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ORIGINAL ARTICLE

T-cell reprogramming through targeted CD4-coreceptor and T-cell receptor expression on maturing thymocytes by latent *Circoviridae* family member porcine circovirus type 2 cell infections in the thymus

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Although porcine circovirus type 2 (PCV2)-associated diseases have been evaluated for known immune evasion strategies, the pathogenicity of these viruses remained concealed for decades. Surprisingly, the same viruses that cause panzootics in livestock are widespread in young, unaffected animals. Recently, evidence has emerged that circovirus-like viruses are also linked to complex diseases in humans, including children. We detected PCV2 genome-carrying cells in fetal pig thymi. To elucidate virus pathogenicity, we developed a new pig infection model by *in vivo* transfection of recombinant PCV2 and the immunosuppressant cofactor cyclosporine A. Using flow cytometry, immunofluorescence and fluorescence *in situ* hybridization, we found evidence that PCV2 dictates positive and negative selection of maturing T cells in the thymus. We show for the first time that PCV2-infected cells reside at the corticomedullary junction of the thymus. In diseased animals, we found polyclonal deletion of single positive cells (SPs) that may result from a loss of major histocompatibility complex class-II expression at the corticomedullary junction. The percentage of PCV2 antigen-presenting cells correlated with the degree of viremia and, in turn, the severity of the defect in thymocyte maturation. Moreover, the reversed T-cell receptor/CD4-coreceptor expression dichotomy on thymocytes at the $CD4^+CD8^{interm}$ and CD4SP cell stage is viremia-dependent, resulting in a specific hypo-responsiveness of T-helper cells. We compare our results with the only other better-studied member of Circoviridae, chicken anemia virus. Our data show that PCV2 infection leads to thymocyte selection dysregulation, adding a valuable dimension to our understanding of virus pathogenicity.
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INTRODUCTION

Life is dependent on a fine-tuned immune system that balances self-tolerance and recognition of foreign antigens. T-cell maturation in the thymus is central to these distinctions. Any disturbance of this system during differentiation and maturation renders affected individuals susceptible to infection, autoimmunity, allergies, tumors and even aging. In the thymus, alphabeta- and a minority of gammadelta-T cells express the T-cell receptor (TCR) and the associated CD3 chains, including CD3ε. Alphabeta-T cells additionally express the CD4 and/or CD8 coreceptors that, together with the TCR, form the signaling module central to their maturation and peripheral T-cell function.[1] CD8-coreceptor-expressing T cells interact with major histocompatibility complex (MHC) class-I (MHC-I) presented ligands, and CD4-coreceptor expressing T cells interact with MHC class-II (MHC-II) presented ligands. During thymocyte maturation, CD4 and CD8 coreceptor double-positive (DP) T cells mature by migrating from the thymic cortex to the corticomedullary junction, leading to coreceptor single-positive (SP) T cells in the medulla. Thymocytes with the appropriate self-reactive signaling-module avidity survive by positive selection, and thymocytes with strong avidity are generally eliminated by negative selection.[2] Thymocytes that receive insufficient signals undergo death by neglect.[2] The signal strength is mostly dependent on the signaling module interaction with self-ligand-loaded MHC presented by thymic epithelial cells (TECs) and dendritic cells that migrate into the thymus.[3] Notably, persistent TCR and coreceptor signals favor CD4SPs and the cessation of coreceptor signaling results in CD8SPs.[4,5] Both lineages mature through the $CD4^+CD8^{interm}$ or even CD4SP stage, as described in the kinetic signaling model.[4,5] These naive T cells [6] with thymic predetermined T-cell specificity are tested again peripherally for self-reactivity. Self-ligand loaded MHCs causing persistent or strong signals through the signaling

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