet xenotransplantation have been responsible

for the discovery of important immune mechanisms involved in causing posttransplantation graft damage.

5.1. Hyperacute rejection

As previously stated, the difference in evolutionary diversity between pigs and monkeys cause a different hyperacute rejection process than in the pig-to-rodent model. The carbohydrate alpha Gal is accepted to be the epitope responsible for immediate xenograft destruction of porcine islets in nonhuman primates [22].

Alpha Gal is expressed by all animal species, including pigs, and many bacterial species. However, in humans and old world monkeys, the evolutionary loss of enzyme alpha 1,3-galactosyltransferase has led to the inability to synthesize alpha Gal. It is hypothesized that exposure to microorganisms shortly after birth cause humans and old world monkeys to synthesize anti-alpha Gal antibodies [27, 28]. These antibodies remain in blood circulation and are thought to be responsible for the destruction of Gal-expressing porcine NPCC within minutes of transplantation [22].

As discussed above, porcine NPCC show the most promise in islet transplantation. Unfortunately, porcine NPCC have a higher expression of alpha Gal when compared to adult porcine islets, which express alpha Gal only minimally [16]. The use of genetically modified pigs that have the enzyme alpha 1,3-galactosyltransferase knocked out (GTKO) remains a large area of research interest for this reason. However, even with the use of GTKO porcine NPCC, acute rejection still occurs (though to a lesser extent) [29]. This suggests that more porcine NPCC antigens are recognized by antibodies in nonhuman primates. Two have been identified: N-glycolylneuraminic acid (NeuGc) and β1,4 N-acetylgalactosaminyltransferase (B4GALNT2) [29]. Interestingly, Stewart et al. have demonstrated that treating nonhuman primates with alpha adrenergic agonist clonidine inhibits the production of these additional "antinon-Gal" antibodies [30].

5.2. IBMIR

Porcine NPCC are susceptible to IBMIR when exposed to the blood of nonhuman primates [22]. This reaction involves platelet activation, complement cascade activation, and mononuclear cell infiltration in the first hours to days following transplantation [2]. Alpha Gal is also thought to be implicated in this inflammatory response. When Komoda et al. developed a transgenic pig that overexpresses an enzyme, which prevented the formation of alpha Gal and transplanted NPCC from this pig to diabetic nonhuman primates, the transplant did not undergo hyperacute rejection and showed less activation of the complement cascade [31].

This reaction occurs regardless of immune cell-mediated rejection and is thought to involve tissue factor production, but the specific pathways behind this event are poorly understood. It has been shown that even with the depletion of the components of the complement system in nonhuman primates, IBMIR still occurs, although the destruction of the islet graft is decreased [11].

Innate immune cells have also been implicated in islet xenograft rejection. In nonhuman primate models, neutrophils and macrophages have been temporally associated with the failure of porcine NPCC grafts [2].

5.3. Adaptive immune response

Aside from the antibody-mediated rejection of xenografts that was mentioned during the discussion of hyperacute rejection, the response of the adaptive immune system to porcine NPCC in the nonhuman primate model has not been studied to the extent that it has been in the rodent model.

Available research shows through analysis of transcript levels in inadequately immunosuppressed nonhuman primates that a T cell-dependent antibody response occurs posttransplantation, resulting in high levels of antiporcine IgG [22]. The results of other studies support this idea, and have demonstrated that immunosuppressive agents that result in a blockade of T cell costimulation maintain normoglycemia in diabetic monkeys for over a year [22].

6. Immune response in human

The immune response to porcine NPCC in human models is poorly understood, due to a lack of a suitable experimental model. Because it is not possible to assess the human immune response *in vivo* in a research setting, scientists rely on *in vitro* experiments and experiments with animals that have been reconstituted with a human immune system.

6.1. Hyperacute rejection

Because of the evolutionary similarity between nonhuman primates and humans, the immune response to porcine NPCC transplant may be very similar in both species. As in nonhuman primates, the carbohydrate alpha Gal is accepted to be the epitope responsible for the hyperacute destruction of porcine NPCC when exposed to human blood *in vitro* [32].

