

# The immunology of PCV2 diseases

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## Immunosuppressive characteristics of PCV2 infections

It is intriguing that although PCV2 represents one the smallest viruses with a tiny genome size of only approximately 1% of herpes viruses, it has successfully established itself in the pig population. Nevertheless, experimental infection of weaned pigs does mostly not result in disease or persistent infections, relating to the fact that in the field 99% of PWMS cases reported have been found to be associated with co-infections, such as PRRSV, M. hyopneumoniae, bacterial septicemia and pneumonia, influenza and parvovirus. One possible explanation for this could be an increased susceptibility of PCV2-infected animals to secondary infections as a consequence of immunosuppression, which is supported by studies from various disciplines (Table 1). Of particular importance are pathological studies of pigs with PWMS describing that severe lesions are always within the primary and secondary lymphoid tissue. The nature of these lesions with disintegration of lymphoid structures, lymphocyte depletion and macrophage infiltration will certainly result in severe suppression of immune responses.

**Table 1: Evidence for immunosuppressive characteristics of PCV2**

Epidemiological	99% of PWMS cases are associated with co-infection: PRRSV, M. hyopneumonia, bacterial pneumonia or septicemia, Influenza and other infections
Pathological	Primary lesions in lymphatic system including lymph nodes, spleen, gut mucosal lymphoid tissue with destruction of lymphoid architecture. Lymphopenia Thymus atrophy
Vaccinological	Reduction of anti-PRRSV vaccine efficiency observed
Virological	Persistent infection of macrophages and dendritic cells Infection of activated lymphocytes
Immunological	Inhibition of IFN- $\alpha$ , IL-6, IL-12 and TNF- $\alpha$ secretion from natural interferon producing cells Induction of tolerogenic IL-10 from monocytic cells

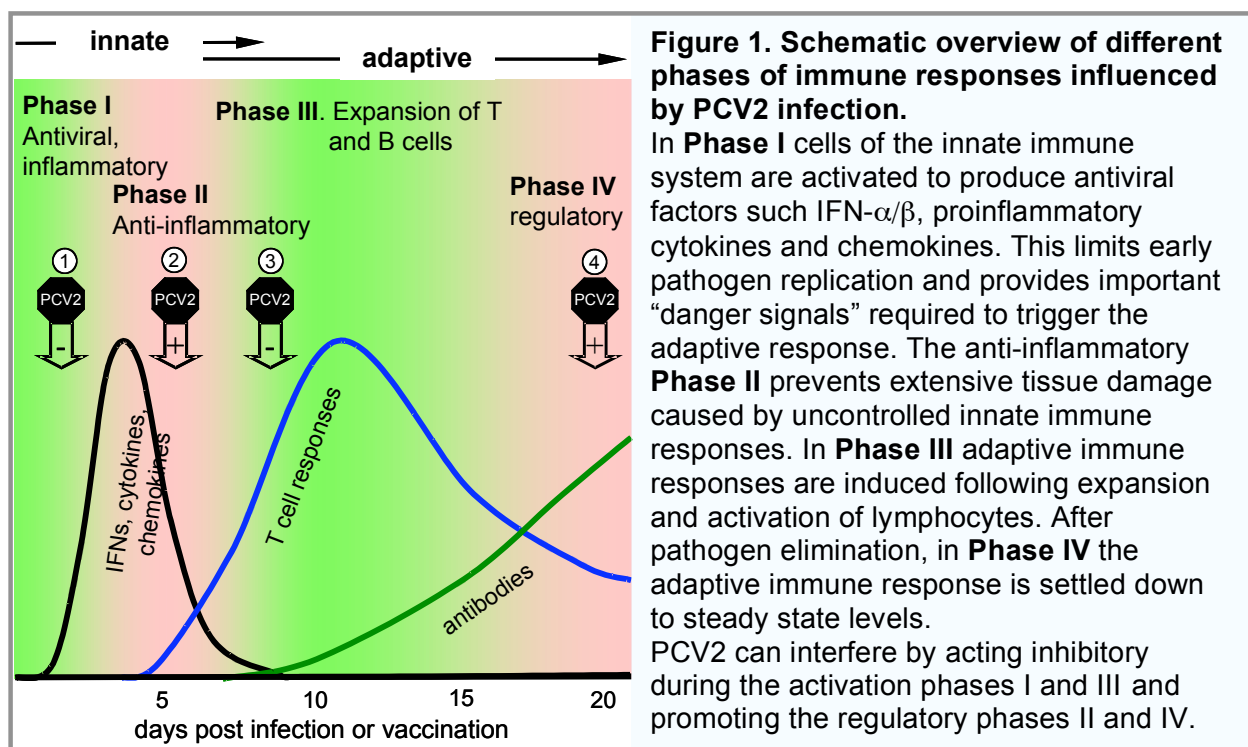
Based on this information researchers have studied how the virus interacts with the immune system. Although PCV2 probably replicates mainly in epithelial and endothelial cells, it can also infect cells of the immune system, including macrophages (M $\Phi$ ), dendritic cells (DC) and activated lymphocytes. While virus replication is inefficient M $\Phi$  and DC, PCV2 can persist and modulate the function of these cells, which play a central role in the immune system. DC and M $\Phi$  sense invading pathogens, mediate innate immune responses to suppress pathogen replication by cytokines, phagocytosis and reactive oxygen and nitrogen release. As "professional" antigen presenting cells, DC not only initiate adaptive immune responses but also control and regulate T-cell activity through production of stimulatory or inhibitory cytokines. M $\Phi$  are more important in phagocytic clearance, inflammatory reactions and for the control of tissue homeostasis.

