Cell tropism and entry of porcine circovirus 2

Debanjan Saha

Virus research

Virus Research 164 (2012) 43–45

Contents lists available at SciVerse ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Review

Cell tropism and entry of porcine circovirus 2

H.J. Nauwynck[a,*], R. Sanchez[b], P. Meerts[c], D.J. Lefebvre[d], D. Saha[a], L. Huang[a], G. Misinzo[e]

[a] Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[b] College of Veterinary Medicine, University of the Philippines Los Banos, Laguna 4031, Philippines
[c] Ablynx nv, Technologiepark 21, 9052 Zwijnaarde, Belgium
[d] CODA-CERVA, Groeselenberg 99, 1180 Ukkel, Belgium
[e] Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, Sokoine University of Agriculture, Morogoro, Tanzania

ARTICLE INFO

Article history:
Available online 11 November 2011

Keywords:
Circovirus
Tropism
Entry
Reproductive failure
PMWS
PCVAD

ABSTRACT

Porcine circovirus 2 (PCV2) may induce reproductive failure (return to oestrus, embryonic death, mummification, weak- and stillborn piglets) and postweaning multisystemic wasting syndrome (PMWS). Furthermore, it may modulate the immunity in such a way that it aggravates the outcome of many bacterial and viral infections. In the present paper, the cellular tropism and entry of PCV2 are described and linked with the pathological and clinical consequences.

Contents

1. PCV2 target organs and cells

Porcine circovirus 2 infections may occur in swine prenatally at different stages of embryonic and foetal development and postnatally at different ages, resulting in variable outcomes (Segales et al., 2005). Although the embryonic cells are from the very beginning susceptible, embryos are resistant to infection as long as they are inside the zona pellucida (Mateusen et al., 2004). After hatching, this barrier disappears and the embryos may become infected. The general infection of all embryonic cells seems to be the result of an absence of a defence mechanism within these embryos. The extensive replication in embryos leads to death and resorption in utero (Mateusen et al., 2007). As a result of the embryonic death, the sow will return to oestrus. In young foetuses of 40–70 days of gestation, the virus replicates mainly in the heart, followed by liver, lymphoid organs and lungs (Sanchez et al., 2001; Saha et al., 2010). The main target cells at that stage are cardiomyocytes, hepatocytes and cells of the monocytic lineage (mø) (Sanchez et al., 2003). Replication in the heart results in heart failure and death of the foetus. This foetus will mummify (Pensaert et al., 2004). Upon infection at later stages of foetal development, the replication decreases considerably with increasing age of the foetus. Two important explanations for this finding are the presence of an adaptive humoral immune response starting in pigs from 70 days of gestation and the reduction of mitosis rate during the progress of gestation. Indeed, circoviruses fully depend on cellular polymerases for their replication (Gassmann et al., 1988). Postnatally, PCV2 loses its tropism for heart cells. It mainly focuses on lymphoblasts and mø. Mø are mainly taking up virus particles but this rarely leads to productive viral infection. In contrast, lymphoblasts are fully susceptible targets (Sanchez et al., 2004; Lefebvre et al., 2008b)). The larger the number of blasts, the faster and higher the primary replication of PCV2 is in its host. This induces automatically a quick immune response. High blastogenesis activity is present after vaccination and during co-infections

* Corresponding author. Tel.: +32 9 264 7373; fax: +32 9 264 7495.
E-mail address: hans.nauwynck@ugent.be (H.J. Nauwynck).

0168-1702/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2011.11.003

with other pathogens and may in part explain why postweaning multisystemic wasting syndrome (PMWS) or severe systemic PCV-associated disease (PCVAD) can more easily be reproduced in these immune-stimulated animals (Allan et al., 2004). This has also been reproduced by a general aspecific T-lymphocyte blastogenesis by Concanavalin A (Lefebvre et al., 2008b). In a more sterile or low-microbial environment (e.g. gnotobiotic pigs, SPF, high health status), the virus has much more difficulties to grow and only during the activation of the immunity from 7 days post-infection onwards, the virus is helped to replicate by the presence of T- and B-lymphoblasts. Only in lymphoid tissues where PCV2 grows to high virus titers (follicles of lymph nodes and periarteriolar lymphocyte sheaths in spleen), lymphocyte depletion can be found, in part due to lysis of infected cells (Sanchez et al., 2004). Monocytes infiltrate into the lymphocyte-depleted areas to clear the virus and cell debris. However, they have problems to do so. The conditions that decide why some pigs develop PMWS is up till now not elucidated.

