VIRAL IMMUNOLOGY
Volume 18, Number 2, 2005

Pp. 333–341

Correlation Between Type of Adaptive Immune Response Against Porcine Circovirus Type 2 and Level of Virus Replication

P. MEERTS,[1] S. VAN GUCHT,[1] E. COX,[2] A. VANDEBOSCH,[3] and H.J. NAUWYNCK[1]

ABSTRACT

Porcine circovirus 2 (PCV2) replication is characterized by high variation among infected pigs. This study investigated the role of immunologic responses in causing this variation. Twelve gnotobiotic pigs were inoculated with PCV2. Four of these pigs were treated with cyclosporin A (CysA) to monitor the effect of the adaptive immunity on the development of the PCV2 infection. Through lymph node biopsies at 10, 15, and 21 days postinoculation (DPI), PCV2 replication in lymphoid tissues was monitored. The production of total PCV2-specific and PCV2-neutralizing antibodies was followed, together with interferon-γ (IFN-γ) mRNA expression levels in peripheral blood monocytes as a marker for cellular immunity. In general, the CysA-treated pigs showed the highest PCV2 titers, indicating that the adaptive immunity is necessary to restrain PCV2 replication. Three different PCV2 replication patterns were observed in non–CysA-treated pigs. Pattern 1: In two pigs, PCV2 was not detected. They had the highest neutralizing antibody titers, appearing from 15 DPI. In these pigs a good cellular response was indicated by a peak in IFN-γ mRNA at 15 DPI. Pattern 2: Five pigs contained low to moderate PCV2 titers at 15 DPI, remaining constant or decreasing towards 21 DPI. Lower neutralizing antibody titers were observed and no rise in IFN-γ was detected. Pattern 3: In one pig, a low PCV2 titer at 15 DPI dramatically increased toward 21 DPI. Although an antibody response against PCV2 was mounted, no PCV2-neutralizing antibodies were detected. This pig also showed no rise in IFN-γ. The study findings indicate that variation in the onset of the adaptive immunity may account for variation in PCV2 replication among pigs. Absence of PCV2-neutralizing antibodies may be an important factor in the development of an increased virus replication.

INTRODUCTION

PORCINE CIRCOVIRUS 2 (PCV2), a small single-stranded DNA virus, is a member of the family of *circoviridae*. Among various other animal viruses, the *circoviridae* also contain some recently identified human viruses (32). PCV2 is a widespread virus. In pig-dense regions, this virus is known to infect all weaned pigs in conventional farms. In farms affected by the postweaning multisystemic wasting syndrome (PMWS), a disease that has been associated with PCV2 infection (8,26), the majority of PCV2 infections remain subclinical. In subclini-

[1]Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Belgium.
[2]Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium.
[3]Department of Applied Mathematics and Computer Science, Faculty of Science, Ghent University, Belgium.

cally infected pigs, a humoral immune response is induced as shown by the production of antibodies (18,22) and the virus is efficiently cleared from the body. In some cases, in conditions that have not yet been elucidated, the infection of susceptible pigs will lead to the development of PMWS (3). A characteristic feature of this disease is the presence of high amounts of PCV2 in lymphoid and non-lymphoid organs of affected pigs (4,36), while at the same time non-affected littermates are virologically negative or contain very low titers of PCV2 in their organs. The presence of high amounts of PCV2 in lymphoid organs of naturally PMWS-affected pigs is correlated with the presence of B and T lymphocyte depletion and monocyte infiltration (6,30), possibly clue factors in the pathogenesis of the disease.

During experimental inoculations of the virus, similar observations were made. When susceptible pigs were inoculated with PCV2, high variation was observed in the level of PCV2 replication. In some pigs the virus was hardly detectable, whereas in others high titers of PCV2 were detected several weeks after inoculation (1,17,31,34). Only in pigs with a high level of PCV2 replication were the typical histopathological lesions, described above, observed (27,35). When the clinical signs of PMWS could be reproduced in some of these studies, they were always found in pigs with high levels of PCV2 replication (17). The combined results of field and experimental studies suggest that pigs that experience high PCV2 replication are predisposed to develop PMWS, whereas pigs with low PCV2 replication are very unlikely to develop the disease. The elucidation of the mechanism that forms the basis of this event could be an important step in further understanding the pathogenesis of PCV2 infection and possibly of PMWS.

Because not all experimentally inoculated pigs with high PCV2 replication developed clinical symptoms, research has been focused on identifying co-factors that influence the reproduction of the disease. Until now, a synergy has been described between PCV2 and two other viruses: porcine reproductive and respiratory disease virus (PRRSV) (1) and porcine parvovirus (PPV) (2). A general stimulation of the immune system has also been shown to influence the clinical outcome of the infection in gnotobiotic pigs (15). The mechanism of the synergistic effect between PCV2 infection, on one hand, and other viruses or immune stimulation, on the other hand, is not clarified. At present, the onset of the adaptive immune response against PCV2 and its influence on the kinetics of the PCV2 replication in the host have only partially been characterized. Starting from 10–14 days post-inoculation (DPI), PCV2-specific antibodies (Ab) have been detected (17,33). The appearance of the Ab coincided with a drop in PCV2 DNA copies in the blood of the host (17,28). Neutralizing antibodies have been described only starting from 28 DPI (29). The onset of the cellular immunity upon PCV2 infection and its influence on PCV2 replication remain undocumented. Therefore, the aims of this study were to monitor the humoral and cellular immune responses upon PCV2 infection and to examine which immunological mechanism determines the development of the PCV2 replication kinetics in the pig and thereby possibly the clinical outcome of the infection.

MATERIALS AND METHODS

Pigs. Sixteen gnotobiotic piglets were derived from two Belgian Landrace sows at 114 days of gestation by cesarean section. They were raised individually in a sterile environment in Horsfall-type isolators. At 19 days of age, they were randomly divided into four groups (group A, four pigs; group B, eight pigs; group C, two pigs; group D, two pigs).

Virus inoculation. At 19 days of age, the pigs in group A (A1, A2, A3, A4) and group B (B1, B2, B3, B4, B5, B6, B7, B8) were oronasally and intraperitoneally inoculated with $10^{4.3}$ $TCID_{50}$ of PCV2 strain 1121 (21). This PCV2 stock was propagated by four passages in PK-15 cells. The pigs in group C (C1, C2) and group D (D1, D2) were mock-inoculated with equal volumes of cell culture medium.

Cyclosporin treatment. Starting from 12 h before the PCV2 or mock inoculation up to the end of the study, the pigs in groups A and C were treated with cyclosporin A (CysA) (Neoral-Sandimmun, Novartis Pharma, Vilvoorde, Belgium) at a dose of 23 mg/kg/day (10) administered in two feedings per day, diluted in milk. By artificially suppressing the onset of the adaptive immune response on the PCV2 infection with CysA, the influence of the adaptive immunity on the development of PCV2 infection was monitored.

Clinical monitoring and sampling. During the study, the pigs were monitored for clinical signs that have been described in association with PMWS; these were cachexia, depression, respiratory distress, and jaundice (3). At the end of the study, the pigs were weighed and the average weight of each group was calculated and compared as a marker for the general clinical condition of the pigs throughout the study.

To follow the replication of PCV2 longitudinally in the inguinal lymph nodes of the pigs, the lymph nodes were surgically sampled. At 10 DPI a biopsy sample was taken from the left inguinal lymph node and at 15 DPI, a biopsy sample was taken from the right inguinal lymph node. To take lymph node biopsy samples the pigs were anesthetized by intramuscular injection of 2.2 mg tiletamine and 2.2 mg zolazepam (Zoletil®, Virbac) dissolved in

0.22 ml 2% Xylazine (XYL-M® 2%, VMD) per kilogram of body weight. An incision was made in the overlaying skin and half the inguinal lymph node was excised. The wound was closed by mersilene sutures (Mersutures®, Ethicon, Somerville, NJ). At 21 DPI, all pigs were euthanized and the remaining parts of both left and right inguinal lymph nodes were collected.

At 0, 10, 15, and 21 DPI, blood was taken (with 15 U/ml heparin) from all pigs to monitor the onset and evolution of both humoral and cellular immunity against PCV2.

All animal experiments performed in this study were approved by the ethical committee of the Faculty of Veterinary Medicine, Ghent University, Belgium.

Longitudinal monitoring of PCV2 replication in inguinal lymph nodes. Because it has been shown that the PCV2 titer in the inguinal lymph nodes is very well correlated with the titer in other lymphoid and non-lymphoid target organs (17,34), the titer in the inguinal lymph node can be considered to be a good marker for the general replication of the virus in the body of the host. At 10, 15, and 21 DPI, PCV2 titers were determined in the biopsy samples and inguinal lymph nodes of all pigs. Ten percent of tissue suspensions were made of a part of the biopsy samples and lymph nodes, and the PCV2 titer was determined by virus titration on PK-15 cells as described elsewhere (33). The detection limit of this assay was $10^{2.4}$ $TCID_{50}$ PCV2 per gram of lymph node tissue.

Monitoring of the adaptive immune response. Peripheral blood mononuclear cells (PBMCs) were separated from blood, collected at different time points, by density centrifugation at 750 *g* on Ficoll-Paque® (Amersham Pharmacia Biotech AB, Upsalla, Sweden) to monitor the cellular immune response. Plasma was collected to determine the humoral immune response against PCV2.