As previously stated, evolutionary loss of enzyme alpha 1,3-galactosyltransferase in humans and old world monkeys led to the inability of either species to synthesize alpha Gal. It is hypothesized that exposure to microorganisms shortly after birth causes humans and old world monkeys to synthesize anti-alpha Gal antibodies [27, 28]. This becomes an issue especially with the use of porcine NPCC, as they express alpha Gal on their surface to a significant extent [16].

6.2. IBMIR

Much like is seen in the immune response in nonhuman primates, islet grafts undergo IBMIR once exposed to human blood. Studies have shown that this damage affects the integrity and viability of the cell membranes within the islet cell cluster and leads to an initial 25% loss of transplanted islets in *in vitro* models [33]. IBMIR involves platelet activation,

complement cascade activation, and mononuclear cell infiltration in the first hours to days following transplantation.

In studies involving the exposure of porcine NPCC to human blood, activation of the coagulation cascade produced proinflammatory thrombin at high concentrations, which exacerbated the destruction of the transplanted islet cells [34]. In reconstituted animal models, complement activation was demonstrated by the increase in concentration of complement proteins in the serum of transplant recipients [35]. Specifically, complement proteins C4d and C5b-9 have been implicated in IBMIR, implicating the classical complement pathway in xenograft destruction [36]. Complement protein Bb, a marker of the alternative complement pathway, also appears to be involved in IBMIR-related graft destruction, but not when islets from genetically engineered pigs (GTKO/CD46) are used [36].

In addition, activated neutrophils interacting with components of the coagulation cascade appear to be essential in the early loss of xenograft function in human models [34]. It has been demonstrated that the mechanisms by which this loss occurs include phagocytosis and secretion of reactive oxygen species (ROS) and proteinases by neutrophils [34].

6.3. Adaptive immune response

The response of the adaptive immune system to porcine NPCC in the human model is poorly studied, aside from what is understood about hyperacute rejection. What is known, however, is that the rejection of porcine NPCC xenografts *in vitro* is predominantly T cell-mediated [37]. This has also been demonstrated by Yi et al., who showed that the reconstitution of mice with regulatory T cells, which suppress effector T cells, prior to reconstitution with a human immune system prevented xenograft rejection [38].

Little research has been done into the specific pathways of T cell rejection of porcine NPCC due to the lack of a suitable experimental model. Murray et al. demonstrated that islet rejection likely occurs via a CD4+ T cell-directed response, with NK cell and CD8+ T cell-mediated injury of the xenograft not contributing to islet loss [37]. Additionally, a study by Lalain et al [39]. examined the adaptive immune response *in vitro* to adult porcine islets in type 1 diabetic and healthy human subjects. It was shown that the immune response to porcine islet cells involves dominantly CD4+ T cells activated through the indirect pathway, as well as CD8+ T cells activated through the direct pathway [39].

Our preliminary results suggest that there are significant differences in the strength and kinetics of *in vitro* proliferation of human peripheral blood mononuclear cells (PBMCs) from individuals with or without type 1 diabetes when stimulated with mitogen, neonatal porcine PBMCs, or porcine NPCC (**Figure 2**). Whether these results could be translated *in vivo* remains to be determined. We initially performed adoptive transfer experiments to identify the human immune cells that are infiltrating the porcine NPCC grafts. Examination of the infiltrating cells showed numerous CD45 positive human leukocytes in porcine NPCC grafts of NOD. SCID gamma mice injected with human PBMCs from donors with or without type 1 diabetes (**Figure 3**). The extent of this infiltration appeared to be similar; however, further quantification is necessary to confirm this observation. In addition, we also found the presence of M2

