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REVIEW ARTICLE

Porcine cytomegalovirus/porcine roseolovirus (PCMV/PRV): A threat for xenotransplantation?

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Abstract

The potential for a donor-derived transmission of porcine cytomegalovirus/porcine roseolovirus (PCMV/PRV) to the recipient has been recognized since pigs were considered candidate donors for xenotransplantation. This review gives a short description of the viral properties and summarizes the current evidence of the effects of PCMV/PRV transmission in preclinical xenotransplantation. Despite evidence that PCMV/PRV does not infect human and non-human primate cells, activation in the transplanted organ and detrimental systemic complications have been described. As PCMV/PRV is a herpesvirus able to establish latency, the importance of adequate screening of donor pigs is emphasized, as no efficient treatment is available. Furthermore, easy and successful ways of elimination of PCMV/PRV from pig herds are indicated.

KEYWORDS
herpes viruses, PCR methods, porcine cytomegalovirus, porcine roseolovirus, Western blot analysis, xenotransplantation

1 | VIRUS CHARACTERIZATION

The porcine cytomegalovirus (PCMV) is actually not a cytomegalovirus in the genus *cytomegalovirus* of the *Herpesviridae* family, but a porcine roseolovirus (PRV) belonging to the genus *Roseolovirus*.[1,2] In order to underline this, we will call it PCMV/PRV. The official name given by the International Committee on Taxonomy of Viruses (ITCV) is suid betaherpesvirus 2 (SuBHV2), indicating that it belongs to the subfamily *Betaherpesvirinae*.[3] The naming certainly was partially based on the appearance of cytomegalic cells with characteristic basophilic intranuclear inclusion bodies in the mucosal glands of turbinates of pigs,[4] but first sequence comparisons made it clear that PCMV/PRV is closely related to the human roseoloviruses herpes virus-6 (HHV-6) and HHV-7.[1] HHV-6A, HHV-6B, and HHV-7 are ubiquitous in the human population, with over 90% of the human population infected within the first 3 years of life in the case of HHV-6B. HHV-6A occurs more frequently in the immunocompromised host, in contrast, HHV-6B is the etiologic agent of the childhood illness exanthema subitem (roseola infantum).[5] Like HHV-6A, HHV-6B and HHV-7 and the murine roseolovirus (MRV), PCMV/PRV is also widely distributed, even in breeding colonies used for xenotransplantation over 95% of the animals were PCMV/PRV-positive.[6] The virus has a linear double-stranded DNA genome 12837 bp long and containing 79 open reading frames (ORFs).[1] Of these ORFs, 69 have counterparts in HHV-6A, HHV-6B, and HHV-7, and two ORFs are homologous to other members in the subfamily *Betaherpesvirinae*.[1] The genome is a Direct repeat (DR)—unique (U)—DR type, similar to HHV-6A, HHV-6B, and HHV-7, but the PCMV/PRV DR is shorter and lacks predicted genes and telomere-like sequences. The telomere-like

sequences are used by HHV-6 to incorporate into the human genome.[7] The PCMV/PRV particle is enveloped, with a diameter of 150–200 nm. The morphogenesis of PCMV/PRV is marked by intranuclear formation of nucleocapsids arranged in crystalline arrays and the outer envelope of cytoplasmic and extracellular particles had projections and showed a clear unit membrane structure when examined by electron microscopy in the nasal mucosa of piglets and in lung cultures[8,9]

2 | PREVALENCE AND PATHOLOGY

PCMV/PRV is ubiquitously found in wild and domestic swine. A recent study detected PCMV/PRV in 5–82% of wild boars in Italy and Germany, when both PCR- and Western blot-based assays were applied.[10] PCMV/PRV has repeatedly been found in herds specifically bred for xenotransplantation purposes.[11] The natural course of infection is believed to be an early infection, likely after weaning of protection by maternal immunization.[12] The clinical picture is mild, recovery is the rule. An exception are pigs born to PCMV/PRV-naive mothers, when intrauterine or early infection (first 5 weeks of life) occur. Here, a more severe course has been described, with clinical symptoms ranging from signs of respiratory diseases to death.[12] Despite this potential for a severe disease course, PCMV/PRV has never been in the focus of commercial swine rearing, as losses are small. Usually, a herd immunity develops, and infection is not clinical apparent.

