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COMMENTARY

Xenotransplantation of pig islet cells: Potential adverse impact of virus infections on their functionality and insulin production

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Type 1 diabetes is a major health problem throughout the world.[1] Xenotransplantation using pig islet cells is under development to alleviate the shortage of human pancreata and human islet cells for the treatment of diabetes. First preclinical and clinical trials were carried out with encouraging results: Transplantation of porcine islets into diabetic non-human primates with immunosuppression showed insulin independence for more than 900 days.[2–5] To improve the outcome of pig islet cell xenotransplantation, several approaches were pursued, including strategies developing new transplantation sites other than the portal vein, potent immunosuppressive regimens, and incorporation of regulatory T cells.[4–6] In addition, genetic engineering and gene editing strategies to overcome humoral and cellular rejections of pig islet were introduced.[7–9] Encapsulation was shown to be an effective strategy to overcome the need for immunosuppression. Microencapsulated pig islet cells were transplanted in preclinical and clinical trials[10,11] and macroencapsulated human islet cells were successfully used to treat patients.[12] Macroencapsulated pig islet cells were tested in rhesus monkeys.[13]

Porcine viruses had not been transmitted to the recipients in some preclinical trials transplanting pig islet cells into non-human primates were a virus screening was performed[10,14–16] and in all clinical trials using pig islet cells performed until now.[17–25] In one preclinical trial,[10] and in most clinical trials,[17–21,23] microencapsulated islet cells from Auckland Island pigs were used, a pig strain, which is well characterized microbiologically. This explains the absence of virus transmission to the recipients. However, other preclinical trials were performed without screening for virus transmission.[7,26–31]

Numerous studies have been performed to select the optimal source (neonatal or adult pigs) and the optimal conditions for isolating the islet cells for future transplantations. However, in these comparative studies, nobody was screening for pig viruses, despite it is well known that different viruses influence the functionality of islet cells even in subclinical concentrations. As will be discussed in more detail below, especially picornaviruses have been shown to impair the function of islet cells.[32,33] Furthermore, double-stranded (ds) RNA, produced by many virus infections, acting via toll-like receptor (TLR) binding induced a decrease in insulin production.[34] When screening for the best source of pig islets, it was found that adult porcine pancreata yield, on average, more than five times the amount of islets than do neonatal pancreata, the high price of adult pigs led to the cost per islet being more than twice that of neonatal islets.[35] The variability of isolation outcome depends on age and breed of the animals and the quality of the initial organ and this represents a major obstacle to pig islet cell xenotransplantation.[36–42] In most of these investigations analyzing the optimal conditions for islet cell preparations for transplantation, for example, comparing neonatal with adult islet cells and designing methods for their isolation, no screening for porcine viruses had been performed. For example, when islet cells from Chicago Medical School miniature pigs were analyzed, extremely high yields of well-functioning islets were isolated from adult animals.[41] The animals were bred in a barrier-sustained specified pathogen free/gnotobiotic facility; however, a list of analyzed viruses was not published. When Wuzhishan (WZS) miniature pigs were compared with slaughterhouse pigs, the islet yield of the WZS miniature pigs' pancreata was significantly higher than that of the market pigs and WZS islets appeared to be of higher quality.[42] The reason may be the infection of slaughterhouse pigs with different viruses.[43,44] When adult porcine islets from genetically unmodified pigs were transplanted intraportally into streptozotocin-diabetic, immunosuppressed cynomolgus macaques, reversal of diabetes for more than 100 days was observed; however, the virus status of the animals was not stated.[26]

To note, the consensus statements of the International Xenotransplantation Association (IXA) analyzed the requirements to ensure safe pig islet cells transplantations and presented guidelines regarding virus

testing of the donor pigs,[45–47] but did not discuss the problem of virus infections when analyzing the functionality of the islet cells.

It has to be considered that pig viruses and viruses able to replicate in pigs impair the function of pig islet cells. Coxsackie viruses belong to the Picornaviridae family. The human Coxsackie virus B5 (CVB5) effectively infects cultured porcine islet cells. Infection with CVB5 resulted in a persistent productive infection with minimal evidence of cell lysis.[48] Fetal pig islet cells have been shown to be susceptible to six serotypes of CVB and the islet function was subsequently lost, accompanied by a complete destruction of the cells by 3 days post infection.[32] Insulin release responses on CBV-infected pig islet cells began to deteriorate 1 day after infection; after 1 week, islet cells infected with CVB-1 and CVB-6 did not secrete any measurable insulin.[32] A slowly progressing subclinical infection of islets could lead to increased beta cell apoptosis.[48,49] This data demonstrates that CVB impairs the islet cell function in CVB-infected pigs and that it may be a major risk factor in pig to human islet cell xenotransplantation.

Virus infections in human and mouse islet cells are better studied and this data serves as evidence for a general effect of viruses on islet cells in all species and they may help to screen for related viruses in pigs which had not been investigated and which may impair pig islet cells.

