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Examining the potential for porcine derived islet cells to harbour viral pathogens

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Abstract:

With an onus on safety in the potential use of porcine islet cells as a treatment for diabetes, the use of animals lacking exogenous pathogens is clearly important and multi-level screening strategies have been presented on testing animals and the product.

In this study, we wished to investigate whether islet cells indeed harboured the same viral pathogens of concern in the source animal. PMBC and islet cells from both adult and neonatal source animals were directly compared and tested for PCMV, PLHV, PCV2, PPV and HEV using both molecular and serological assays.

Adult PBMC were found positive for all viruses with the exception of PCV2 and HEV. Neonatal PBMC were only found positive for PCMV and HEV. All animals were found negative for HEV antibodies. Interestingly, islet cells were negative for all viruses tested regardless of status in the animal derived PBMC. Given that other laboratories have demonstrated the lack of virus detection during culture of islets, this study also demonstrates that the hygiene status of the herd may not reflect the status of the product. This is important for establishing guidelines for any risk evaluation and mitigation process utilised during product manufacture.

Introduction:

The risk of zoonotic disease transmission to human recipients during xenotransplantation is considered one of the barriers to xenotransplantation's transition to clinical practice[1]. Such risks can be difficult to eliminate, particularly in the case of Porcine Endogenous Retrovirus. However this risk may be reduced by the selection of low PERV expressing donor pigs or eliminated by the creation of PERV free pigs via genetic manipulation[2].The risk posed by exogenous pathogens can be minimised by thorough screening of donor animals from barrier reared herds[3]. However, it is clear that certain pathogens can be difficult to detect, particularly for viruses that form latent infections, are highly divergent or for which sensitive and specific assays are not available[4,5].

The encapsulation of porcine islets has been evaluated as a therapeutic modality for the treatment of diabetes and, despite the lack of evidence to date of the transmission of specific exogenous viruses to both animal models and humans, evaluation of the microbiological safety of porcine islets is still required. Indeed current methods have been evaluated[6].

It is clear that different cells, tissues and organs vary in their potential to harbour infectious pathogens and this variation may play an important factor in assessing the risk of zoonotic infection in a particular xenotransplantation trial[3,6]. A number of reports have characterised the pathogen status of pigs[7,8], however, whether an animal's pathogen profile obtained through blood screening is reflective of the pathogen profile of its tissues or organs has not been previously investigated. It is important to note that pathogens detected in the blood may not be present in the transplanted tissue and vice versa. Given the progression of porcine islet xenotransplantation towards clinical application, we felt it prudent to examine the potential for porcine islet cells to harbour exogenous pathogens in comparison to peripheral blood mononuclear cell (PBMC) samples from the same animal for several key viruses considered to have zoonotic potential[9]; Porcine Cytomegalovirus (PCMV), Porcine Parvovirus (PPV), Hepatitis E Virus (HEV), Porcine Lymphotropic Herpesvirus 1-3 (PLHV1-3) and Porcine Circovirus 2 (PCV-2).

Materials and Methods:

Pigs (Belgian landrace) were provided by Rattlerow-Seghers Genetics (Ooigem, Belgium). Piglets were directly delivered to the islet isolation facility in Brussels. Adult pigs were housed in the A. de Marbaix center (Louvain-la-Neuve, Belgium). All experiments were conducted in accordance with the local ethical committee and carried out in accordance to EU Directive 2010/63/EU for animal experiments. Two age category of animal were tested; neonatal (14-21days) and adult (1-2 years, 100-300kg). Neonatal animals had not yet undergone weaning from the sow. Blood samples were taken to prepare PBMCs. Briefly, 50 mL heparinized blood were centrifuged at room temperature over Histopaque 1077 from Sigma (Darmstadt, Germany). The opaque interface containing PBMCs was then aspirated, washed in PBS, pelleted and stored at -70°C. Pancreatic islet isolation was carried out as detailed elsewhere[10] using a method based on that described by Korbutt et al[11]. Collagenase V from Sigma was used for piglet pancreas digestion and collagenase NB8 from Serva (Heidelberg, Germany) was used for adult pancreas. We obtained 2087±151 islet equivalents (IEQ)/g pancreas (purity >75%) from neonatal piglets and 234±16 IEQ/g pancreas (purity >90%) from adult pigs (mean±SEM). Islet samples were taken after overnight culture in the case of adult islets and after 8 days in the case of neonatal islets. Corresponding serum samples were also collected from each animal and analysed using the swine HEV IgG ELISA (Wanti Beijing, Beijing, People's Republic of China). DNA was isolated from the PBMC and islet samples using the DNeasy mini kit (Qiagen, Crawley, UK). RNA was isolated from islet samples using the RNeasy mini kit (Qiagen, Crawley, UK). Viral RNA was isolated from serum using the Viral RNA mini kit (Qiagen, Crawley, UK). PCR assays were performed as previously described; PCMV[12], PLHV1[13], PLHV2[14], PLHV3[15], PCV2[16], PPV [17] and HEV [18], the sensitivity of these assays was determined to be 2copies, 10 copies, 2 copies, 10copies, 5copies, 5 copies and 2.5IU per reaction, respectively. For DNA virus detection, each reaction contained 250ng of DNA. All samples were assessed for integrity and RNA was validated in serum and confirmed without contamination as described previously[19]. All animals had received a