Humoral immune response. The presence of PCV2-specific antibodies in plasma was determined by an immunoperoxidase monolayer assay (IPMA) as described earlier (33). This assay detects all antibodies that react with PCV2 antigens and does not discriminate between neutralizing or non-neutralizing antibodies. This discrimination was made by using a seroneutralization assay adapted from that described by Delputte *et al.* (7). Briefly, $10^{3.7}$ $TCID_{50}$ PCV2 was incubated for 1 h with 25% of plasma at 37°C. Subsequently this mixture of virus and plasma was inoculated on PCV-negative PK-15 cells. After 1 h of incubation, the inoculum was removed, cell cultures were washed twice, and new medium was added. At 36 h post-inoculation, after the first replication cycle of PCV2 was completely finished (23), the cultures were fixed and PCV2-infected PK-15 cells were stained by an immunoperoxidase reaction using polyclonal anti-PCV2 antibodies. The number of infected cells was counted by light microscopy. The PCV2-neutralizing capacity of the plasma was determined by calculating the reduction in PCV2-infected cells compared to cells inoculated with PCV2 incubated with plasma of the same pig from day 0 (i.e., before PCV2-inoculation).

IFN-γ response. In this study, the level of IFN-γ mRNA in freshly isolated PBMCs was determined by real-time reverse transcriptase–polymerase chain reaction (RT-PCR) as described by Verfaillie *et al.* (37). Briefly, RNA from PBMCs was extracted with the Trizol® reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. In a next step, RNA was transformed into cDNA by means of reverse transcriptase (Superscript™ II reverse transcriptase; Invitrogen). In a final step, the number of cDNA copies coding for IFN-γ and cyclophilin was determined by real-time PCR using the Lightcycler FastStart DNA Master SYBR Green I kit (Roche Applied Science, Penzberg, Germany) in a Roche LightCycler (Roche applied science, Penzberg, Germany). The level of IFN-γ mRNA was compared with the level of mRNA transcription of the household gene cyclophilin that served as an internal control. The level of IFN-γ mRNA was determined by calculating the ratio of IFN-γ mRNA to cyclophilin mRNA. To circumvent individual variation between pigs, the results of this test were expressed as the IFN-γ mRNA expression level at the day of testing divided by the level at day 0.

Statistical analysis. The statistical significance of differences between medians of different groups was tested by applying two-sided Mann-Whitney tests. Bonferroni corrections for multiple comparisons at different time points were used. Differences were considered significant at values of $p < 0.05$.

RESULTS

Clinical monitoring of pigs. During the study, no clinical signs were observed in pigs in group B (non–CysA-treated, PCV2-inoculated) and group D (non–CysA-treated, non–PCV2-inoculated). All pigs in groups A and C (respectively, CysA-treated, PCV2-inoculated and CysA-treated, non–PCV2-inoculated) showed diarrhea starting between the days 5 and 10 of CysA treatment and lasting until the end of the study. At the end of the study these pigs showed moderate jaundice. Other clinical signs such as respiratory distress, anorexia, or depression were not observed during the study. The excision of lymph node biopsy samples did not cause noticeable discomfort to the pigs. At the end of the study, no major differences were observed among the mean

body weights of pigs in different groups (group A, 5.1 kg; group B, 4.9 kg, group C, 5.3 kg; group D, 5.4 kg) or among the body weights of individual pigs in the same group.

Longitudinal monitoring of PCV2 replication in inguinal lymph nodes. The results of the evolution of PCV2 replication in inguinal lymph nodes of the pigs are shown in Fig. 1. In the inguinal lymph nodes of the non–PCV2-inoculated pigs (groups C and D), PCV2 was never detected. At 10 DPI, PCV2 could not be detected in the inguinal lymph nodes of any of the PCV2-inoculated pigs. Starting from 15 DPI, PCV2 was detected in inguinal lymph nodes of 10 of 12 PCV2-inoculated pigs, and different evolutions in PCV2 titers over time were observed. The pigs in group A (CysA-treated, PCV2-inoculated) showed significantly higher median titers at 15 and 21 DPI compared to the pigs from group B. Two of these pigs (A2 and A3), the pigs with the highest titers at 15 DPI (respectively $10^{6.2}$ and $10^{5.2}$ $TCID_{50}$ per gram of lymph node tissue) reached the maximal level of PCV2 replication at this time point; the two other pigs in this group (A1 and A4) showed an increase in PCV2 titer from 15 DPI ($10^{3.7}$ $TCID_{50}$ per gram of lymph node tissue) to 21 DPI ($10^{5.2}$ and $10^{5.4}$ $TCID_{50}$ per gram of lymph node tissue).

In group B (non–CysA-treated, PCV2-inoculated), three different patterns in PCV2 replication were observed. From the lymph nodes of two of eight pigs in this group (B1 and B5), PCV2 could not be isolated during the study (pattern 1). PCV2 could be isolated from the inguinal lymph nodes of the six other pigs in this group at various amounts. Five of these pigs (B2, B4, B6, B7, B8) showed a maximal PCV2 titer at 15 DPI (between $10^{2.7}$ and $10^{3.7}$ $TCID_{50}$ per gram of lymph node tissue), after which the titer remained similar or decreased toward 21 DPI (pattern 2). In one pig (B3) a very distinct evolution in PCV2 replication was observed; at 15 DPI this pig showed one of the lowest PCV2-titers of group B ($10^{2.7}$ $TCID_{50}$ per gram of lymph node tissue). A dramatic increase in PCV2 replication was observed towards 21 DPI ($10^{4.9}$ $TCID_{50}$ per gram of lymph node tissue) (pattern 3). The evolution in PCV2 replication in this pig was comparable to the evolution in pigs A1 and A4 (CysA-treated, PCV2-inoculated), in which a steep increase in the PCV2-titer also was observed between 15 and 21 DPI. At 21 DPI, the PCV2 titer in the inguinal lymph nodes of this pig was between 1.6 and 2.2 log 10 times higher compared to the titers in the other pigs in group B, and was situated closer to the mean of the titers of the artificially immunosuppressed pigs in group A than it was to the mean of group B.

Monitoring of the PCV2-specific adaptieve immune response.

Humoral immune response. The IPMA Ab titers against PCV2, as determined in the plasma of pigs at different time points during the study, are shown in Fig. 2. The non–PCV2-inoculated pigs in groups C and D did not show PCV2-specific antibodies during the study. This comfirms that they were not infected with PCV2. All PCV2-inoculated pigs showed seroconversion against PCV2 during the study. In all pigs in group B (non–CysA-

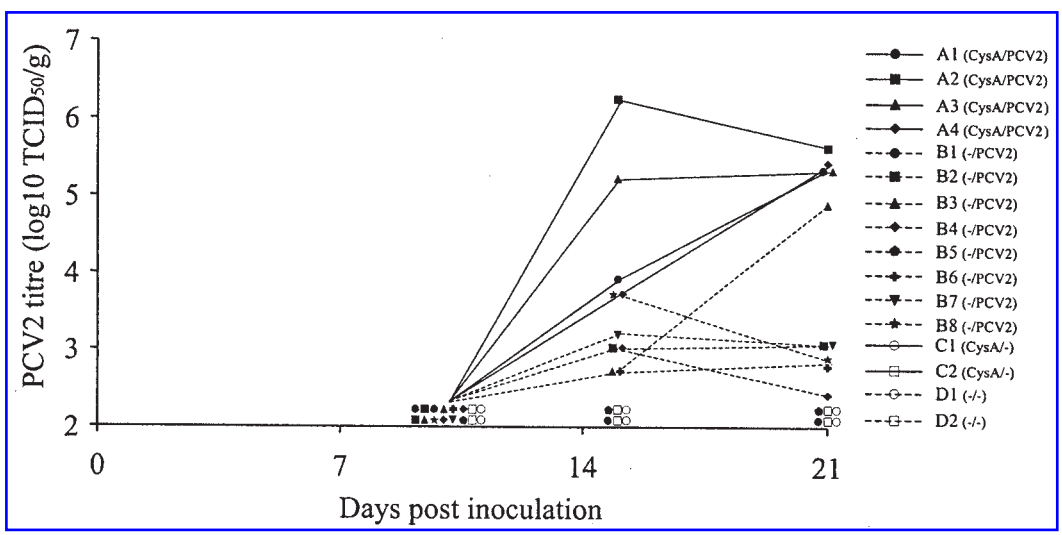

FIG. 1. Evolution of porcine circovirus 2 (PCV2) titers in inguinal lymph nodes of PCV2-inoculated and non–PCV2-inoculated pigs. The PCV2 titers at 10 and 15 days post-inoculation (DPI) are titers in the lymph node biopsy samples exised on those days. The titers at 21 DPI are the average titers of the remaining parts of the left and right lymph nodes collected when pigs were euthanized.

