

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

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Forty-one 8- to 12-week-old wasted pigs were selected from several conventional farms with histories of postweaning multisystemic wasting syndrome (PMWS) and classified into two groups according to their porcine circovirus type 2 (PCV2) infection status, as determined by *in situ* hybridization (ISH). Twenty-four pigs tested positive for PCV2 (PCV2-positive group), while 17 pigs tested negative for PCV2 (PCV2-negative group). In addition, eight uninfected healthy pigs from an experimental farm were used as controls. Heparinized blood samples were taken to obtain peripheral blood mononuclear cells. The CD4⁺, CD8⁺, CD4⁺ CD8⁺ (double-positive [DP]), and immunoglobulin M-positive (IgM⁺) cell subsets were analyzed by flow cytometry with appropriate monoclonal antibodies. Histopathological studies were done to evaluate the apparent degrees of lymphocyte depletion in different lymphoid organs (superficial inguinal and mesenteric lymph nodes, Peyer's patches, tonsils, and spleen) and to determine the viral load of the PCV2 genome by using an ISH technique. Animals of the PCV2-positive group showed a significant downshift of the CD8⁺ and DP cell subsets compared to the other groups ($P < 0.05$). Moreover, in PCV2-positive pigs, the amount of PCV2 genome in lymphoid tissues was related to the degree of cell depletion in those tissues ($P < 0.05$) as well as to the relative decrease in IgM⁺ and CD8⁺ cells in peripheral blood. These data support the notion that PCV2-positive pigs might have an impaired immune response.

Since its first description in 1996 by Clark and Harding (Abstr. Proc. West Can. Assoc. Swine Pract., abstr. 21 to 25, 1996), postweaning multisystemic wasting syndrome (PMWS) has been reported worldwide (2, 4, 9, 11, 13, 22; S. C. Kyriakis, S. Kennedy, K. Saoulidis, S. Lekkas, C. C. Miliotis, G. C. Balkamos, and P. A. Papoutsis, Abstr. 16th Int. Pig Vet. Soc. Congr., p. 633, 2000). This syndrome, which affects weaned and fattening pigs (8), is characterized by progressive weight loss or unthriftiness, dyspnea, enlarged lymph nodes, and, less frequently, pallor, jaundice, and diarrhea (5, 8). Viral detection techniques and nucleotide sequence analyses have shown an association between porcine circovirus type 2 (PCV2) and the presence of a characteristic histopathologic pattern of PMWS (7, 8, 12).

Nowadays, PCV2 is included with *Chicken anemia virus*, *Beak and feather disease virus*, and PCV1 in the family *Circoviridae*. Members of this family are small, nonenveloped viruses characterized by circular, single-stranded-DNA genomes (6, 16, 24). Infections caused by circoviruses have been associated with damage to lymphoid tissues resulting in extensive lymphocyte depletion (18, 25). For instance, chicken anemia virus infects the hemocytoblasts in the bone marrow and precursor T lymphocytes in the thymus (25), and beak and feather disease

virus infects macrophages and causes atrophy of lymphoid tissue in the thymus and bursa of Fabricius (25). Particularly, PMWS-affected pigs show histiocytic infiltration and lymphocyte depletion of both follicle centers and parafollicular zones, symptoms associated with the presence of PCV2 (18). Consequently, members of this viral family have been supposed to be immunosuppressive (1, 10, 14).

The effects of PCV2 on the pig immune system are not yet fully known, but it has been reported that the main target cells for PCV2 replication are the monocyte/macrophage lineage cells as well as other antigen-presenting cells such as follicular dendritic cells (18). In addition, PCV2 antigen in the nuclei of some lymphocyte subsets has been described (23). Recent studies have suggested that PCV2 infects dividing cells, macrophages, and B lymphocytes, inducing apoptosis of the B cells that leads to the depletion of the lymphoid organs (23). Moreover, studies of lymphocyte subsets showed that pigs suffering from PMWS had lower proportions of CD4⁺ and immunoglobulin M-positive (IgM⁺) cells in blood than healthy, uninfected pigs did (20). Taken together, these facts have led some to suggest that PCV2 infection might cause immunosuppression (20, 23). However, little is known regarding the immunological response of pigs suffering from PMWS and the mechanism by which PCV2 infection might result in the development of PMWS.

The aim of this study was to evaluate by means of flow cytometry the changes in lymphocyte subsets of peripheral blood of naturally PCV2-infected pigs suffering from PMWS as

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