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REVIEW ARTICLE

Mouse models of porcine circovirus 2 infection

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Abstract

PCV2 is considered the main pathogen of porcine circovirus diseases and porcine circovirus-associated diseases (PCVD/PCVAD). However, the exact mechanism underlying PCVD/PCVAD is currently unknown. Mouse models of PCV2 are valuable experimental tools that can shed light on the pathogenesis of infection and will enable the evaluation of antiviral agents and vaccine candidates. In this review, we discuss the current state of knowledge of mouse models used in PCV2 research that has been performed to date, highlighting their strengths and limitations, as well as prospects for future PCV2 studies.

KEYWORDS
animal model, mouse (*Mus musculus*), porcine circovirus 2 (PCV2)

1 | INTRODUCTION

Porcine circovirus (PCV) belongs to the genus *Circovirus* of the family Circoviridae and contains a single-stranded 1.7-kb circular DNA.[1-4] There are two types of PCV: porcine circovirus type 1 (PCV1) and porcine circovirus type 2 (PCV2). PCV1 is nonpathogenic, whereas PCV2 is considered the main pathogen of porcine circovirus diseases and porcine circovirus-associated diseases (PCVD/PCVAD), including a number of different syndromes and diseases in pigs, such as post-weaning multisystemic wasting syndrome (PMWS), porcine respiratory disease complex (PRDC), reproductive failure, granulomatous enteritis, necrotizing lymphadenitis, exudative epidermitis, and congenital tremor.[1,4-6] Furthermore, many of the syndromes associated with PCVD/PCVAD are a result of coinfection with PCV2 and other swine pathogens, such as porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), *Mycoplasma hyopneumoniae*, bacterial septicemia or pneumonia, and swine influenza virus (SIV).[4,7-9]

To date, the exact mechanism of PCVD/PCVAD is currently unknown, and there are no approved effective therapeutics for PCV2 infection. Although several commercial vaccines based on PCV2a are effective in protecting pigs against challenge with PCV2a,[9] they cannot protect pigs against the PCV2b genotype that is prevalent worldwide, as well as other PCV2 genotypes. Moreover, some PCV2-infected pigs can develop severe disease while many pigs in the same herd and farm remain asymptomatic. Therefore, an in vivo infection model is critical for understanding the pathogenesis during PCV2 infection and coinfection with other swine pathogens.

The mouse has been widely used as an infection model to elucidate the in vivo behaviors of virus-host interactions.[10-12] Although some research groups have reported that mouse models provide only limited utility in advancing the understanding of PCVD/PCVAD[13-15] and that the ORF3 protein has very limited pathogenicity in its primary host,[16] mouse models are useful for the study of cellular responses to PCV2 in the context of an animal host. The purpose of this review is to discuss the current state of knowledge

of mouse models used in PCV2 research that has been performed to date, highlighting their strengths and limitations as well as prospects for future PCV2 studies.

2 | PATHOGENESIS OF PCV2 IN MICE

PCV2 has been shown to replicate and spread in BALB/c[14,17-19] and Kunming mice.[20,21] PCV2 nucleic acids can be detected in lymphoid tissues, the liver, epithelial cells, and the thymus.[17] Furthermore, the virus can be transmitted directly from mouse to mouse by contact, and it causes vertical infection through the placenta.[20,22-24] Microscopic lesions in PCV2-infected mice are characterized by the expansion of germinal centers in lymphoid organs, with large numbers of histiocytic cells and lymphoblasts, apoptosis of histiocytic cells in germinal centers, and mild lymphoid depletion of the paracortex.[17] Moreover, PCV2 can also cause lesions of spermatocytes and oocytes prior to zygote formation; Leydig cells in the testes and granulosa cells in the ovaries were degenerated, and a small number of spermatocytes and oocytes underwent apoptosis.[25]