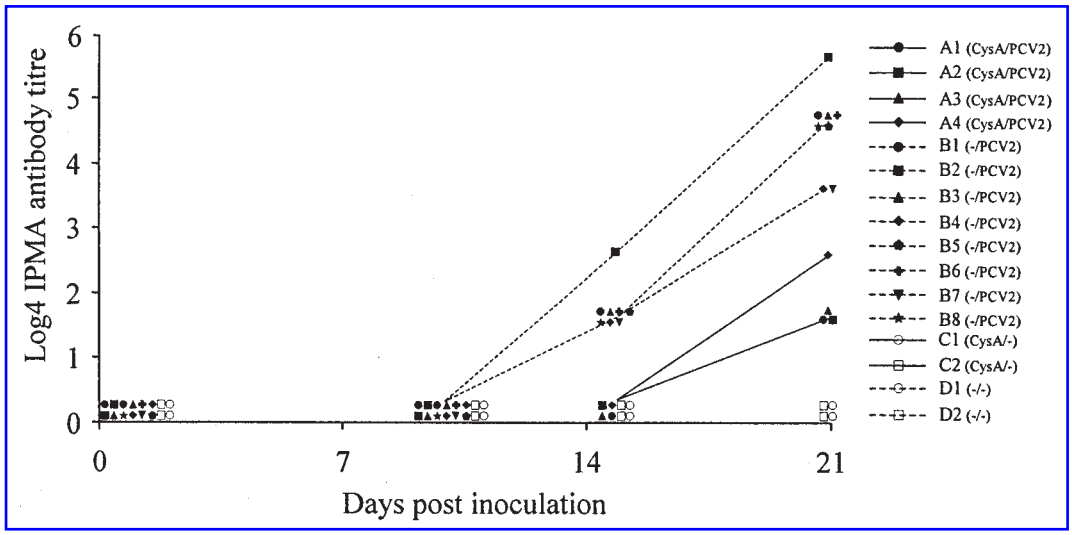

FIG. 2. Evolution of anti–porcine circovirus 2 (PCV2) antibodies (IPMA) in plasma of PCV2-inoculated and non–PCV2-inoculated pigs.

treated, PCV2-inoculated), anti-PCV2 Ab were detected for the first time at 15 DPI (IPMA titers ranging from $4^{1.7}$ to $4^{2.7}$), clearly showing that all pigs in group B became infected upon PCV2 inoculation. The IPMA Ab titer increased in these pigs toward 21 DPI (IPMA titers ranging from $4^{3.7}$ to $4^{5.7}$). The pigs in group A (CysA-treated, PCV2-inoculated) also seroconverted against PCV2 but Ab were detected for the first time at 21 DPI. At this time point the Ab titers in these pigs were similar to the Ab titers in pigs in group B at 15 DPI (IPMA titers ranging from $4^{1.7}$ to $4^{2.7}$).

The evolution of PCV2-neutralizing Ab (neutralization of PCV2 infection) in the plasma is shown in Fig. 3. Pigs in groups C and D (non–PCV2-inoculated pigs) did not contain neutralizing Ab in their plasma during the study. At 10 DPI, the median outcome of the PCV2-inoculated pigs did not differ significantly from the median of the non–PCV2-inoculated pigs, indicating that at this time no evidence for the presence of PCV2-neutralizing Ab in the plasma of PCV2-inoculated pigs was found. At 15 and 21 DPI a significant difference was found between the median outcome of non–PCV2-inoculated and PCV2-inoculated pigs, indicating the presence of PCV2-neutralization in the sera of PCV2-inoculated pigs. Starting from 15 DPI, PCV2-neutralizing Ab were detected in 10 of 12 PCV2-inoculated

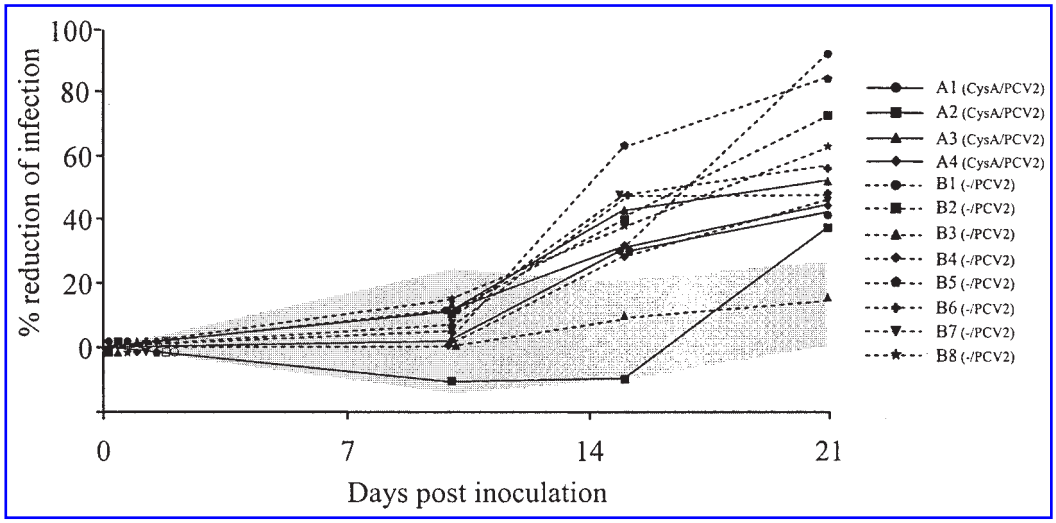

FIG. 3. Porcine circovirus 2 (PCV2)–neutralizing activity in plasma of PCV2-inoculated and non–PCV2-inoculated pigs. Lines represent values of individual PCV2-inoculated pigs. Gray area represents range (minimal and maximal values) in which values of the four non–PCV2-inoculated pigs were situated

pigs (all except A2 and B3). The PCV2-neutralization at this time point ranged from 30% to 62%, increasing toward 21 DPI (ranging from 41% to 93%). At 21 DPI, neutralization was also found in plasma of pig A2 (neutralization of 38%). In the plasma of one pig in group B, pig B3 (the pig with the strong increase in PCV2 replication between 15 and 21 DPI), PCV2 neutralization could not be detected during the course of the study. The highest PCV2 neutralization at 21 DPI was found in plasma of pigs B1 and B5, the pigs from which no PCV2 could be isolated during the study.

IFN-γ response. In all pigs, a basal level of IFN-γ gene transcription in PBMCs was detected before PCV2 inoculation. The evolution of IFN-γ mRNA expression levels in PBMCs of individual pigs during the study is shown in Fig. 4. Because of poor RNA-quality after isolation, the results of some pigs at some time points were not available (C1: 10 DPI; A2: 10 and 21 DPI; A4: 21 DPI). Non–PCV2-inoculated pigs (groups C and D) generally showed low IFN-γ mRNA expression levels (between 0.02 and 1). All pigs in group A (CysA-treated, PCV2-inoculated) and six of eight pigs from group B (non–CysA-treated, PCV2-inoculated) showed IFN-γ mRNA expression levels ranging between 0.1 and 2.3. In these pigs, no clear peak in IFN-γ mRNA expression levels in PBMCs was observed during the study. Two pigs in group B (B1 and B5) showed a peak in IFN-γ mRNA at 15 DPI (respectively to 5.8 and 4.6), decreasing (B1) or remaining at the same level (B5) toward 21 DPI. These pigs were the two inoculated pigs from which no PCV2 could be isolated during the study.

DISCUSSION

In the present study, an effect of the PCV2-adaptive immune response on the replication of the virus was observed. A detectable humoral and cellular immune response was observed in some pigs and was correlated with a replication level of the virus below the detection limit of the assay used. On the other hand, a correlation was described between a complete absence of PCV2-neutralizing antibodies and the presence of an increased PCV2 replication in one pig. This study was performed in gnotobiotic piglets that did not encounter any immunologic stimulation before PCV2 infection, and therefore their immune response might deviate to some extent from pigs raised in a more conventional environment (24).

In this study, PCV2 replication in lymphoid organs was followed for the first time using a method of inguinal lymph node biopsy samples. In this way, titers could be obtained at different time points in the same pig and PCV2 replication kinetics could be compared among individual pigs. Through this technique, the number of pigs necessary to demonstrate significant differences could be reduced. At 10 DPI, a discrepancy was detected between the experimental inoculation performed in the present study and similar inoculations performed previously in our laboratory (34). In experimentally inoculated piglets studied previously, PCV2 titers could already be detected starting from 10 DPI. At this time, the titers in the internal organs of the piglets (including the inguinal lymph nodes) were relatively high. In the present study, PCV2 could not be detected in the inguinal lymph nodes of the inoculated piglets at 10 DPI despite identical procedures.

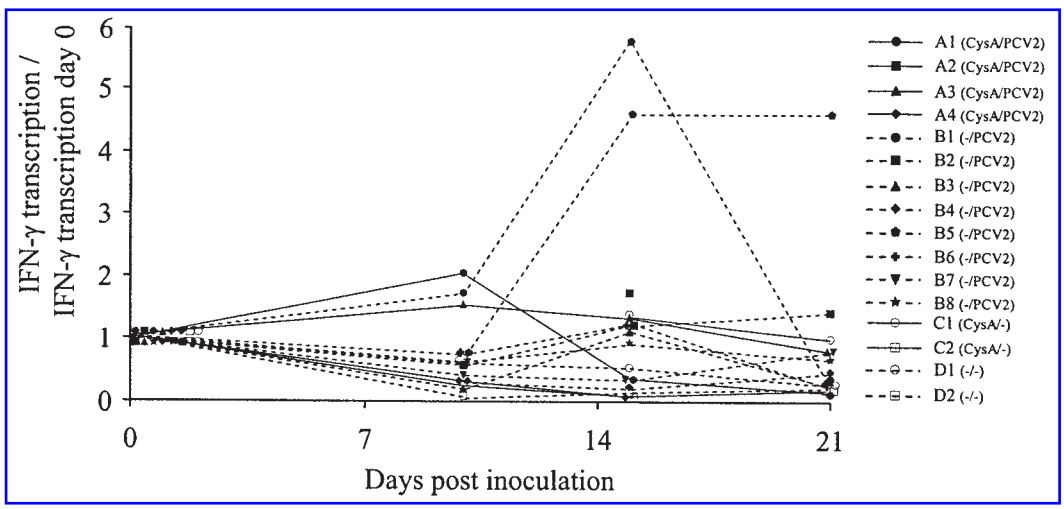

FIG. 4. Interferon-γ (IFN-γ) mRNA expression levels in peripheral blood mononuclear cells of porcine circovirus 2 (PCV2)–inoculated and non–PCV2-inoculated pigs.