2. Binding of PCV2 to its target cell (Fig. 1)

In general, primary virus attachment often involves interactions with glycosaminoglycans (GAGs), like heparan sulphate or other carbohydrate structures such as sialic acids on the cell surface (Marsh and Helenius, 2006). On the basis of attachment competition experiments with GAGs, glycosidase digestion, affinity chromatography and infection experiments using wild type and mutant CHO-derived cells lacking either heparan sulphate or all GAGs, it was shown that heparan sulphate and chondroitin sulphate B are PCV2 attachment receptors on host cells (Misinzo et al., 2006). These two GAGs are largely linked to glycoproteins anchored in the plasma membrane of all cell types. PCV2 sticks in large amounts to cells reaching saturation already at 30 min post-incubation (Misinzo et al., 2005, 2009). Analysis of the amino acid sequence of the PCV2 capsid protein revealed a putative heparin-binding motif (^{98}IRKVKV^{103}). Because this motif is not located on the surface of the capsid protein, its role as a binding region is questioned (Khayat et al., 2011). Other more exposed regions with lysine and arginine residues may be more suitable targets (Esko, 1999).

3. Internalization of PCV2 in its target cell (Fig. 1)

Internalization of PCV2 via endocytosis is very inefficient and the kinetics and mechanism completely depend on the host cell type. In the monocytic cell line 3D4/31, internalization is found in only 50% of the cells and the uptake of particles takes hours (70% of the particles at 3 h). The process is clathrin dependent (Misinzo et al., 2005). In the epithelial cell lines PK15, SK and ST, the virus may follow two internalization pathways: a clathrin-mediated one and a dynamin-and cholesterol-independent, but actin- and small GTPase-dependent one. The former pathway does not lead to a full infection, while the latter does. In monocytes, macrophages and monocytic-derived and bone marrow-derived dendritic cells, PCV2 enters the cell but the precise mechanism is not characterized (Misinzo, unpublished results; Meerts et al., 2005a,b; Steiner et al., 2008; Vincent et al., 2003).

4. Disassembly of PCV2 in its target cell (Fig. 1)

Just like the internalization, the disassembly process of PCV2 fully depends on the host cell used. In the monocytic cell line 3D4/31, PCV2 is released from the endosome after acidification of the endosomes (Misinzo et al., 2005). In the epithelial cell lines PK15, SK and ST and primary porcine kidney cells, the opposite was found since blocking the pH drop enhances PCV2 replication. By using protease inhibitors, it could clearly be shown that