3 | LATENCY

Like most herpesviruses PCMV/PRV establishes a lifelong latency in infected pigs, which can be reactivated, for example, by stress.[13] During latency the viral DNA of herpesviruses is stably maintained in the nucleus of the infected cells as multiple copies of circular chromosomes. Herpesviruses that maintain latency in dividing cells express viral proteins to guarantee that episomes will be partitioned to daughter cells. The virus must limit protein expression in latently infected cells to avoid detection by the immune system. Many herpesviruses express long noncoding viral RNA that contribute to the establishment of latency. Herpesvirus genomes are associated with cellular histones to regulate gene expression and avoid damage by host proteins.[13] In addition, viral microRNAs (miRNA) are expressed during latency to inhibit expression of lytic genes. Whereas these processes are well studied for some human herpesviruses such as varizella zooster virus (VZV, human herpesvirus 3, HHV-3) and HCMV, they are mainly unknown for HHV-6, HHV-7, and PCMV/PRV. Reactivation allows herpesviruses to be transmitted, reactivation happens usually during periods of extreme stress.[13] HCMV reactivation can be induced by stimulation with allogenic cells, which partially explains the reactivation during transplantation.[14] Reactivation of PCMV/PRV through allogenic stimulation [15] and incubation of pig peripheral blood mononuclear cells (PBMCs) in culture medium [16] was described.

4 | DETECTION METHODS

The traditional histology-based diagnosis looking at inclusion bodies (hence the initial name inclusion body rhinitis), has since been replaced by direct virus detection methods based on PCR-based assays. They are very reliable in a disease setting, and can be performed in any laboratory familiar with PCR techniques.[6,17,18] Detection of PCMV/PRV in non-invasively taken samples from piglets is also possible.[19] Despite their high specificity and sensitivity, they can however yield negative results if used for screening, given that replication is usually absent in the latent phase and the virus is hiding in some organs such as the spleen. Therefore, serological detection methods must supplement any screening effort.[20] Developing such assays is more demanding, and validation quite complex. So far, such serological detection methods have been developed in the context of research projects and are not widely available nor easy to implement. A recent paper highlights the importance of the combined use of the two methods.[21] As the disease never has been perceived to be a major danger for commercial breading, the interest to develop widely available and validated assays has been very limited. The recent transmission of PCMV/PRV to the first patient transplanted with a pig heart,[3] but also the previous transplantations of pig hearts and kidneys into non-human primates [22–24] have clearly shown the need to reliably diagnose PCMV/PRV.

5 | PCMV/PRV IN THE IMMUNOSUPPRESSED SETTING: PRECLINICAL XENOTRANSPLANTATION

The potential for zoonotic infections through transmission of porcine pathogens has been recognized since many years.[25,26] Latent viruses, and those present in the genome, such as porcine endogenous retroviruses (PERVs), are of special interest, as they are transmitted by default with the transplanted organ, which in turn is then exposed to an immunosuppressive setting and an immunological naïve environment. The potential harm inflicted by PCMV/PRV is evident from a series of studies of preclinical xenotransplantation. The first report showed activation of PCMV/PRV and baboon cytomegalovirus (BCMV) in pig-to primate organ kidney xenotransplantation.[6] PCMV/PRV DNAemia was detected in recipient baboon tissue and particularly in the transplanted porcine graft tissues. Consumptive coagulopathy observed in pig-to baboon kidney xenotransplantation was at least partially connected to PCMV/PRV, as an increase in porcine tissue factor was seen in a model of primary porcine aortic endothelial cells infected in vitro with PCMV/PRV.[27] Improved graft survival was achieved by early weaning of piglets, which eliminated PCMV/PRV.[28] The most compelling evidence on the role of PCMV/PRV was recently published by in 2020.[22] This study demonstrated the deleterious effect of PCMV/PRV an orthotopic heart transplantation model. In baboon recipients of such an organ, IL-6 and TNF-α were increased and tPA-PAI-1 complexes circulated in high concentrations in the recipients, suggesting interference with the cytokine and coagulation system.