The only differences between these pigs were their age at inoculation (1 day of age *versus* 19 days of age in this study) or minor environmental factors that may have occurred without our knowledge. Our findings show that minor factors can have important consequences on the further evolution of PCV2 replication in its host. This may explain the high variation in virological and clinical results from different PCV2 inoculation experiments performed by different research groups (17,34).

Although the eight PCV2-inoculated, non–CysA-treated pigs used in this study were derived from only two litters, three distinct patterns in PCV2 replication were observed. In pattern 1, from the lymph nodes of two of these eight pigs (25%), no PCV2 could be isolated. These pigs did become infected by the virus, as shown by their normal IPMA antibody response. In pattern 2, among five pigs, the majority of the pigs (63%) showed a low to moderate PCV2 replication remaining constant or decreasing toward the end of the study. In pattern 3, a very different evolution was observed in the last pig (one of eight pigs, or 13%), which experienced an intense increase in PCV2 replication from 15 to 21 DPI. To understand fully the pathogenesis of a PCV2 infection in its host, it is important to understand the basis for these different patterns.

An important mechanism contributing to the variation in virus replication seems to be represented by the onset and efficacy of the adaptive immune response of the individual pig against PCV2 infection. This could be concluded from correlating the PCV2 replication patterns with the immunological parameters investigated in this study. During the study, four PCV2-inoculated pigs were treated with CysA to artificially suppress their adaptive immunity (group A). CysA is known to interrupt the T-cell receptor signal transduction pathway and by doing this, inhibiting T-cell proliferation (12,20). By this mechanism, CysA treatment inhibits the onset of the cellular immune response and the thymus-dependent humoral immune response upon virus-infection (5). CysA treatment has already been shown to render pigs more susceptible to PCV2 replication (16). The CysA-treated pigs showed a delay in production of IPMA antibodies against PCV2 and low levels of neutralizing antibodies compared to the non–CysA-treated pigs. The late onset of antibody production indicates that the CysA treatment inhibited the humoral immunity but did not completely block it. Because IFN-γ is produced by activated Th1 cells, its transcription in a virus-inoculated host can be used to monitor the onset of the cellular immunity against infection (14). No rise in IFN-γ mRNA expression was detected in the CysA-treated pigs. At 21 DPI, all four of the CysA-treated piglets contained PCV2 titers that were significantly higher compared to the titers in seven of eight non–CysA-treated, PCV2-inoculated pigs at the same time point, indicating that the adaptive immunity plays an important role in controlling PCV2 infection. When the evolution in PCV2 replication in CysA-treated pigs and in non–CysA-treated pigs was compared, it was clear that the pigs with replication patterns 1 and 2 mounted an adaptive immune response upon PCV2 infection that was able to control the level of PCV2 replication. The PCV2-titer at 21 DPI of the non–CysA-treated pig with pattern 3 did not significantly differ from the titers of the CysA-treated pigs, indicating that the virus was able to replicate as intensively in this pig as in the pigs with artificial immunosuppression. The pigs with pattern 1 mounted the most efficient adaptive immune response against the virus. This was shown by the peak in IFN-γ mRNA in PBMCs, indicating a Th1-mediated immune response, and by the very high PCV2-neutralizing antibody levels in these pigs. The efficient immunological response in these animals coincided with strong protection against the virus. In contrast, the pig with pattern 3 did not show a peak in IFN-γ mRNA, nor did it produce any PCV2-neutralizing antibodies in its plasma. This coincided with weak virological protection. In between these opposites, the pigs with pattern 2 did not show an IFN-γ mRNA peak but did have PCV2-neutralizing antibodies. Differences in IFN-γ mRNA expression levels were most likely not induced by other infectious agents. Bacteriological examinations of internal organs and feces demonstrated that the pigs were negative, and serological examinations confirmed that the pigs remained seronegative to various common porcine viruses throughout the study (data not shown). From our study it cannot be concluded whether the pigs in which an increase in IFN-γ mRNA expression level in PBMCs was not detected mounted no cellular immune response at all. It is possible that an increase in IFN-γ mRNA could have been present in lymphocytes in lymph nodes or other target organs. This was not investigated in the present study.

This is the second study describing the existence of PCV2-neutralizing antibodies. In contrast to the previous study (29), in which the neutralizing antibodies were detected starting from 28 DPI, neutralizing antibodies could be detected starting from 15 DPI simultaneously with the onset of the total anti-PCV2 antibody production. The difference between these results is probably based on a difference in sensitivity of the techniques applied. The total absence of neutralizing antibodies in the pig with pattern 3, despite the presence of relatively high levels in other pigs, was a remarkable finding, as the pig with pattern 3 was able to produce IPMA PCV2 antibody titers similar to those of other pigs in the group. A possible explanation for the absence of neutralizing antibodies is a difference in epitope-specific responses as has been shown for other viruses (9,13,19,24). The inability of a

subpopulation of pigs to raise neutralizing antibodies against PCV2 may have an important effect on the further evolution of the infection and perhaps on its clinical outcome. It indicates that high titers of IPMA anti-PCV2 antibodies do not always represent a good protective immunity of the host, a feature that will have to be considered when vaccines against PCV2 are optimized.

In the present study, all CysA-treated pigs developed diarrhea and jaundice during the study. However, these clinical signs were observed in both PCV2-inoculated and non–PCV2-inoculated pigs, indicating that the signs were not caused by the infection. The presence of an increased and prolonged PCV2 replication in one pig did not induce clinical symptoms as described in PMWS-affected pigs during the time of the study. When PMWS was reproduced in PCV2-inoculated animals, the first clinical signs always appeared within the first 2 or 3 weeks (10,17). The absence of clinical signs in the present study suggests that an additional factor is needed for the reproduction of disease. When it is considered that a high PCV2 replication is crucial in the pathogenesis of PMWS, as sufficiently shown in the literature, it can be assumed that pigs that are not able to mount neutralizing antibodies in field conditions will have a higher chance to suffer the consequences of the infection compared to pigs that are able to clear the virus efficiently. To prevent PCV2-related clinical signs and their associated economic losses, two different strategies can be followed. The first strategy implies the identification and elimination of the second factor responsible for the induction of disease upon high PCV2 replication. This might be a complicated matter, especially when this factor would be an enzootic virus, as has been suggested (1,2), or even a general stimulation of the immunity (15). The second strategy involves the ability of the pig to control the PCV2 replication. In the present study, a gradation was observed in the ability of pigs to mount a good adaptive immune response against PCV2 infection. The pig with pattern 3 (that is, high PCV2 titers) was clearly more susceptible to PCV2 replication than the two pigs showing pattern 1 (no PCV2 detected), with the majority of the pigs (five showing pattern 2) caught in between. The differences in susceptibility to PCV2 infection and sustained replication were in this study correlated with the adaptive immune response mounted in the pigs. The results indicate that in the long term, the selection of pigs toward pattern 1 may be the best way to avoid PCV2-related problems.

ACKNOWLEDGMENTS

This research was supported by a grant from the Belgian ministry of Social Affairs and Public Health. The authors acknowledge F. De Backer for assistance in taking care of the animals and G. Labarque and F. Barbé for their assistance during surgery. The authors also acknowledge C. Boone, C. Bracke, and G. De Smet for their excellent technical assistance.