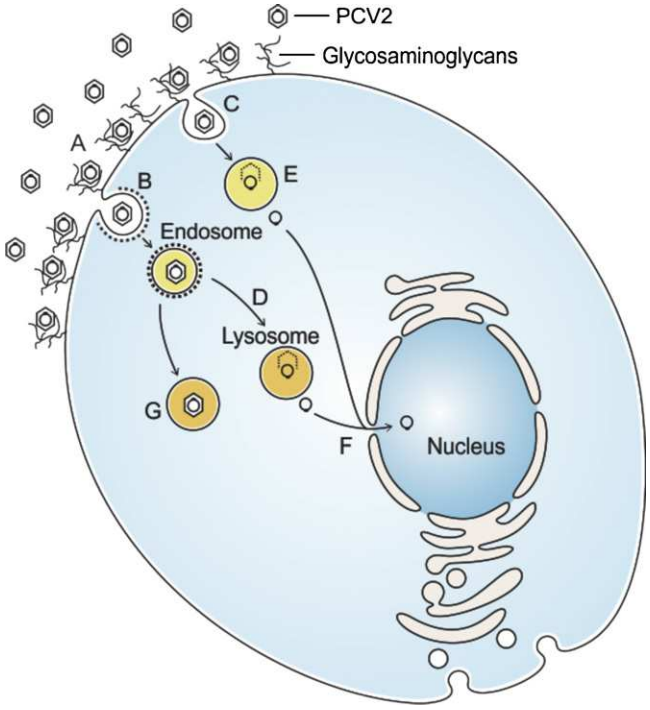

Fig. 1. Attachment, internalization and uncoating of porcine circovirus 2 (PCV2) in target cells. PCV2 attaches to the cell surface of all cell types via glycosaminoglycan receptors (heparan sulphate and chondroitin sulphate B) (A). PCV2 is slowly internalized via clathrin-mediated endocytosis in epithelial cells and cells of the monocytic cell line 3D4/31 (B). In epithelial cells, PCV2 is in addition internalized via a clathrin-, caveolae-, and dynamin-independent small GTPase-regulated pathway (C). The latter leads to a more efficient PCV2 replication while the former seems to trap PCV2 leading to accumulation of the virus within epithelial cells. Inhibition of the endosomal/lysosomal system acidification reduces PCV2 infection of cells of the monocytic cell line 3D4/31, indicating the requirement for a pH drop during PCV2 replication (D). In contrast, inhibiting the pH drop highly increases PCV2 replication in epithelial cells, indicating that uncoating occurs at low pH (E). Serine proteases mediate PCV2 uncoating in both epithelial cells and cells of the monocytic cell line 3D4/31. It is presumed that the serine protease(s), that cleave PCV2 in epithelial cells work optimally at a neutral pH while in cells of the monocyte/macrophage lineage an acidic pH offers the optimal activity. PCV2 transport into the nucleus is not yet determined (F). PCV2 has also been shown to accumulate within monocytes, macrophages and dendritic cells without uncoating (G).

serine proteases are involved in the disassembly of the virus and not aspartyl, cysteine and metalloproteases (Misinzo et al., 2008a). In monocytes, macrophages and monocytic-derived and bone marrow-derived DCs, disassembly is hampered (Misinzo, unpublished results; Vincent et al., 2003). In one way or another the virus escapes from the strong breakdown mechanisms that are present in these cells. Because in different cell types, different pH conditions are important, we believe that different proteases are involved in epithelial cells (active at neutral pH) and the monocytic cell line (active at low pH). Why the PCV2 is not disassembled in porcine mø both in vitro and in vivo is not yet elucidated.

5. Some considerations on PCV2 entry

In general, one can conclude that PCV2 like most viruses binds to sugars (heparan sulphate and chondroitin sulphate B) present on the extracellular domains of plasma membrane anchored glycoproteins. However, in contrast to most other viruses it does not enter via a specific receptor associated with fast internalization. In a viral world of the fittest, this seems somewhat exceptional. Therefore, we believe that the glycoprotein that carries heparan sulphate or chondroitin sulphate B is not only mediating the binding but also the internalization. The unstable binding of virus particles to GAGs

may explain in part the inefficient entry. Recent genetic changes leading to small antigenic changes, detected by monoclonal antibodies, may represent the search for an ideal receptor (Lefebvre et al., 2008a; Saha et al., 2011). The intriguing finding that cholesterol depletion and treatments with IFNα and IFNγ augment the number of PCV2-infected cells in cells in vitro, suggests that they are able to influence the entry mechanism of PCV2. Because this may have in vivo relevance, further studies are required to understand the mechanism (Meerts et al., 2005a,b; Misinzo et al., 2008b).

References

Allan, G.M., McNielly, F., Ellis, J., Krakowka, S., Botner, A., McCullough, K., Nauwynck, H., Kennedy, S., Meehan, B., Charreyre, C., 2004. PMWS: experimental model and co-infections. Vet. Microbiol. 98, 165–168.

Esko, J.D., 1999. Glycosaminoglycan-binding proteins. In: Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., Marth, J. (Eds.), Essentials of Glycobiology. Cold Spring Harbor Laboratory, NY, pp. 441–453.

Gassmann, M., Focher, F., Buhk, H.J., Ferrari, E., Spadari, S., Hübscher, U., 1988. Replication of single-stranded porcine circovirus DNA by DNA polymerases alpha and delta. Biochim. Biophys. Acta 951, 280–289.

Khayat, R., Brunn, N., Speir, J.A., Hardham, J.M., Ankenbauer, R.G., Schneemann, A., Johnson, J.E., 2011. The 2.3-anstrom structure of porcine circovirus 2. J. Virol. 85, 7856–7862.

Lefebvre, D.J., Costers, S., Van Doorsselaere, J., Misinzo, G., Delputte, P.L., Nauwynck, H.J., 2008a. Antigenic differences among porcine circovirus type 2 strains, as demonstrated by the use of monoclonal antibodies. J. Gen. Virol. 89, 177–187.

Lefebvre, D.J., Meerts, P., Costers, S., Misinzo, G., Barbé, F., Van Reeth, K., Nauwynck, H.J., 2008b. Increased porcine circovirus type 2 replication in porcine leukocytes in vitro and in vivo by concanavalin A stimulation. Vet. Microbiol. 25, 74–86.

Marsh, M., Helenius, A., 2006. Virus entry: open sesame. Cell 124, 729–740.

Mateusen, B., Sanchez, R.E., Van Soom, A., Meerts, P., Maes, D.G.D., Nauwynck, H.J., 2004. Susceptibility of pig embryos to porcine circovirus type 2 infection. Theriogenology 61, 91–101.