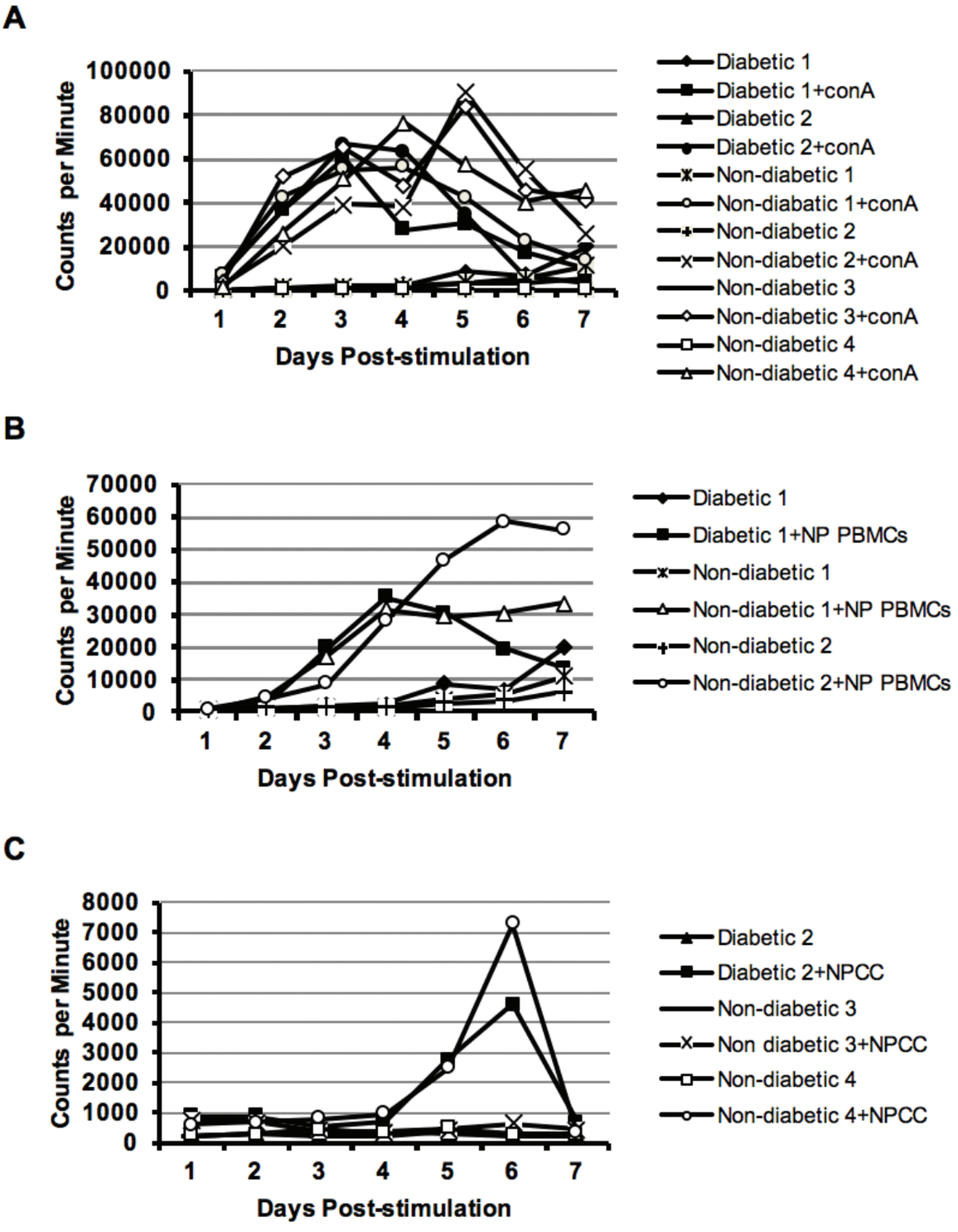

Figure 2. *In vitro*proliferation of PBMCs from human individuals with or without type 1 diabetes after stimulation with (A) mitogen (conA), (B) neonatal porcine PBMCs, or (C) porcine NPCC.