REFERENCES

1. Allan, G.M., F. McNeilly, J. Ellis, et al. 2000. Experimental infection of colostrum deprived pigs with porcine circovirus 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) potentiates PCV2 replication. Arch. Virol. 145:2421–2429.
2. Allan, G.M., F. McNeilly, B.M. Meehan, et al. 2000. A sequential study of experimental infection of pigs with porcine circovirus and porcine parvovirus: immunostaining of cryostat sections and virus isolation. J. Vet. Med. B. 47:81–94.
3. Allan, G.M., J.A. Ellis. 2000. Porcine circoviruses: a review. J. Vet. Diagn. Invest. 12:3–14.
4. Calsamiglia, M., J. Segales, J. Quintana, et al. 2002. Detection of porcine circovirus types 1 and 2 in serum and tissue samples of pigs with and without postweaning multisystemic wasting syndrome. Clin. Microbiol. 40:1848–1850.
5. Charan, S., A.W. Huegin, A. Cerny, et al. 1986. Effects of cyclosporin A on humoral immune response and resistance against vesicular stomatitis virus in mice. J. Virol. 57: 1139–1144.
6. Darwich, L., J. Segales, M. Domingo, E. Mateu. 2002. Changes in CD4(+), CD8(+), CD4(+) CD8(+), and immunoglobulin M-positive peripheral blood mononuclear cells of postweaning multisystemic wasting syndrome-affected pigs and age-matched uninfected wasted and healthy pigs correlate with lesions and porcine circovirus type 2 load in lymphoid tissues. Clin. Diagn. Lab. Immunol. 9:236–242.
7. Delputte, P.L., P. Meerts, S. Costers, H.J. Nauwynck. 2005. Effect of virus-specific antibodies on attachment, internalization and infection of porcine reproductive and respiratory syndrome virus. Vet. Immunol. Immunopathol. 102:179–188.
8. Ellis, J., L. Hassard, E. Clark, et al. 1998. Isolation of circovirus from lesions of pigs with postweaning multisystemic wasting syndrome. Can. Vet. J. 39:44–51.
9. Green, K.Y., K. Taniguchi, E.R. Mackow, A.Z. Kapikian. 1990. Homotypic and heterotypic epitope-specific antibody response in adult and infant rotavirus vaccinees: implications for vaccine development. J. Infect. Dis. 161:667–679.
10. Harms, P.A., S.D. Sorden, P.G. Halbur, et al. 2001. Experimental reproduction of severe disease in CD/CD pigs concurrently infected with type 2 porcine circovirus and-porcine reproductive and respiratory syndrome virus. Vet Pathol. 38:528–539.
11. Huang, C.A., Y. Fuchimoto, Z.L. Gleit, et al. 2001. Post-transplantation lymphoproliferative disease in miniature swine after allogeneic hematopoietic cell transplantation: similarity to human PTLD and association with a porcine gammaherpesvirus. Blood 97:1467–1473.

12. Ho, S., N. Clipstone, L. Timmermann, et al. 1996. The mechanism of action of cyclosporin A and FK506. Clin. Immunol. Immunopathol. 80:S40–S45.

13. Jacobs, L., T.G. Kimman. 1994. Epitope-specific antibody response against glycoprotein E of pseudorabies virus. Clin. Diagn. Lab. Immunol. 5:500–505.

14. Johnsen, C.K., A. Bøtner, S. Kamstrup, et al. 2002. Cytokine mRNA profiles in bronchoalveolar cells of piglets experimentally infected *in utero* with porcine reproductive and respiratory syndrome virus: association of sustained expression of IFN-γ and IL-10 after viral clearance. Viral Immunol. 15:549–556.

15. Krakowka, S., J.A. Ellis, F. McNeilly, S. et al. 2001. Activation of the immune system is the pivotal event in the production of wasting disease in pigs infected with porcine circovirus-2 (PCV-2). Vet. Pathol. 38:31–42.

16. Krakowka, S., J.A. Ellis, F. McNeilly, et al. 2002. Immunologic features of porcine circovirus type 2 infection. Viral. Immunol. 15:567–582.

17. Ladekjær-Mikkelsen, A-S., J. Nielsen, T. Stadejek, et al. 2002. Reproduction of postweaning multisystemic wasting syndrome (PMWS) in immunostimulated and non-immunostimulated 3-week-old pigs experimentally infected with porcine circovirus type 2 (PCV2). Vet. Microbiol. 89:97–114.

18. Labarque, G.G., H.J. Nauwynck, A.P. Mesu, M.B. Pensaert. 2000. Seroprevalence of porcine circovirus types 1 and 2 in the Belgian pig population. Vet. Q. 22:234–236.

19. Lambkin, R., H.J. Dimmock. 1995. All rabits immunized with type A influenza virus have a serum haemagglutination-inhibtion antibody response biased to a single epitope in antigenic site B. J. Gen. Virol. 76:889–897.

20. Matsuda, S., S. Koyasu. 2000. Mechanisms of action of cyclosporin. Immunopharmacology 47:119–125.

21. Meehan, B.M., F. McNeilly, I. McNair, et al. 2001. Isolation and characterization of porcine circovirus 2 from cases of sow abortion and porcine dermatitis and nephropathy syndrome. Arch. Virol. 146:835–142.

22. Meerts, P., H.J. Nauwynck, R.E. Sanchez Jr., et al. 2004. Prevalence of porcine circovirus 2 (PCV2)-related wasting on Belgian farms with or without a history of postweaning multisystemic wasting syndrome Flem. Vet. J. 73:31–38.

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

24. Mehrazar, K., A. Gilman-Sachs, K.A. Knisley, et al. 1993. Comparison of the immune response to Ars-BGG in germfree or conventional piglets. Dev. Comp. Immunol. 17:459–464.

25. Müller, T.F., D. Gicklhorn, T. Jungraitmayr, et al. 2002. Pattern and persistence of the epitope-specific IgM response against human cytomegalovirus in renal transplant patients. J. Clin. Virol. 24:45–56.

26. Morozov, I., T. Sirinarumitr, S.D. Sorden, et al. 1998. Detection of a novel strain of porcine circovirus in pigs with postweaning multisystemic wasting syndrome. J. Clin. Microbiol. 36:2535–2541.

27. Nielsen, J., I.E. Vincent, A. Bøtner, et al. 2003. Association of lymphopenia with porcine circovirus type 2 induced postweaning multisystemic wasting syndrome (PMWS). Vet. Immunol. Immunopathol. 92:97–111.

28. Pensaert, M.B., R.E. Sanchez Jr., A-S. Ladekjaer-Mikkelsen, et al. 2004. Viremia and effect of fetal infection with porcine viruses with special reference to porcine circovirus 2 infection. Vet. Microbiol. 98:175–183.

29. Pogranichnyy, R.M., K.J. Yoon, P.A. Harms, et al. 2000. Characterization of immune response of young pigs to porcine circovirus type 2 infection. Viral Immunol. 13:143–153.

30. Quintana, J., J. Segales, C. Rosell, et al. 2001. Clinical and pathological observations on pigs with postweaning multisystemic wasting syndrome. Vet. Rec. 149:357–361.

31. Rovira, A., M. Balasch, J. Segales, L. et al. 2002. Experimental inoculation of conventional pigs with porcine reproductive and respiratory syndrome virus and porcine circovirus 2. J. Virol. 76:3232–3239.

32. Sagir, A., O. Kirschberg, T. Heintges, et al. 2004. SEN virus infection. Rev. Med. Virol. 14:141–148.

33. Sanchez, R.E. Jr., H.J. Nauwynck, F. McNeilly, et al. 2001. Porcine circovirus 2 infection in swine foetuses inoculated at different stages of gestation. Vet. Microbiol. 83:169–176.

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

35. Sanchez, R.E. Jr., P. Meerts, H.J. Nauwynck, et al. 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. Diagn. Invest. 16:175–185.

36. Segales, J., M. Domingo. 2002. Postweaning multisystemic wasting syndrome (PMWS) in pigs. A review. Vet. Q. 24:109–124.

37. Verfaillie, T., E. Cox, L.T. To, et al. 2001. Comparative analysis of porcine cytokine production by mRNA and protein detection. Vet. Immunol. Immunopathol. 81:97–112.

Address reprint requests to:
Prof. Dr. Hans Nauwynck
Laboratory of Virology
Faculty of Veterinary Medicine,
Salisburylaan 133, 9820 Merelbeke, Belgium

E-mail: Hans.Nauwynck@UGent.be

Received September 29, 2004; accepted December 18, 2004

This article has been cited by:

1. Wilhelm Gerner, Kerstin H. Mair, Selma Schmidt. 2022. Local and Systemic T Cell Immunity in Fighting Pig Viral and Bacterial Infections. *Annual Review of Animal Biosciences* **10**:1, 349-372. [Crossref]
2. Ying Huang, Xianghong Chen, Yunzhi Long, Liu Yang, Wenbo Song, Jinjin Liu, Qianqian Li, Gong Liang, Daobing Yu, Chao Huang, Xibiao Tang. 2021. Epidemiological Analysis From 2018 to 2020 in China and Prevention Strategy of Porcine Circovirus Type 2. *Frontiers in Veterinary Science* **8**. . [Crossref]
3. Hyungmin Um, Siyeon Yang, Taehwan Oh, Hyejean Cho, Kee Hwan Park, Jeongmin Suh, Chanhee Chae. 2021. A field efficacy trial of a trivalent vaccine containing porcine circovirus type 2a and 2b, and Mycoplasma hyopneumoniae in three herds. *Veterinary Medicine and Science* **168**. . [Crossref]
4. Seok-Jin Kang, Sung-Min Bae, Hye-Jeong Lee, Young-Ju Jeong, Min-A Lee, Su-Hwa You, Hyang-Sim Lee, Bang-Hun Hyun, Nakhyung Lee, Sang-Ho Cha. 2021. Porcine Circovirus (PCV) Genotype 2d-Based Virus-like Particles (VLPs) Induced Broad Cross-Neutralizing Antibodies against Diverse Genotypes and Provided Protection in Dual-Challenge Infection of a PCV2d Virus and a Type 1 Porcine Reproductive and Respiratory Syndrome Virus (PRRSV). *Pathogens* **10**:9, 1145. [Crossref]
5. Taehwan Oh, Kee Hwan Park, Siyeon Yang, Hyejean Cho, Jeongmin Suh, Chanhee Chae. 2021. Pathogenicity of Porcine Circovirus Type 2d (PCV2d) in Pigs Infected with PCV2d or Co-infected with Mycoplasma hyopneumoniae and PCV2d or with Porcine Reproductive and Respiratory Syndrome Virus and PCV2d. *Journal of Comparative Pathology* **187**, 75-82. [Crossref]
6. Yongjun Ahn, Siyeon Yang, Taehwan Oh, Kee Hwan Park, Hyejean Cho, Jeongmin Suh, Chanhee Chae. 2021. Efficacy Evaluation of a Bivalent Vaccine Containing Porcine Circovirus Type 2b and Mycoplasma hyopneumoniae Against an Experimental Dual Challenge. *Frontiers in Veterinary Science* **8**. . [Crossref]
7. Seok-Jin Kang, Hyeonjeong Kang, Su-Hwa You, Hye Jeong Lee, Nakhyung Lee, Bang-Hun Hyun, Sang-Ho Cha. 2020. Genetic diversity and different cross-neutralization capability of porcine circovirus type 2 isolates recently circulating in South Korea. *BMC Veterinary Research* **16**:1. . [Crossref]
8. Lili Zhang, Siyu Qiu, Mingqing Lu, Canping Huang, Yingjun Lv. 2020. Nuclear transporter karyopherin subunit alpha 3 levels modulate Porcine circovirus type 2 replication in PK-15 cells. *Virology* **548**, 31-38. [Crossref]
9. Guoyang Liu, Xuwen Qiao, Chen Chang, Tao Hua, Jichun Wang, Bo Tang, Daohua Zhang. 2020. Reduction of Postweaning Multisystemic Wasting Syndrome-Associated Clinical Symptoms by Virus-Like Particle Vaccine Against Porcine Parvovirus and Porcine Circovirus Type 2. *Viral Immunology* **33**:6, 444-456. [Abstract] [Full Text] [PDF] [PDF Plus]
10. Liping Huang, Zhenzhao Sun, Deli Xia, Yanwu Wei, Encheng Sun, Chunguo Liu, Hongzhen Zhu, Haiqiao Bian, Hongli Wu, Li Feng, Jingfei Wang, Changming Liu. 2020. Neutralization Mechanism of a Monoclonal Antibody Targeting a Porcine Circovirus Type 2 Cap Protein Conformational Epitope. *Journal of Virology* **94**:9. . [Crossref]
11. Taehwan Oh, Kee Hwan Park, Siyeon Yang, Jiwoon Jeong, Ikjae Kang, Changhoon Park, Chanhee Chae. 2019. Evaluation of the efficacy of a trivalent vaccine mixture against a triple challenge with Mycoplasma hyopneumoniae, PCV2, and PRRSV and the efficacy comparison of the respective monovalent vaccines against a single challenge. *BMC Veterinary Research* **15**:1. . [Crossref]
12. Kee Hwan Park, Taehwan Oh, Siyeon Yang, Hyejean Cho, Ikjae Kang, Chanhee Chae. 2019. Evaluation of a porcine circovirus type 2a (PCV2a) vaccine efficacy against experimental PCV2a, PCV2b, and PCV2d challenge. *Veterinary Microbiology* **231**, 87-92. [Crossref]
13. Bei Huang, Lili Zhang, Mingqing Lu, Jiansheng Li, Yingjun Lv. 2018. PCV2 infection activates the cGAS/STING signaling pathway to promote IFN-β production and viral replication in PK-15 cells. *Veterinary Microbiology* **227**, 34-40. [Crossref]
14. Pattama Mutthi, Sirin Theerawatanasirikul, Sittiruk Roytrakul, Atchara Paemanee, Chalermpol Lekcharoensuk, Payuda Hansoongnern, Nantawan Petcharat, Nattarat Thangthamniyom, Porntippa Lekcharoensuk. 2018. Interferon gamma induces cellular protein alteration and increases replication of porcine circovirus type 2 in PK-15 cells. *Archives of Virology* **163**:11, 2947-2957. [Crossref]
15. Lianna R. Walker, Taylor B. Engle, Hiep Vu, Emily R. Tosky, Dan J. Nonneman, Timothy P. L. Smith, Tudor Borza, Thomas E. Burkey, Graham S. Plastow, Stephen D. Kachman, Daniel C. Ciobanu. 2018. Synaptogyrin-2 influences replication of Porcine circovirus 2. *PLOS Genetics* **14**:10, e1007750. [Crossref]
16. Marcus Rebouças Santos, Viviane Sisdelli Assao, Fabiana de Almeida Araújo Santos, Rafael Locatelli Salgado, Ana Paula Carneiro, Juliana Lopes Rangel Fietto, Gustavo Costa Bressan, Márcia Rogéria de Almeida, Zelia Inês Portela Lobato, Carlos Ueira-Veira, Luíz Ricardo Goulart, Abelardo Silva-Júnior. 2018. Utilization of phage display to identify antigenic regions in the PCV2 capsid protein for the evaluation of serological responses in mice and pigs. *Archives of Virology* **163**:7, 1877-1887. [Crossref]
17. Bei Huang, Jiansheng Li, Xinchen Zhang, Qiling Zhao, Mingqing Lu, Yingjun Lv. 2017. RIG-1 and MDA-5 signaling pathways contribute to IFN-β production and viral replication in porcine circovirus virus type 2-infected PK-15 cells in vitro. *Veterinary Microbiology* **211**, 36-42. [Crossref]

18. Shanshan Zhu, Chunyan Zhang, Jing Wang, Li Wei, Rong Quan, Jiayu Yang, Xu Yan, Zixuan Li, Ruiping She, Fengjiao Hu, Jue Liu. 2016. Immunity Elicited by an Experimental Vaccine Based on Recombinant Flagellin-Porcine Circovirus Type 2 Cap Fusion Protein in Piglets. *PLOS ONE* **11**:2, e0147432. [Crossref]
19. Changhoon Park, Jiwoon Jeong, Kyuhyung Choi, Chanhee Chae. 2016. Efficacy of a new bivalent vaccine of porcine circovirus type 2 and Mycoplasma hyopneumoniae (Fostera™ PCV MH) under experimental conditions. *Vaccine* **34**:2, 270-275. [Crossref]
20. Zanotti Cinzia, Martinelli Nicola, Lelli Davide, Amadori Massimo. 2015. Correlates of Protection Following Vaccination with Inactivated Porcine Circovirus 2 Vaccines. *Viral Immunology* **28**:10, 600-608. [Abstract] [Full Text] [PDF] [PDF Plus] [Supplementary Material]
21. Tuija Kekarainen, Joaquim Segalés. 2015. Porcine circovirus 2 immunology and viral evolution. *Porcine Health Management* **1**:1. . [Crossref]
22. Joaquim Segalés. 2015. Best practice and future challenges for vaccination against porcine circovirus type 2. *Expert Review of Vaccines* **14**:3, 473-487. [Crossref]
23. Kristi L. Helke, Paula C. Ezell, Raimon Duran-Struuck, M. Michael Swindle. Biology and Diseases of Swine 695-769. [Crossref]
24. Hwi Won Seo, Changhoon Park, Kiwon Han, Chanhee Chae. 2014. Effect of porcine circovirus type 2 (PCV2) vaccination on PCV2-viremic piglets after experimental PCV2 challenge. *Veterinary Research* **45**:1. . [Crossref]
25. Taylor B Engle, Erin E Jobman, Timothy W Moural, Autumn M McKnite, Justin W Bundy, Sarah Y Barnes, Emily H Davis, Judith A Galeota, Thomas E Burkey, Graham S Plastow, Stephen D Kachman, Daniel C Ciobanu. 2014. Variation in time and magnitude of immune response and viremia in experimental challenges with Porcine circovirus 2b. *BMC Veterinary Research* **10**:1. . [Crossref]
26. Changhoon Park, Hwi Won Seo, Su-Jin Park, Kiwon Han, Chanhee Chae. 2014. Comparison of porcine circovirus type 2 (PCV2)-associated lesions produced by co-infection between two genotypes of PCV2 and two genotypes of porcine reproductive and respiratory syndrome virus. *Journal of General Virology* **95**:11, 2486-2494. [Crossref]
27. E. Pileri, M. Cortey, F. Rodríguez, M. Sibila, L. Fraile, J. Segalés. 2014. Comparison of the immunoperoxidase monolayer assay and three commercial ELISAs for detection of antibodies against porcine circovirus type 2. *The Veterinary Journal* **201**:3, 429-432. [Crossref]
28. Yeonsu Oh, Hwi Won Seo, Changhoon Park, Chanhee Chae. 2014. Comparison of sow and/or piglet vaccination of 3 commercial porcine circovirus type 2 (PCV2) single-dose vaccines on pigs under experimental PCV2 challenge. *Veterinary Microbiology* **172**:3-4, 371-380. [Crossref]
29. Hwi Won Seo, Jeehoon Lee, Kiwon Han, Changhoon Park, Chanhee Chae. 2014. Comparative analyses of humoral and cell-mediated immune responses upon vaccination with different commercially available single-dose porcine circovirus type 2 vaccines. *Research in Veterinary Science* **97**:1, 38-42. [Crossref]
30. Seydou Sylla, Yan-Long Cong, Yi-Xue Sun, Gui-Lian Yang, Xue-Mei Ding, Zhan-Qing Yang, Yu-Long Zhou, Minnan Yang, Chun-Feng Wang, Zhuang Ding. 2014. Protective immunity conferred by porcine circovirus 2 ORF2-based DNA vaccine in mice. *Microbiology and Immunology* **58**:7, 398-408. [Crossref]
31. Dipongkor Saha, Uladzimir U. Karniychuk, Liping Huang, Marc Geldhof, Merijn Vanhee, David J. Lefebvre, Peter Meerts, Richard Ducatelle, Jan V. Doorsselaere, Hans J. Nauwynck. 2014. Unusual outcome of in utero infection and subsequent postnatal super-infection with different PCV2b strains. *Virologica Sinica* **29**:3, 176-182. [Crossref]
32. Hwi Won Seo, Su-Jin Park, Changhoon Park, Chanhee Chae. 2014. Interaction of porcine circovirus type 2 and Mycoplasma hyopneumoniae vaccines on dually infected pigs. *Vaccine* **32**:21, 2480-2486. [Crossref]
33. Hwi Won Seo, Kiwon Han, Changhoon Park, Chanhee Chae. 2014. Clinical, virological, immunological and pathological evaluation of four porcine circovirus type 2 vaccines. *The Veterinary Journal* **200**:1, 65-70. [Crossref]
34. A. M. McKnite, J. W. Bundy, T. W. Moural, J. K. Tart, T. P. Johnson, E. E. Jobman, S. Y. Barnes, J. K. Qiu, D. A. Peterson, S. P. Harris, M. F. Rothschild, J. A. Galeota, R. K. Johnson, S. D. Kachman, D. C. Ciobanu. 2014. Genomic analysis of the differential response to experimental infection with porcine circovirus 2b. *Animal Genetics* **45**:2, 205-214. [Crossref]
35. A. Bielanski, J. Algire, A. Lalonde, A. Garceac, J.W. Pollard, C. Plante. 2013. Nontransmission of porcine circovirus 2 (PCV2) by embryo transfer. *Theriogenology* **80**:2, 77-83. [Crossref]
36. Paolo Borghetti, Marina Morganti, Roberta Saleri, Luca Ferrari, Elena De Angelis, Valeria Cavalli, Antonio Cacchioli, Attilio Corradi, Paolo Martelli. 2013. Innate pro-inflammatory and adaptive immune cytokines in PBMC of vaccinated and unvaccinated pigs naturally exposed to porcine circovirus type 2 (PCV2) infection vary with the occurrence of the disease and the viral burden. *Veterinary Microbiology* **163**:1-2, 42-53. [Crossref]