It was reported that the ORF3 protein of PCV2 is critical in viral pathogenesis and apoptosis in vitro.[4] Using a mouse model, the role of the ORF3 protein in viral pathogenesis were evaluated in vivo. The results showed that the ORF3 protein plays an important role in viral pathogenesis.[26] Furthermore, ORF3 expedites the spread of the virus by inducing the early release of the virus from the infected cells.[27] ORF3 protein activates caspases 8 and 3 by interacting with pPirh2, resulting in apoptosis in the spleens of infected mice.[19,26,28,29] ORF3-induced apoptosis also aids in recruiting macrophages to phagocytose the infected apoptotic cells, leading to the systemic dissemination of the infection in PCV2-infected mice, which promotes the spread of the virus.[27]

3 | IMMUNOLOGY OF PCV2 IN MICE

Compared with control mice, PCV2 infection significantly enhanced TNF-α secretion and markedly decreased IFN-α secretion.[30] $MHCII^+$ $CD40^-$ and $CD137L^-$ $CD80^+/CD86^+$ DCs increased significantly in PCV2-infected mouse spleens.[30] Secretion of IFN-γ by $CD4^+$ and $CD8^+$ T cells and of IL-12 by $CD8^+$ T cells was significantly lower in PCV2-infected mice, while secretion of IL-4 by $CD4^+$ T cells was remarkably higher.[30] These results indicate that PCV2 modulates cytokine secretion and costimulatory molecule expression by DCs and alters the activation of $CD4^+$ and $CD8^+$ T cells by DCs, which might be related to the host's immune dysfunction and persistent infection with PCV2.[30] Moreover, the Rep and ORF3 proteins of PCV2 may interfere with the cellular, humoral, and protective immunity of the Cap protein in vivo.[31] ORF4 from PCV2 is not essential for the viral replication but inhibits caspase activity and regulates $CD4^+$ and $CD8^+$ T lymphocytes during viral infection in mice.[32] In contrast, the relative proportions of $CD4^+$ and $CD8^+$ T cells were more greatly decreased in ORF4-deficient PCV2-infected mice compared with wild-type PCV2-infected mice.[33]

CD44 is a widely expressed class I transmembrane glycoprotein in immunological and inflammatory responses.[34] Upon infection with PCV2, the CD44 mRNA level in the lung tissue was upregulated, while CD44 deficiency resulted in decreased proinflammatory cytokine production in lungs of the PCV2-infected mice, suggesting that CD44 plays a role in the development of the pneumonia response to PCV2 infection.[34]

4 | VACCINE EVALUATION IN MICE

PCV2 is divided into 5 genotypes according to the Cap gene sequence: PCV2a, b, c, d, and e.[35] Additionally, genotype PCV2b is subclassified into 3 clusters, 1A-1C, and genotype PCV2a is subdivided into 5 clusters, 2A-2E.[35-37] Although vaccination is the main method to prevent and control PCV2, few vaccines are available for these genotypes. Therefore, it is urgent to develop effective PCV2 vaccines.

Cap protein, encoded by the ORF2 gene of PCV2, is the only structural protein of PCV2, and thus it contains the main antigenic determinant of the virus.[4] Thus, recombinant Cap protein expressed in baculovirus/insect cell, yeast, adenovirus and *Escherichia coli* expression systems were purified and injected into mice to produce antibodies against PCV2. Importantly, mice inoculated with secreted Cap developed a significantly higher level of neutralizing antibodies.[38] Furthermore, a transcriptional enhancer element consisting of the largest intron of the human cytomegalovirus (Intron A) and woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) significantly improved the expression of the Cap protein in an adenovirus vector system and enhanced the immune responses in mice.[39] Moreover, the flagellin-Cap fusion protein elicited a stronger PCV2-specific IgG antibody response, higher neutralizing antibody levels, milder histopathological changes and lower viremia, as well as increased secretion of cytokines such as TNF-α and IFN-γ that conferred better protection against PCV2 challenge than those in the mice inoculated with recombinant Cap alone.[40] Additionally, the efficacy of a DNA vaccine expressing Cap protein can be improved by the simultaneous expression of porcine cytokines such as IL-6, IL-15, IL-2, GM-CSF, and IFN-γ, resulting in significantly higher humoral and cellular immune responses in mice.[41-44]