CVBs, which had been shown to impair the functionality of pig islets (see above), have also been described to directly infect and kill human beta cells.[32,50] Beta cells are the major cell type in pancreatic islets; they synthesize and secrete insulin. Beta cells make up to 50–70% of the cells in human islets. CVBs are not only able to kill beta cells, but they are also able to induce moderate beta cell damage and through this may incite the beta cell immunity.[51] It was shown that isolated human pancreatic islets infected with enteroviruses from newly diagnosed diabetes patients supported virus replication. The viral isolates varied in their ability to cause destruction of the islets. Furthermore, the ability to secrete insulin in response to high glucose was reduced in all infected islets as early as 3 days post infection, before any difference in viability was observed.[33] Using human clonal beta cell lines and human islet cells, enterovirus infections were shown to inhibit the autophagy machinery, resulting in increased virus replication.[52] Coxsackievirus B4 (CVB4) infection has been shown to lyse primary human pancreatic ductal cells or to persist in these cells, which resulted in impairment of differentiation into insulin-producing cells.[53] This agrees with the view that non-cytopathic infection of human beta cells by enterovirus leads to decreased glucose-induced insulin secretion.[54,55] There is evidence that enteroviruses, mainly CVB4, can infect beta cells in patients with type 1 diabetes and that infection is associated with inflammation and functional impairment. In addition, isolated virus was able to infect beta cells from nondiabetic multiorgan donors in vitro, causing beta cell dysfunction characterized by impaired glucose-stimulated insulin release.[54]

Infection of human pancreatic islets with epidemic strains of echovirus, which also belong to the picornaviruses, resulted either in severe damage or proceeded without visible changes in infected islets. Two strains did replicate in human pancreatic beta cell lines and resulted in a pronounced cytopathic effect within 3 days following infection. The insulin release in response to high glucose stimulation was hampered in all infected cells when no evidence of cytolysis was present; however, the adverse effect of some strains on insulin secretion appeared to be higher than that of others.[55,56]

In mice, inoculation with a rotavirus closely related to the human rotavirus and known to infect mouse beta cells in vitro[57] induced a pathogenic effect on the pancreas, which was mediated initially by the innate toll-like receptor 3 (TLR3) for dsRNA. Viral infections have also been shown to induce cytokine release by human beta islet cells.[58]

Of great interest are the experiments with polyinosinic-polcytidylic acid (polyI:C). This is a simulation of an infection with all viruses able to be detected in the infected cell by the dsRNA pattern recognition receptor TLR3, independent of the animal species. Poly I:C is a synthetic dsRNA, and interacting with the TLR3, it mimics an effect of all infections with dsRNA viruses. When poly I:C was transfected into human beta cells in order to simulate a virus infection, this led to a decrease in insulin production.[34] Most virus infections including infections by enteroviruses result in the formation of dsRNA,[59] which is recognized by TLR3. Analysis of the gene expression showed a decrease in beta cell specific gene expression, for example, a dedifferentiation of the cells. This suggests that viral infection of human islet cells leads to a decrease in insulin production rather than beta cell death. Taken together, this data demonstrates that different viruses may influence the functionality and insulin production of islet cells of humans and mice, and therefore, the presence of related viruses in donor pigs and isolated islet cells should be analyzed and their impact on pig islet cell function should be studied.

The viruses impairing the function of human and murine islet cells, mainly picornaviruses, are the most common viruses found in the pig virome in healthy animals (up to 86%),[60] indicating that they may play the same role in pigs and that appropriate detection methods should be developed.

Considering the risk of virus transmission to the recipient, the situation with xenotransplantation of pig islet cells slightly differs from that of xenotransplantation of solid organs: Even when donor pigs were positive for porcine parvovirus (PPV), porcine cytomegalovirus/porcine roseolovirus (PCMV/PRV), and porcine lymphotropic herpesviruses (PLHV) in their peripheral blood mononuclear cells (PBMCs), their islet cells were found negative for these viruses, showing that the viral status of the product can be better than that of the donor pig.[61] However, in other studies, the islet cells were also positive for PCMV/PRV.[26] Furthermore, it has been shown that islets encapsulated in an alginate patch do not release porcine endogenous retroviruses (PERVs).[62]

Virological screening was neglected when pig islet cells were analyzed for high yield, easy purification, and excellent insulin production. As shown here, numerous viruses, especially picornaviruses and rotaviruses, have been shown to influence these parameters in porcine, human, and murine islet cells. Based on this result, it is important to develop sensitive detection systems[63] for these viruses based on polymerase chain reactions (PCR) and immunological methods and to screen the animals produced for islet cell preparation. In summary, viruses affecting the functionality and insulin production of pig islet cells such as picornaviruses and rotaviruses, and viruses posing a risk for the recipient, such as HEV and PCMV/PRV, should be eliminated

from pigs to be used in xenotransplantation. Pigs and islet cell preparations should be tested for both groups of viruses before performing comparative analyses concerning the optimal source (neonatal or adult pigs) and the optimal conditions for isolating the islet cells and they should be tested before transplantation.

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CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

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