(Mac-2) macrophages among the cells infiltrating the porcine NPCC and their role in the rejection of porcine NPCC needs to be elucidated. Further characterization of human immune cells that infiltrated the porcine NPCC are ongoing and continued research in this area is necessary to enhance our understanding on the immune mechanisms involved in the rejection of porcine NPCC in human recipients with type 1 diabetes.

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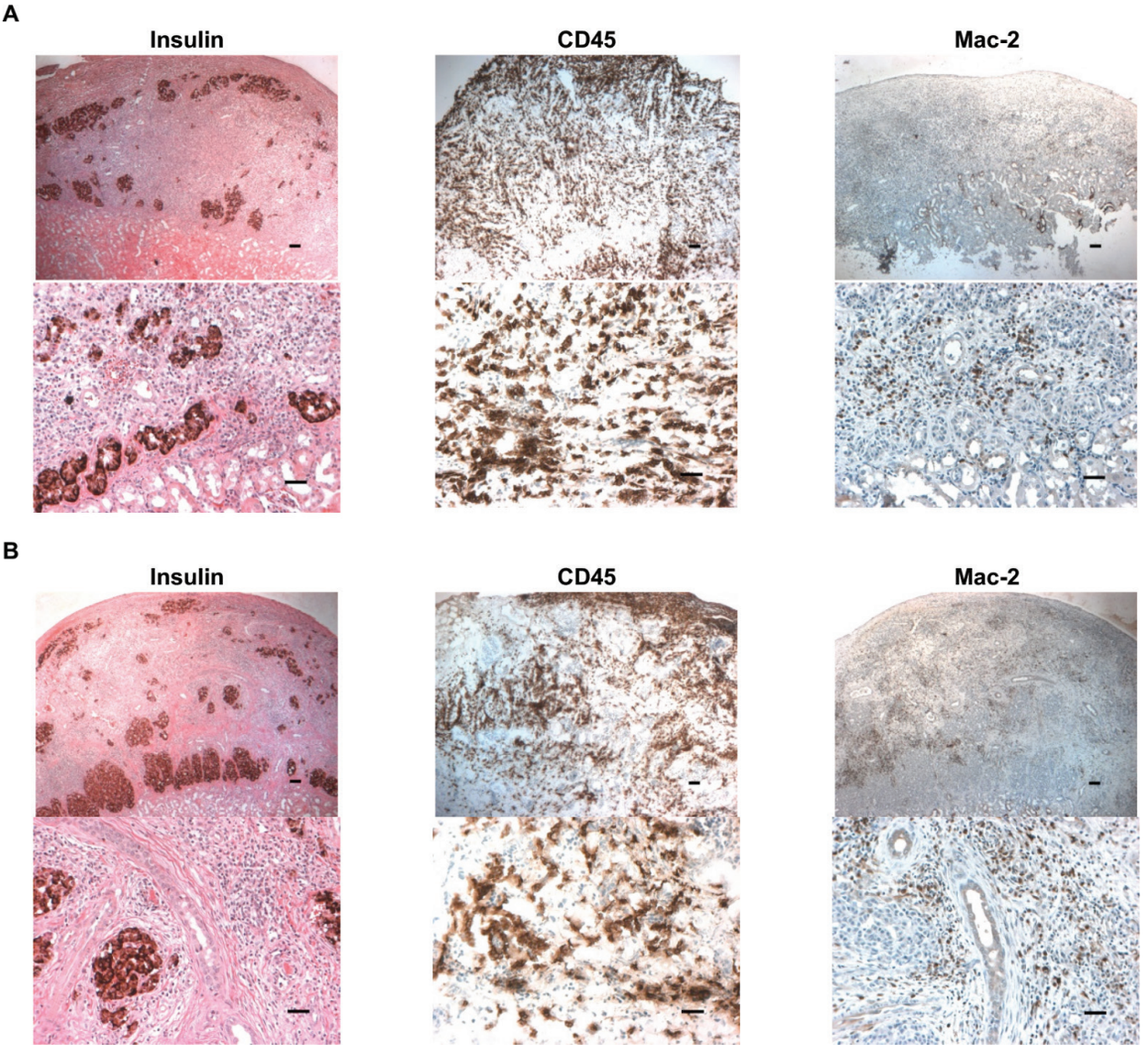