Virus-like particles (VLPs) constitute versatile tools in vaccine development due to their favorable immunological characteristics, such as their size, repetitive surface geometry, and ability to induce both innate and adaptive immune responses.[45] The full-length PCV2 Cap protein expressed in *E. coli* can self-assemble into VLPs 25-30 nm in diameter that can stimulate specific immune responses to PCV2 in mice.[46] Aguilera and colleagues constructed chimeric VLPs using papaya ringspot virus (PRSV) as an epitope presentation scaffold.[47] They found that the chimeric VLPs induced high levels of immunoglobulin G against PCV2 epitopes in immunized BALB/c mice.[47] Furthermore, VLPs can be used as transfer vehicles carrying

foreign proteins or antigenic epitopes to produce chimeric VLPs.[48] The GP5 epitope B from PRRSV was inserted into loop CD of the PCV2 Cap, resulting in chimeric PCV2 VLPs.[48] The results showed that chimeric PCV2 VLPs induced strong humoral (neutralizing antibodies against PCV2 and PRRSV) and cellular immune responses in mice.[48] The somatostatin (SS) gene was fused to the 3′-terminal of the Cap protein, self-assembled into VLPs in Sf9 cells, and immunized into mice, followed by challenge with PCV2.[49] The results demonstrated that body weight gain and anti-SS antibody in the rCap-SS group was obviously higher than that of the control group 28 and 42 days postinoculation (dpi).[49]

In addition to vaccines, different adjuvants have also been investigated for the production of antibodies from PCV2 vaccine-immunized mice. Inactivated PCV2 vaccine conjugated with chitosan oligosaccharide, *Lycium barbarum* polysaccharides or *Epimedium* polysaccharide-propolis flavone liposomes can remarkably enhance both humoral and cellular immunity against PCV2 by promoting T lymphocyte proliferation, initiating a Th1/Th2 response, and increasing the production of PCV-2-specific antibodies and the secretion of inflammatory cytokines in mice.[50-52] These natural polysaccharides from plant, bacterial, yeast, and synthetic sources are safer and biodegradable, without the tissue deposits observed for aluminum adjuvants.[53] Moreover, adjuvant cytokines or DNA, such as porcine CD40 ligand (CD40L), granulocyte-macrophage colony-stimulating factor (GM-CSF), ubiquitin, the N-terminus of porcine heat shock protein Gp96, and a CpG motif could also significantly enhance humoral immune responses, PCV2-specific antibody titer, and neutralizing activities in mice.[54-57] Additionally, a CpG motif can also reduce immune organ damage in mice,[55] while CD40L and GM-CSF could synergistically enhance the protective immune responses of PCV2 adenovirus vaccine.[54]

5 | EVALUATING PROTECTIVE CHEMICALS AND ANTIVIRAL AGENTS IN MICE

To determine whether dietary supplementation with protective chemicals or antiviral agents can offer protection against virus infection, mice were treated with different chemicals or antivirals, followed by PCV2 infection. The results showed that dietary supplementation with aluminosilicate,[58] selenium yeast,[59] arginine,[60] proline,[61] and L-glutamine[62,63] can enhance the immune system and confer mice with antiviral protection against PCV2.[4] Moreover, dietary supplementation with arginine, L-glutamine, and proline can also improve pregnancy outcomes in PCV2-infected mice.[4,61-63]