To understand how PCV2 modulates the immune system it is most important to point out that all immune responses are tightly regulated in terms of a balance between immunostimulating and immunosuppressant triggers. For the latter, immunologists use the term

immunoregulation, and it has become unambiguous that this is important to avoid immune-mediated damage, such as severe uncontrolled inflammation, allergies or autoimmune diseases.

**Figure 1** illustrates how this process of immunoregulation generally operates during virus infections and where PCV2 interferes. The first phase (Phase I) of the innate immune response is triggered directly after infection through recognition of viral structures by pathogen recognition receptors in cells of the innate immune system including MΦ and DC. These cells produce antiviral factors such as interferon $\alpha/\beta$  (IFN $\alpha/\beta$ ), proinflammatory cytokines such as interleukins (IL-1 $\beta$ , IL-6, IL-12), tumor-necrosis-factor $\alpha$  (TNF $\alpha$ ), and chemokines to attract more leukocytes to the site of infection. Phase I is not only essential to limit early pathogen replication but also provides the immune system with the important “danger signals” required to trigger the adaptive response. If uncontrolled, this inflammatory response may convert into a “cytokine storm” which can cause extensive tissue damage and even be lethal. Therefore, the immune system will activate anti-inflammatory pathways in Phase II. Adaptive immune responses including T- and B-cell responses can only be detected a few days after innate immune responses, a consequence of the time consuming clonal expansion of very rare antigen specific lymphocytes during phase III. After pathogen elimination or control, the adaptive immune response is settled down to steady state levels in phase IV. This is resulting from apoptosis of pathogen specific lymphocytes and activation of regulatory cells of the immune system.

Current research indicates that PCV2 interferes with all four phases of the immune response in that it acts inhibitory during the activation phases and promotional during the pacification phases of both the innate and adaptive immune responses.

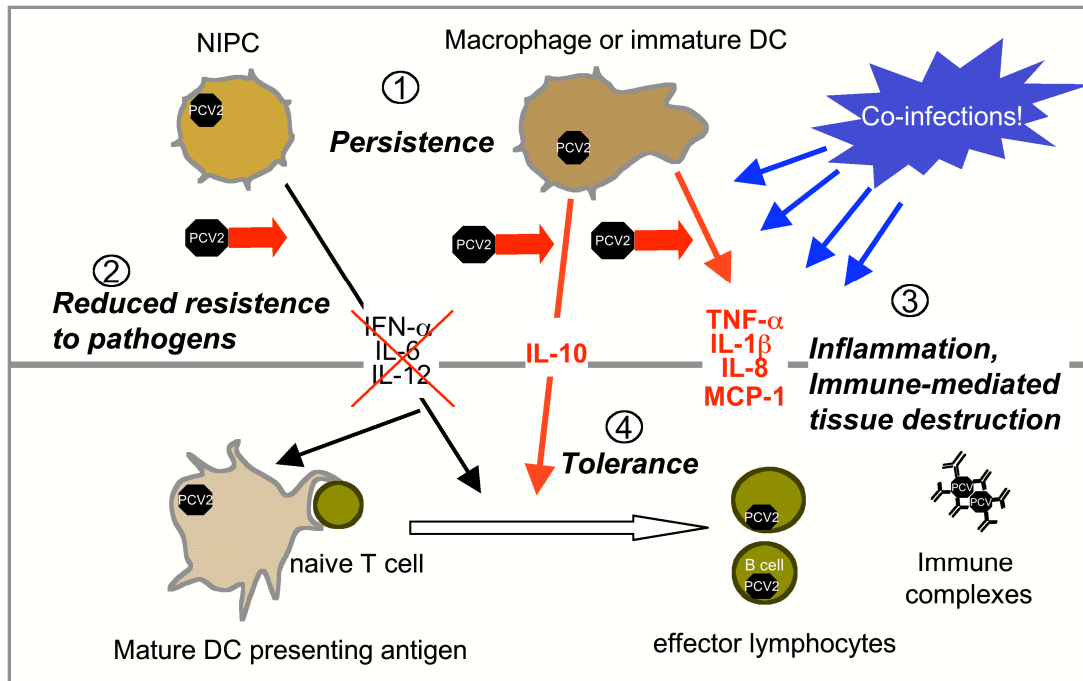


### Mechanisms of PCV2 compromise of immune responses

The concept proposed above is based on in vitro and ex vivo studies on cytokine response modulation by PCV2 summarized in **Figure 2**. It is important to note that this response is cell-type specific and will also be peculiar to a particular phase of the immune response

during infection. Furthermore, it will be critically influenced by co-infections with other pathogens.