Figure 3. Porcine NPCC xenografts in NOD.SCID gamma mice reconstituted with PBMCs from human donors with (A) or without (B) type 1 diabetes. The presence of insulin-positive beta cells, human CD45-, and Mac-2-positive immune cells are shown as brown structures. Islet xenografts were collected 14 days postcell reconstitution. Images were taken at 2.5× and 10× objectives. Scale bar represents 100 μm.

7. Strategies and recommendations

There are many potential strategies to improve the viability and the function of islet xenografts. The following are a selection of strategies that do not involve an immunosuppressive regimen, and instead involve making changes to the islets themselves or the posttransplant environment that attempts to minimize damage from the recipient's immune system.

7.1. Immune modulation *in vitro*

Modulating the immune response before transplantation of the porcine NPCC into the recipient may allow for increasing the viability and function of the xenograft without the need for

more immunosuppressive drugs. One example of this strategy would be the culturing of porcine NPCC with protective agents, such as antioxidants, prior to transplantation.

It has been shown that oxidative stress is a likely contributor to cellular damage in the post-transplant environment [40]. Luca et al. have demonstrated that treating porcine NPCC with antioxidants vitamin D3 and E results in increased *in vitro* function of the islets for a significantly longer time than untreated controls [40]. In addition, morphologic examination of the porcine NPCC at 16 days after exposure to antioxidants showed increased porcine NPCC size and viability [40].

7.2. Immune modulation *in vivo*

Immunoisolation, or attempting to prevent exposure of the transplant to the recipient's immune system, is another method that can be utilized in islet xenotransplantation. Microencapsulation, macroencapsulation, and immunosuppressive scaffolding are all variants of this strategy.

Encapsulation involves placing islets inside a protective barrier, or capsule, prior to transplantation. Microencapsulation and macroencapsulation differ only in the number of islets inside each capsule. Microcapsules contain a single islet or very few islets, whereas macrocapsules contain a greater number of islets. Ideally, these capsules protect porcine NPCC from immune-mediated damage but allow for exchange of oxygen, nutrients, and waste. Advancement in the materials used for encapsulation, which now include cellulose, agarose, alginate, and protamine-heparin complex [19], have resulted in prolonged survival of islet xenografts in mice [20] and nonhuman primates [19]. Similarly, scaffolding involves transplanting islets on a porous, biodegradable material. This offers some of the protection of encapsulation with evidence for longer term function and survival of islet xenografts compared to encapsulation [41].

Other strategies involve administering immunomodulatory (but not immunosuppressive) agents to transplant recipients to prolong the life of the xenograft. As previously mentioned, Stewart et al. have demonstrated that treating nonhuman primates with alpha adrenergic agonist clonidine inhibits the production of anti-pig antibodies by the transplant recipient [30].

7.3. Manipulation of islet cell donors

Genetically modifying the pig donors allows for a minimization of hyperacute rejection and the IBMIR-related damage and cell loss that occurs shortly after transplant. An example of this has already been mentioned. Porcine NPCC from GTKO pigs can prevent anti-Gal-mediated damage to the islet graft [29, 32]. Additional genetic engineering of donor pigs can knockout other antigens present on NPCC, such as NeuGc [32]. Donor pigs can also be engineered to knockout tissue factor, a factor needed in coagulation [19], in order to successfully prevent IBMIR.

In addition to knocking out harmful genes, genetic engineering can also be used to add helpful genetic material. Komoda et al. performed a study in which porcine NPCC from *N*-acetylglucosaminyltransferase III (GnT-III) transgenic pigs were transplanted into diabetic cynomolgus

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monkeys. GnT-III pigs are bred with an additional residue in the complex *N*-linked sugars that are implicated in "antinon-Gal" antibody formation. The study demonstrated that the engineered porcine NPCC showed a reduced antigenicity and increased survival time compared to the wild-type islet transplants [31].