Previously, we found that statin, an inhibitor of HMG CoA reductase (HMGCR), significantly stimulated PCV2 replication in vitro.[64] Using the mouse models, we further evaluated the effect of statin on PCV2 infection in mice. The results showed that mice treated with atorvastatin during PCV2 infection had reduced body weights.[18] PCV2 antigens were mainly immunolocalized to the cytoplasms and plasma membranes of cells in the lymph nodes of PCV2-inoculated mice.[18] These results further confirmed that HMGCR is negatively associated with PCV2 infection.[18,64] Furthermore, CD44 has been reported to play an antiviral role in response to PCV2 infection.[34] Astragalus polysaccharide (APS) treatments can reduce the pathological injury of tissues, inhibit PCV2 infection and decrease glucose-regulated protein 78 and GADD153/CHOP gene mRNA and protein expression significantly by inhibiting endoplasmic reticulum stress.[65] Additionally, intraperitoneal injection of 200 μg/kg arctigenin (ACT) significantly inhibited PCV2 proliferation in the lungs, spleens, and inguinal lymph nodes of mice, demonstrating the effectiveness of ACT as an antiviral agent against PCV2 in vivo.[66] 17-Dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG), an inhibitor of Hsp90, is highly effective in suppressing PCV2 replication in BALB/c mice.[67] Nitric oxide-generating compound *S*-nitrosoglutathione (GSNO) treatment reduced the percentages of PCV2-positive serum and tissue samples, as well as the viral DNA copies in the serum samples, indicating the reduction of PCV2 infection progression in mice.[68] GSNO also improved the growth performance and immune organs (spleens and thymuses) of the PCV2-infected mice.[68] *Lactobacillus reuteri* significantly decreased the amount of PCV2 in the feces and in the ileum in mice, upregulated the gene expression of chemokines, interferon (IFN)-γ, IgA and PIgR in the ileum, and significantly increased the percentage of $CD19^+$ lymphocytes in the mesenteric lymph nodes and natural killer cells, indicating that probiotic *L. reuteri* has an antiviral effect on PCV2 in the intestine via stimulation of the local gut immune response.[69] Therefore, these compounds may have the potential to serve as drugs for protection of pigs against PCV2 infection.[66]

PCV2 infection leads to a significant decrease in the thymus and spleen indices, elevation of xanthine oxidase (XOD) and myeloperoxidase (MPO) activities, reduction of the glutathione (GSH) level and GSH to oxidized glutathione (GSSG) ratio, and decreased superoxide dismutase (SOD) activity, indicating the formation of immunosuppression and oxidative stress.[70] It has been reported that oxidative stress plays an important role in the pathogenesis of virus infection, and antioxidants are becoming promising candidates as therapeutic agents.[70] Total flavonoids of *Spatholobus suberectus* Dunn (TFSD) treatment recovered the alteration of the viscera index, antioxidant content and activities of oxidative-associated enzymes to a level similar to controls.[70] Carboxymethylpachymaran (CMP) can significantly improve the spleen or thymus index, promote the proliferation of T or B lymphocytes, and increase the production of glutathione, the superoxidase dismutase capacity, and the total antioxidant capacity in the spleen or thymus of PCV2-infected mice.[71] Treatment of PVC2-inoculated mice with CMP resulted in the upregulation of IL-2 and IFN-α or the downregulation of IL-10 levels in the serum, suggesting that CMP has potential applications in regulating immunological functions to overcome the immunosuppression caused by PCV2 infection in mice.[71] Therefore, the critical role of these chemicals against PCV2 infection is to regulate immune function and inhibit oxidative stress in mice.[66-68,70,71]

6 | CONCLUSIONS AND FUTURE PERSPECTIVES

Some groups have reported that infection of mice with PCV2 results in different diseases that vary in severity depending on the virus dose and viral genotype, mouse strain, and age, indicating differences in the pathogenesis of PCV2, as well as innate and adaptive immune response, between pigs (the natural host) and mice (an unnatural animal model).[6,14,22,72] However, mouse models might be useful for antiviral agent and vaccine research due to the commercial availability of mouse-specific immunological reagents and the ease of genetic manipulation in mice. Furthermore, mouse-adapted virus strains may improve our understanding of the pathogenesis and mechanisms of immunity to PCV2.

In many cases, PCV2 evokes a subclinical infection, without any obvious symptoms, in pigs.[73] PCVD/PCVAD and PCV2 infection are often accelerated by concurrent viral or bacterial infections.[72] However, the mechanisms involved are poorly understood. Therefore, mouse models for coinfections of PCV2 with other swine pathogens still need more research.

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ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

XHL and TO wrote the original draft of the manuscript. LZR and HSO revised the manuscript and act as guarantors. All authors critically read and contributed to the manuscript, approving its final version.

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