37. Bernhard Heißenberger, Christiane Weissenbacher-Lang, Isabel Hennig-Pauka, Mathias Ritzmann, Andrea Ladinig. 2013. Efficacy of vaccination of 3-week-old piglets with Circovac® against porcine circovirus diseases (PCVD). *Trials in Vaccinology* **2**, 1-9. [Crossref]
38. Xiang-Jin Meng. 2013. Porcine Circovirus Type 2 (PCV2): Pathogenesis and Interaction with the Immune System. *Annual Review of Animal Biosciences* **1**:1, 43-64. [Crossref]
39. Hwi Won Seo, Yeonsu Oh, Kiwon Han, Changhoon Park, Chanhee Chae. 2012. Reduction of porcine circovirus type 2 (PCV2) viremia by a reformulated inactivated chimeric PCV1-2 vaccine-induced humoral and cellular immunity after experimental PCV2 challenge. *BMC Veterinary Research* **8**:1. . [Crossref]
40. T.E. Cecere, X.J. Meng, K. Pelzer, S.M. Todd, N.M. Beach, Y.Y. Ni, T. LeRoith. 2012. Co-infection of porcine dendritic cells with porcine circovirus type 2a (PCV2a) and genotype II porcine reproductive and respiratory syndrome virus (PRRSV) induces CD4+CD25+FoxP3+ T cells in vitro. *Veterinary Microbiology* **160**:1-2, 233-239. [Crossref]
41. Chanhee Chae. 2012. Commercial porcine circovirus type 2 vaccines: Efficacy and clinical application. *The Veterinary Journal* **194**:2, 151-157. [Crossref]
42. Yeonsu Oh, Hwi Won Seo, Kiwon Han, Changhoon Park, Chanhee Chae. 2012. Protective effect of the maternally derived porcine circovirus type 2 (PCV2)-specific cellular immune response in piglets by dam vaccination against PCV2 challenge. *Journal of General Virology* **93**:7, 1556-1562. [Crossref]
43. Benjamin R. Trible, Alejandro Ramirez, Andrew Suddith, Alexandra Fuller, Maureen Kerrigan, Richard Hesse, Jerome Nietfeld, Baoqing Guo, Eileen Thacker, Raymond R.R. Rowland. 2012. Antibody responses following vaccination versus infection in a porcine circovirus-type 2 (PCV2) disease model show distinct differences in virus neutralization and epitope recognition. *Vaccine* **30**:27, 4079-4085. [Crossref]
44. Chanhee Chae. 2012. Porcine circovirus type 2 and its associated diseases in Korea. *Virus Research* **164**:1-2, 107-113. [Crossref]
45. Laila Darwich, Enric Mateu. 2012. Immunology of porcine circovirus type 2 (PCV2). *Virus Research* **164**:1-2, 61-67. [Crossref]
46. Nathan M. Beach, Xiang-Jin Meng. 2012. Efficacy and future prospects of commercially available and experimental vaccines against porcine circovirus type 2 (PCV2). *Virus Research* **164**:1-2, 33-42. [Crossref]
47. P.C. Gauger, K.M. Lager, A.L. Vincent, T. Opriessnig, M.E. Kehrli, A.K. Cheung. 2011. Postweaning multisystemic wasting syndrome produced in gnotobiotic pigs following exposure to various amounts of porcine circovirus type 2a or type 2b. *Veterinary Microbiology* **153**:3-4, 229-239. [Crossref]
48. Lester J. Pérez, Heidy Díaz de Arce, Martí Cortey, Patricia Domínguez, Maria I. Percedo, Carmen L. Perera, Joan Tarradas, Maria T. Frías, Joaquim Segalés, Llilianne Ganges, José I. Núñez. 2011. Phylogenetic networks to study the origin and evolution of porcine circovirus type 2 (PCV2) in Cuba. *Veterinary Microbiology* **151**:3-4, 245-254. [Crossref]
49. KwangSoo Lyoo, HanSoo Joo, Brian Caldwell, HyeunBum Kim, Peter R. Davies, Jerry Torrison. 2011. Comparative efficacy of three commercial PCV2 vaccines in conventionally reared pigs. *The Veterinary Journal* **189**:1, 58-62. [Crossref]
50. H. G. Shen, S. Schalk, P. G. Halbur, J. M. Campbell, L. E. Russell, T. Opriessnig. 2011. Commercially produced spray-dried porcine plasma contains increased concentrations of porcine circovirus type 2 DNA but does not transmit porcine circovirus type 2 when fed to naïve pigs. *Journal of Animal Science* **89**:6, 1930-1938. [Crossref]
51. Chun-Ming Lin, Chian-Ren Jeng, Shih-Hsuan Hsiao, Jen-Pei Liu, Chih-Cheng Chang, Ming-Tang Chiou, Yi-Chieh Tsai, Mi-Yuan Chia, Victor Fei Pang. 2011. Immunopathological characterization of porcine circovirus type 2 infection-associated follicular changes in inguinal lymph nodes using high-throughput tissue microarray. *Veterinary Microbiology* **149**:1-2, 72-84. [Crossref]
52. D. Kim, C.H. Kim, K. Han, H.W. Seo, Y. Oh, C. Park, I. Kang, C. Chae. 2011. Comparative efficacy of commercial Mycoplasma hyopneumoniae and porcine circovirus 2 (PCV2) vaccines in pigs experimentally infected with M. hyopneumoniae and PCV2. *Vaccine* **29**:17, 3206-3212. [Crossref]
53. Sumathy Puvanendiran, Suzanne Stone, Wanqin Yu, Craig R. Johnson, Juan Abrahante, Liza Garcia Jimenez, Theodor Griggs, Charles Haley, Bruce Wagner, Michael P. Murtaugh. 2011. Absence of porcine circovirus type 1 (PCV1) and high prevalence of PCV 2 exposure and infection in swine finisher herds. *Virus Research* **157**:1, 92-98. [Crossref]
54. Liping Huang, Yuehua Lu, Yanwu Wei, Longjun Guo, Changming Liu. 2011. Development of a blocking ELISA for detection of serum neutralizing antibodies against porcine circovirus type 2. *Journal of Virological Methods* **171**:1, 26-33. [Crossref]
55. S. Ramamoorthy, T. Opriessnig, N. Pal, F.F. Huang, X.J. Meng. 2011. Effect of an interferon-stimulated response element (ISRE) mutant of porcine circovirus type 2 (PCV2) on PCV2-induced pathological lesions in a porcine reproductive and respiratory syndrome virus (PRRSV) co-infection model. *Veterinary Microbiology* **147**:1-2, 49-58. [Crossref]
56. Huigang Shen, Chong Wang, Darin M. Madson, Tanja Opriessnig. 2010. High prevalence of porcine circovirus viremia in newborn piglets in five clinically normal swine breeding herds in North America. *Preventive Veterinary Medicine* **97**:3-4, 228-236. [Crossref]