Mateusen, B., Maes, D., Van Soom, A., Lefebvre, D., Nauwynck, H.J., 2007. Effect of a porcine circovirus type 2 infection on embryos during early pregnancy. Theriogenology 68, 896–901.

Meerts, P., Misinzo, G., McNeilly, F., Nauwynck, H.J., 2005a. Replication kinetics of different porcine circovirus 2 strains in PK-15 cells, fetal cardiomyocytes and macrophages. Arch. Virol. 150, 427–441.

Meerts, P., Misinzo, G., Nauwynck, H.J., 2005b. Enhancement of porcine circovirus 2 replication in porcine cell lines by interferon-gamma pre- and post-treatment and by IFN-alpha post-treatment. J. Interf. Cytok. Res. 25, 684–693. Misinzo, G., Meerts, P., Bublot, M., Mast, J., Weingartl, H.M., Nauwynck, H., 2005. Binding and entry characteristics of porcine circovirus 2 in cells of the porcine monoocytic line 3D4/31. J. Gen. Virol. 86, 2057–2068.

Misinzo, G., Delputte, P., Meerts, P., Lefebvre, D.J., Nauwynck, H., 2006. Porcine circovirus 2 uses heparan sulfate and chondroitin sulfate B glycosaminoglycans as receptors for its attachment to host cells. J. Virol. 80, 3487–3494.

Misinzo, G., Delputte, P., Nauwynck, H.J., 2008a. Inhibition of endosomal-lysosomal system acidification enhances porcine circovirus 2 infection of porcine epithelial cells. J. Virol. 82, 1128–1135.

Misinzo, G., Delputte, P., Lefebvre, D.J., Nauwynck, H.J., 2008b. Increased yield of porcine circovirus-2 by a combined treatment of PK-15 cells with interferon-gamma and inhibitors of endosomal-lysosomal system acidification. Arch. Virol. 153, 337–342.

Misinzo, G., Delputte, P., Nauwynck, H., 2009. Porcine circovirus 2 infection of epithelial cells is clathrin-, caveolae- and dynamin-independent, actin and Rho-GTPase-mediated, and enhanced by cholesterol depletion. Virus Res. 139, 1–9.

Pensaert, M.B., Sanchez, R.M., Ladekjær-Mikkelsen, A-S., Allan, G.M., Nauwynck, H.J., 2004. Viremia and effect of fetal infection with porcine viruses with special reference to porcine circovirus 2 infection. Vet. Microbiol. 98, 175–183.

Saha, D., Lefebvre, D., Van Doorsselaere, J., Atanasova, K., Barbé, F., Geldhof, M., Karniychuk, U.U., Nauwynck, H.J., 2010. Pathological and virological outcome in mid-gestational porcine foetuses after experimental inoculation with PCV2a and PCV2b. Vet. Microbiol. 145, 62–68.

Saha, D., Huang, L., Bussalleu, E., Lefebvre, D.J., Fort, M., Van Doorsselaere, J., Nauwynck, H.J., 2011. Antigenic subtyping and epitopes' competition analysis of porcine circovirus type 2 using monoclonal antibodies. Vet. Microbiol., in press.

Sanchez, R., Nauwynck, H.J., Pensaert, M.B., 2001. Porcine circovirus 2 infection in swine foetuses inoculated at different stages of gestation. Vet. Microbiol. 83, 169–176.

Sanchez, R., Meerts, P., Nauwynck, H., Pensaert, M.B., 2003. Change of porcine circovirus 2 target cells in pigs during development from fetal to early postnatal life. Vet. Microbiol. 95, 15–25.

Sanchez, R., Meerts, P., Nauwynck, H.J., Pensaert, M.B., 2004. Characteristics of porcine circovirus 2 replication in lymphoid organs of pigs inoculated in late-gestation or postnatally and possible relation to clinical and pathological outcome of infection. J. Vet. Diag. Invest. 16, 168–178.

Segales, J., Allan, G.M., Domingo, M., 2005. Porcine circovirus diseases. Anim. Health Res. Rev. 6, 119–142.

Steiner, E., Balmelli, C., Herrmann, B., Summerfield, A., McCullough, K., 2008. Porcine circovirus type 2 displays pluripotency in cell targeting. Virology 378, 311–322.

Vincent, I.E., Carrasco, C.P., Herrmann, B., Meehan, B.M., Allan, G.M., Summerfield, A., McCullough, K.C., 2003. Dendritic cells harbor infectious porcine circovirus type 2 in the absence of apparent cell modulation or replication of the virus. J. Virol. 77, 13288–13300.