6 | ROLE OF PCMV/PRV IN THE FIRST PIG-TO-HUMAN HEART XENOTRANSPLANTATION

The detection of PCMV/PRV in the blood of the first human recipient of a pig heart transplanted by the team of the University of Maryland at Baltimore has rekindled the interest in this zoonosis.[3] The initial success was followed by a deterioration of the patient ultimately resulting in the demise. It is important to note that the exact role of PCMV/PRV is unclear, and hopefully additional studies will shed some light on the specific contribution of PCMV/PRV to the course of the patient. Given the limited data so far, all discussion on the cause (or causes) remain speculative.[29–31] However, the clinical features described in the patient resemble the features observed in the baboons, which received a PCMV/PRV-positive heart.[22] This pioneering work, despite the outcome, did however yield some important insights for the management of PCMV/PRV. Testing by PCR only does not reliably exclude donors latently infected. Screening has to be complemented by serological assays. Elimination as described in chapter 8 is feasible, but is a challenge in the context of rearing genetically-modified pigs since it may be possible that PCMV/PRV is introduced by somatic cell nuclear transfer (SCNT).[32]

7 | ANTIVIRALS AND ANTISERUM

There are effective antiviral drugs against the HCMV such as ganciclovir (GCV) and acyclovir. PCMV/PRV was relatively resistant to GCV and acyclovir, only cidofovir and foscarnet had a therapeutic efficacy for PCMV/PRV in vivo in achievable concentrations,[33] although these agents often carry significant toxicity in transplant recipients, GCV and other antiviral agents have limited activities against PCMV/PRV in vitro.[33] There is no vaccine against PCMV/PRV. Due to the sequence homology with HHV-6, there are cross-reacting antibodies against the glycoproteins B (gB) of PCMV in healthy humans.[34] Whether these antibodies protect humans from PCMV/PRV infections is unclear.

8 | ELIMINATION BY EARLY WEANING AND OTHER METHODS

PCMV/PRV can be easily eliminated from a pig herd by early weaning.[28,35,36] This means the piglets were not allowed to suck milk from the infected mother. In the most recent experiment, piglets were early-weaned at the age of 24 hours and transferred to a commercially available Rescue Deck system dedicated to motherless rearing of piglets. Sows were removed from the facility. The PCMV/PRV status of F1-generation animals was determined at regular intervals over a period of 14 months by a sensitive real-time PCR-based detection method testing blood, nasal swabs and cultured PBMCs.[36] No transmission was observed by the colostrum. Although transmission of the virus via the placenta was reported in an experimental setting,[37,38] this was not seen under natural conditions.[11] However, if the early weaning is not effective, piglets can be treated by colostrum deprivation or even Caesarean section or embryo transfer to avoid virus transmission.[2]

9 | CONCLUSION

The transmission of the porcine herpes virus PCMV/PRV during the first transplantation of a pig heart into a patient and previous transmissions of PCMV/PRV in preclinical trials transplanting pig organs into non-human primates, which were associated with a significant reduction of the survival time, indicated that PCMV/PRV may pose a risk for clinical xenotransplantation. However, there are effective methods to detect the virus in the donor pigs even when it is in its latent stage and there are strategies how to eliminate the virus from pig breeds, for example by early weaning. Based on this, future clinical trials can be performed safely. This requires the involvement of experienced virologists in the study design and FDA and EMEA have to make sure that the appropriate virus screening methods will to be used.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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