8. Future research directions

There are several directions for future research that have become apparent throughout this chapter. Firstly, a better understanding of the human immune response to porcine NPCC is needed, both *in vitro* and *in vivo*. This must include an examination of the antibodies involved in hyperacute rejection and identification of the epitopes present on porcine NPCC. In addition to simply a greater number of studies needing to be performed, most *in vitro* and reconstituted animal *in vivo* studies of the human immune response to porcine NPCC are performed with blood products from healthy subjects. As a result, little is known specifically about the immune response of patients with type 1 diabetes. Additionally, while there have been *in vivo* studies in nonhuman primates [11, 31] using genetically engineered porcine NPCC, *in vitro* and *in vivo* human studies using transgenic porcine NPCC are needed.

There is also no clear consensus on the best transplant sites for optimizing xenograft function and minimizing immune-mediated damage to the islets. Injection of porcine NPCC into the portal vein, traditionally accepted as the site of islet transplantation, causes a sizable and immediate immune response and results in islet loss [33]. More recently, porcine NPCC have been transplanted into the peritoneal cavity in an attempt to minimize the recipient's immune response; however, it is hypothesized that this transplant site leads to a lag time between islets sensing blood glucose levels and releasing insulin, resulting in poorer glycemic control [7]. Other transplant sites, such as within the omentum or under the skin, have been proposed but not investigated.

Scaffolding and encapsulation allows for protection of the islet grafts from the recipient's immune system without the need for additional immunosuppressive drugs. There are challenges with long-term survival of islet transplants with encapsulation, as encapsulation can prevent revascularization and remodeling posttransplantation. Scaffolding can address these issues, as scaffolds are porous and allow for tissue ingrowth and revascularization. While studies with encapsulated islets have been performed in mice [10, 20, 24, 25], nonhuman primates [19], and humans [7] with success, fewer experiments have been performed using scaffolds and more research is needed into their utility.

Lastly, little research has been done into multiple dose transplantation. Because long-term survival of islet xenotransplantation has not yet been achieved, multiple dose transplantation becomes an important consideration. There are implications for the immunosuppression regime necessary if multiple islet xenotransplants are needed in a single patient, as it is reasonable to assume that the recipient would develop immunological memory to xenoantigens present in the islet graft. This is an important area of future research that has major implications in using islet transplantation as a clinical treatment of type 1 diabetes.

9. Conclusion

Islet xenotransplantation addresses the shortage of available human islet donors for clinical islet transplantation and has the potential to become a viable treatment of type 1 diabetes. Porcine islets remain the best option for islet transplantation, due to their ease of acquisition and similar physiology to human islet cells. Neonatal pigs appear to be the best source for transplantable islets because of their resilience to ischemic damage and growth potential postcollection. Like islet allotransplantation, islet xenotransplantation has been limited by posttransplant graft destruction from the recipient's immune response.

The immune response in rodent, nonhuman primate, and human models can be separated into hyperacute rejection, IBMIR, and adaptive immune responses. While each species group has a slightly different immune response to porcine NPCC xenografts, there are also some similarities. Antigens present on the porcine NPCC, such as alpha Gal, are largely responsible for hyperacute rejection in humans and nonhuman primates. However, other xenoantigens that need to be identified also may contribute to this response. In addition, it appears that the indirect pathway of T cell activation is an essential part of xenograft rejection in all species groups, with CD4+ T cells dominating the rejection process.

There are several strategies that can be utilized in islet xenotransplantation to improve the viability and function of the islet grafts that do not involve immunosuppressive drug regimens. These include culturing the islets with immune-modulating agents pretransplantation, transplanting encapsulated or scaffolded islets, or genetically modifying the islet cell donors to dampen the recipient's immune response. Future research directions include eliciting the specific mechanism of islet xenotransplant rejection in the human model, ideally *in vivo*.

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