

The effect of consecutive infection with *Toxoplasma gondii* strains on the IFN- γ production and parasitic load in tissues of experimentally infected pigs

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Background

T. gondii is an intracellular protozoan parasite of humans and animals. Severe clinical symptoms follow the infection, together with an important economic impact in wealth care and animal production. Main source of disease in humans is the consumption of raw or undercooked meat. The objective of this study was to determine the influence of subsequent infections of pigs with different *T. gondii* strains on parasitic load of tissues, and to study the accompanying immune response.

Methods

Two groups of seronegative pigs were consecutively inoculated with tissue cysts of *IPB-Gangji* strain and *IPB-LR* strain (group1: Gangji/LR, group2: LR/Gangji, respectively) and re-infected with the two respective strains at d60 PI. Group3 (Gangji ½ t, n=3) was inoculated with tissue cysts of *IPB-Gangji* strain at d60 PI. PBMC's were isolated and subsequently stimulated with total lysate antigen and concanavalin A to determine IFN- γ production upon infection by qRT-PCR. The parasitic load was determined by qPCR in the following tissues: brain, heart, spleen, skeletal muscles and diaphragm.

In another experiment two groups of seronegative pigs were inoculated with a single dose of *IPB-Gangji* strain (group4) or *IPB-LR* strain (group5). The PBMCs were isolated and cultured as described above. T-cell populations and intracellular IFN- γ production were detected and analyzed by flow cytometry.

Results

In all groups, brain and heart samples showed the highest parasitic load. In group1 most skeletal muscles contained parasitic DNA, while in group2 only few muscles tested positive. Moreover, the amount of DNA found in these samples was remarkably lower than in positive tissues of group1. Group3 showed a high and a moderate parasitic load in brain and two muscle samples, respectively. Interestingly, these positive tissues contained less parasite-derived DNA than the same tissues of group1 but more than those of group2, suggesting parasitic clearance.

IFN- γ detection by qRT-PCR showed an increased production in group1 and2, compared to control animals, but did not reach the maximum level in group3. In group4 and 5 the involvement of T-cell populations in cellular response was investigated by flow cytometry. The CD3⁺CD8^{high+} T-cells and, to a

lesser extent, the CD3⁺CD4⁺ population produced IFN- γ , whereas CD3⁺CD8^{intermediate+} showed fewest IFN- γ positive cells.

Conclusions

This study and previous data suggest that *IPB-Gangji* strain can induce a clearance of the infected tissues, irrespective of the strain of the initial inoculation. The cellular response to parasitic infection was demonstrated by the time-dependent and strain-independent increased IFN- γ production by mainly CD3⁺CD8^{high+} T cells.