57. Lester J. Pérez, Heidy Díaz de Arce, María T. Frías. 2010. Genetic characterization and phylogenetic analysis of porcine circovirus type 2 strains present in Cuban swine herds. *Research in Veterinary Science* **89**:2, 301-305. [Crossref]

58. Maria Fort, Marina Sibila, Miquel Nofrarías, Eva Pérez-Martín, Alex Olvera, Enric Mateu, Joaquim Segalés. 2010. Porcine circovirus type 2 (PCV2) Cap and Rep proteins are involved in the development of cell-mediated immunity upon PCV2 infection. *Veterinary Immunology and Immunopathology* **137**:3-4, 226-234. [Crossref]

59. T. Kekarainen, K. McCullough, M. Fort, C. Fossum, J. Segalés, G.M. Allan. 2010. Immune responses and vaccine-induced immunity against Porcine circovirus type 2. *Veterinary Immunology and Immunopathology* **136**:3-4, 185-193. [Crossref]

60. Mette S. Hansen, Charlotte K. Hjulsager, Vivi Bille-Hansen, Svend Haugegaard, Kitt Dupont, Peter Høgedal, Lars Kunstmann, Lars E. Larsen. 2010. Selection of method is crucial for the diagnosis of porcine circovirus type 2 associated reproductive failures. *Veterinary Microbiology* **144**:1-2, 203-209. [Crossref]

61. Tanja Opriessnig, Abby R. Patterson, Darin M. Madson, Narinder Pal, Sheela Ramamoorthy, Xiang-Jin Meng, Patrick G. Halbur. 2010. Comparison of the effectiveness of passive (dam) versus active (piglet) immunization against porcine circovirus type 2 (PCV2) and impact of passively derived PCV2 vaccine-induced immunity on vaccination. *Veterinary Microbiology* **142**:3-4, 177-183. [Crossref]

62. Tanja Opriessnig, John R. Prickett, Darin M. Madson, Hui-Gang Shen, Nicole M. Juhan, Roman M. Pogranichniy, Xiang-Jin Meng, Patrick G. Halbur. 2010. Porcine circovirus type 2 (PCV2)-infection and re-inoculation with homologous or heterologous strains: virological, serological, pathological and clinical effects in growing pigs. *Veterinary Research* **41**:3, 31. [Crossref]

63. Anna Tomás, Lana T. Fernandes, Armand Sánchez, Joaquim Segalés. 2010. Time course differential gene expression in response to porcine circovirus type 2 subclinical infection. *Veterinary Research* **41**:1, 12. [Crossref]

64. Per Wallgren, Inger Marit Brunborg, Gunilla Blomqvist, Gunnar Bergström, Frida Wikström, Gordon Allan, Caroline Fossum, Christine Monceyron Jonassen. 2009. The index herd with PMWS in Sweden: Presence of serum amyloid A, circovirus 2 viral load and antibody levels in healthy and PMWS-affected pigs. *Acta Veterinaria Scandinavica* **51**:1. . [Crossref]

65. Lakshman N.A. Gamage, John Ellis, Sidney Hayes. 2009. Immunogenicity of bacteriophage lambda particles displaying porcine Circovirus 2 (PCV2) capsid protein epitopes. *Vaccine* **27**:47, 6595-6604. [Crossref]

66. S. Ramamoorthy, F.F. Huang, Y.W. Huang, X.J. Meng. 2009. Interferon-mediated enhancement of in vitro replication of porcine circovirus type 2 is influenced by an interferon-stimulated response element in the PCV2 genome. *Virus Research* **145**:2, 236-243. [Crossref]

67. J. Gillespie, T. Opriessnig, X.J. Meng, K. Pelzer, V. Buechner-Maxwell. 2009. Porcine Circovirus Type 2 and Porcine Circovirus-Associated Disease. *Journal of Veterinary Internal Medicine* **23**:6, 1151-1163. [Crossref]

68. L. T. Fernandes, A. Tomás, A. Bensaid, M. Pérez-Enciso, M. Sibila, A. Sánchez, J. Segalés. 2009. Exploratory Study on the Transcriptional Profile of Pigs Subclinically Infected with Porcine Circovirus Type 2. *Animal Biotechnology* **20**:3, 96-109. [Crossref]

69. Sheela Ramamoorthy, Xiang-Jin Meng. 2009. Porcine circoviruses: a minuscule yet mammoth paradox. *Animal Health Research Reviews* **10**:1, 1-20. [Crossref]

70. T. Opriessnig, A. R. Patterson, D. M. Madson, N. Pal, M. Rothschild, D. Kuhar, J. K. Lunney, N. M. Juhan, X. J. Meng, P. G. Halbur. 2009. Difference in severity of porcine circovirus type two-induced pathological lesions between Landrace and Pietrain pigs1. *Journal of Animal Science* **87**:5, 1582-1590. [Crossref]

71. Anna Tomás, Lana T. Fernandes, Oliver Valero, Joaquim Segalés. 2008. A meta-analysis on experimental infections with porcine circovirus type 2 (PCV2). *Veterinary Microbiology* **132**:3-4, 260-273. [Crossref]

72. D.J. Lefebvre, P. Meerts, S. Costers, G. Misinzo, F. Barbé, K. Van Reeth, H.J. Nauwynck. 2008. Increased porcine circovirus type 2 replication in porcine leukocytes in vitro and in vivo by concanavalin A stimulation. *Veterinary Microbiology* **132**:1-2, 74-86. [Crossref]

73. T. Opriessnig, S. Ramamoorthy, D. M. Madson, A. R. Patterson, N. Pal, S. Carman, X. J. Meng, P. G. Halbur. 2008. Differences in virulence among porcine circovirus type 2 isolates are unrelated to cluster type 2a or 2b and prior infection provides heterologous protection. *Journal of General Virology* **89**:10, 2482-2491. [Crossref]

74. F. Madec, N. Rose, B. Grasland, R. Cariolet, A. Jestin. 2008. Post-Weaning Multisystemic Wasting Syndrome and Other PCV2-Related Problems in Pigs: a 12-Year Experience. *Transboundary and Emerging Diseases* **55**:7, 273-283. [Crossref]

75. Chun-Ming Lin, Chian-Ren Jeng, Hui-Wen Chang, Ing-Cherng Guo, Yu-Liang Huang, Yi-Chieh Tsai, Mi-Yuan Chia, Victor Fei Pang. 2008. Characterization of porcine circovirus type 2 (PCV2) infection in swine lymphocytes using mitogen-stimulated peripheral blood lymphocytes from healthy PCV2-carrier pigs. *Veterinary Immunology and Immunopathology* **124**:3-4, 355-366. [Crossref]

76. J. Gillespie, N.M. Juhan, J. DiCristina, K.F. Key, S. Ramamoorthy, X.J. Meng. 2008. A genetically engineered chimeric vaccine against porcine circovirus type 2 (PCV2) is genetically stable in vitro and in vivo. *Vaccine* **26**:33, 4231-4236. [Crossref]

77. Vicky Fachinger, Ralf Bischoff, Samir Ben Jedidia, Armin Saalmüller, Knut Elbers. 2008. The effect of vaccination against porcine circovirus type 2 in pigs suffering from porcine respiratory disease complex. *Vaccine* **26**:11, 1488-1499. [Crossref]

78. Danielle Gava, Eraldo L. Zanella, Nelson Morés, Janice R. Ciacci-Zanella. 2008. Transmission of porcine circovirus 2 (PCV2) by semen and viral distribution in different piglet tissues. *Pesquisa Veterinária Brasileira* **28**:1, 70-76. [Crossref]

79. D. J. Lefebvre, S. Costers, J. Van Doorsselaere, G. Misinzo, P. L. Delputte, H. J. Nauwynck. 2008. Antigenic differences among porcine circovirus type 2 strains, as demonstrated by the use of monoclonal antibodies. *Journal of General Virology* **89**:1, 177-187. [Crossref]

80. Tanja Opriessnig, Xiang-Jin Meng, Patrick G. Halbur. 2007. Porcine Circovirus Type 2?Associated Disease: Update on Current Terminology, Clinical Manifestations, Pathogenesis, Diagnosis, and Intervention Strategies. *Journal of Veterinary Diagnostic Investigation* **19**:6, 591-615. [Crossref]

81. Leanne S. Stevenson, Deirdre F. Gilpin, Alastair Douglas, Francis McNeilly, Irene McNair, Brian M. Adair, Gordon M. Allan. 2007. T Lymphocyte Epitope Mapping of Porcine Circovirus Type 2. *Viral Immunology* **20**:3, 389-398. [Abstract] [PDF] [PDF Plus]

82. Peter Meerts, Gerald Misinzo, David Lefebvre, Jens Nielsen, Anette Bøtner, Charlotte S Kristensen, Hans J Nauwynck. 2006. Correlation between the presence of neutralizing antibodies against porcine circovirus 2 (PCV2) and protection against replication of the virus and development of PCV2-associated disease. *BMC Veterinary Research* **2**:1. . [Crossref]

83. Peter Meerts, Gerald Misinzo, Prof. Hans J. Nauwynck. 2005. Enhancement of Porcine Circovirus 2 Replication in Porcine Cell Lines by IFN-γ Before and After Treatment and by IFN-α After Treatment. *Journal of Interferon & Cytokine Research* **25**:11, 684-693. [Abstract] [PDF] [PDF Plus]