Regulatory T cells in draining lymph nodes of Lawsonia intracellularis infection in pigs

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Lawsonia intracellularis infection in pigs cause diarrhoea and poor performance in growing pigs and is an important contributor to the high antibiotic usage in pig production. Experimentally, a primary subclinical L. intracellularis infection can induce protection against a secondary challenge infection. Although, immune responses to L. intracellularis infection have been investigated to a certain level, with IFN-γ being a key factor for development of protection, the role of Tregs is unknown. Activation of suppressive Tregs may play a role in the ability of L. intracellularis to survive in the infected host.

Four pigs were challenged twice with L. intracellularis infectious material, with four weeks interval. Lack of faecal shedding after the second challenge indicated the pigs were protected. The pigs developed L. intracellularis specific IgG responses and CMI responses in PBMCs confirmed Tc cells (CD3⁺CD4⁻CD8β⁺) and memory Th cells (CD3⁺CD4⁻CD8α⁻) being main producers of IFN-γ. Pigs were slaughtered 8 week after the second challenge and ileocacal lymph node cells (iLNC) and PBMCs were prepared and frozen.

With focus on identification and characterisation of Tregs, iLNC were co-cultured with porcine IL-2 and L. intracellularis antigen (Ag), Con A, or IL-2 alone. Before culture iLNC showed 1.4-4.0% Tregs (CD3⁺FoxP3⁺), which were mainly CD25⁺. ILNCs were around 20% CD4⁺CD8α⁺ T cells of which 6.3-10.7% were Tregs, whereas within CD4⁺CD8α⁻ T cells (37%) and CD4⁺CD8α⁺ T cells (35%) the levels of Tregs were 1.7-3.4% and 0.9-1.6%, respectively. The phenotype CD4⁺CD8α⁺ of Tregs may indicate these cells being induced (iTregs) compared to naturally occurring (nTregs) mainly CD4⁺CD8α⁻.

Co-culture for 6 days (CFSE proliferation assay) with IL-2 and Con A identified FoxP3⁺ cells among proliferating cells, however proliferation in Ag-cultures was at same level as without antigen.

Further characterisation of Tregs after L. intracellularis antigen culture of iLNC and PBMC will be performed.