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 T_{regs} is unknown. Activation of suppressive T_{regs} 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 T_C cells ($CD3^+CD4^-CD8\beta^+$) and memory T_H cells ($CD3^+CD4^+CD8\alpha^+$) 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 T_{regs}, 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 $CD25^h$. ILNCs were around 20% $CD4^+CD8\alpha^+$ T cells of which 6.3-10.7% were T_{regs}, whereas within $CD4^+CD8\alpha^-$ T cells (37%) and $CD4^-CD8\alpha^+$ T cells (35%) the levels of T_{regs} were 1.7-3.4% and 0.9-1.6%, respectively. The phenotype $CD4^+CD8\alpha^+$ of T_{regs} may indicate these cells being induced (iT_{regs}) compared to naturally occurring (nT_{regs}) mainly $CD4^+CD8\alpha^-$.
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 T_{regs} after *L. intracellularis* antigen culture of iLNC and PBMC will be performed.