vaccination against mycoplasma (Stellamune, Elanco) at 14 days of age. Adult pigs had received a vaccination against; porcine parvovirus and Erysipelothrix rhusiopathiae (Porcilis ERY-PARVO (Intervet International)) at 6 months of age and again after each farrowing.

Results and discussion:

As indicated, adult and neonatal animals were screened for PCMV, PLHV, PCV, PPV and HEV utilising corresponding PBMC, serum and islet samples. Overall, none of the viruses were found in the islet samples despite their presence in the PBMC or serum samples analysed. PCV-2 was not found in any of the animals tested. PCMV was only detected in the PBMC and the remaining data is summarised in Table 1.

As shown in Table 1, no islet sample tested positive for any herpes virus, despite several animals testing positive in the PBMC for herpes viruses, ranging from 2.9% for PLHV2 to 38.1% for PLHV3. In humans, cases of acute pancreatitis associated with herpes viruses such as CMV[20], Varicella-Zoster virus (VZV)[21] and herpes simplex virus (HSV)[22] do occur but are rare, although the cell type affected within the pancreas is not known. However, several reports have examined CMV in immunosuppressed CMV negative patients receiving islet allografts from CMV positive donors and found a complete absence of CMV transmission in all recipients (n=12 and n=4)[23] supporting our data that islet cells do not commonly harbour herpes viruses.

Hepatitis E has also been associated with acute pancreatitis in humans[24], but as no islet sample tested positive for HEV, and only 1 serum sample tested positive for HEV, it is not possible to draw any conclusions regarding the potential for islet cells to harbour HEV. In addition, all animals were negative for antibodies to HEV.

No neonatal animals tested positive for PPV and only one adult pig tested positive for PPV, although adult animals were vaccinated for PPV. There are several possible explanations for this; possibly this pig was a vaccine non-responder, its immunity had waned since vaccination, the animal had recently been vaccinated and the PCR detected the attenuated vaccine strain or the vaccine the animal received did not protect against the strain the animal was infected with. All animals tested PCV2 negative, suggesting that the virus was not circulating in these herds.

There was a higher prevalence of herpes viruses in the adult population in comparison to the neonatal population. This is concurrent with increased risk of exposure to viruses with increasing age. This data also supports the suggestion of early weaning as a measure to reduce the risk of herpes transmission to neonates. The duration of islet culture following isolation varied between however, as no islet sample tested positive for any virus, it is not possible to determine if the period of culture after isolation could impact on the detection of contaminating viruses.

In conclusion, this data suggests that porcine islet cells do not harbour porcine herpes viruses; PCMV and PLHV1-3. Although further confirmation of this finding via in-vitro culture studies, to examine the permissibility of porcine islet cells to herpesvirus infection, may prove useful. However, despite having used the most sensitive assays available to us, we cannot exclude the possibility of low level infection of islet cells by herpes viruses, below the level of detection of our assays.

This study indicates that the risk of porcine islet pathogen contamination is low, regardless of age of harvest, and that, irrespective of the multi-level testing strategy, testing of the end product will be an important requirement. This report has identified an absence of correlation between the pathogen profiles of porcine PBMCs and islets, indicating that product screening in xenotransplantation, as opposed to ante mortem donor screening, provides a safer and more reliable approach. Updates to current regulations have been suggested[6,25] and information on the relevance of viral pathogens in specific xenotransplant products will aid to advise this.

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