As mentioned above, PCV2 infects and persists in MΦ and DC. Based on the migratory function of these cells to lymphoid tissue it has been proposed that this infection may represent a “Trojan horse” for PCV2 to its predilection sites.



**Figure 2. Overview of current knowledge on PCV2 immunomodulation.**

① PCV2 infects and persists in MΦ and DC and may use these cells a “Trojan horse” for virus transport into lymphoid tissue where it will modulate the function of various cell types.

② PCV2 inhibits the function of natural interferon producing cells (NIPC, also called plasmacytoid DC, pDC) to produce  $IFN_{\alpha/\beta}$ , IL-6 and IL-12. Particularly the inhibition of  $IFN_{\alpha/\beta}$  is important since NIPC are most potent producers of this cytokine plays a prominent role in controlling virus infections. In addition to its direct antiviral effect,  $IFN_{\alpha/\beta}$  together with other cytokines promoted the induction of adaptive immune responses through the induction of DC maturation and by enhancing cytotoxic T-cell and B-cell responses. DC that have not been matured are inefficient in stimulating effector and memory T-cell responses and even may promote antigen tolerance.

③ PCV2 can stimulation the production of pro-inflammatory cytokines ( $TNF_{\alpha}$ , IL-1β) and chemoattractants (IL-8 and MΦ chemotactic protein-1, MCP-1). These cytokines may be responsible for inflammatory responses and destruction found in lymphoid tissue of pigs with PWMS. A chronic stimulation of the immune system with such cytokines could also contribute to immune complex mediated diseases such as PDNS.

④ PCV2 promotes IL-10 secretion. During immune responses, this cytokine has an anti-inflammatory and tolerogenic role.

All these functional modulations are influenced by co-infection representing an important factor in disease development.

PCV2 inhibits the ability of natural interferon producing cells (NIPC, also called plasmacytoid DC) to produce  $TNF_{\alpha}$ , IL-6, IL-12 and  $IFN_{\alpha/\beta}$ . The latter is particularly important since NIPC

are the most important producers of IFN $\alpha/\beta$ , which plays a prominent role in controlling virus infections. In addition to its direct antiviral effect, IFN $\alpha/\beta$  together with other cytokines has a potent effect on the induction of adaptive immune responses through the induction of DC maturation and by promoting cytotoxic T-cell (CTL) responses and B-cell differentiation. DC that have not received such maturation signals are inefficient in stimulating effector and memory T-cell responses and may even promote antigen tolerance.

Although, PCV2 is inhibitory for the function of NIPC it has been reported to induced the stimulation of pro-inflammatory cytokines (TNF $\alpha$ , IL-1 $\beta$ ) and chemoattractants (IL-8 and M $\Phi$  chemotactic protein-1, MCP-1) after infection of M $\Phi$  in vivo and in vitro. These cytokines may be responsible for inflammatory responses and the destruction found in lymphoid tissue of pigs with PWMS. Furthermore, a chronic stimulation of the immune system with such cytokines could also contribute to immune complex mediated diseases such as PDNS.

In apparent opposition to these inflammatory responses is the IL-10 secretion found from leukocytes of pigs with PWMS. This cytokine has anti-inflammatory and tolerogenic effects and could thus have a dual role. On one hand it could limit inflammatory reactions, on the other hand it could suppress antiviral T-cell responses.

### **Protective immune responses**

Despite the immunomodulation described above, experimental PCV2 infection of weaned piglets have an very low morbidity and appear to be controlled by the immune system. Furthermore, vaccination programs against PCVD can be successfully applied. The nature of these protective immune responses is still unclear although the importance of neutralizing antibodies is indicated by studies showing a correlation between protection and the antibody levels. Nevertheless, after infection or vaccination, neutralizing antibodies do not appear before 21 days and it is unclear whether this relatively slow response is caused by the immunosuppressive characteristics of PCV2 or by poorly immunogenic neutralizing viral epitopes. Interestingly, PCV2-specific T cell-responses induced by infection also follow this slow kinetic and are generally weak. Their role in protection is not clear.

### **Summary**

An interdisciplinary look at PCVD strongly suggests that PCV2 can mediate immunosuppression. Nevertheless, the virus has pleiotropic effects on immune functions, which range from triggering to suppression of immune responses. While this is perplexing, it is not surprising considering the functioning of the immune system based on the interaction of highly specialized cell types within a regulatory network. Furthermore, a most important feature of the immune system is its "double-edge sword" nature, which requires a tightly regulated balance between stimulatory and regulatory elements. Our current understanding is that PCV2 has evolved by creeping into this system to hide from immune responses. PCV2 alone appears to be inoffensive but its immunomodulating activity together with co-infections and other husbandry factors contribute to the devastating effects of PCVD.

### **Further reading (reviews)**

Ramamoorthy S, Meng XJ. 2008. Porcine circoviruses: a minuscule yet mammoth paradox. *Anim Health Res Rev* 2:1-20.

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