Mycoplasma hyosynoviae (Mhs) is an extracellular parasitizing bacterium that causes arthritis in swine. The infection is widespread in modern pig production. An effective immunoprophylaxis is not available. In an experimental infection study (Lauritsen, K. T., et al., 2009. 3rd EVIW Poster, Berlin (Germany) September 10-13, 2009), testing a new vaccine (formalin inactivated Mhs and EMULSIGEN-BCL® (MVP Laboratories)), cell-mediated immune response was measured in the vaccine group (n=13) and not in the placebo group (n=13). In an IFN-γ assay, where whole-blood was co-cultured with Mhs-antigen and recombinant IL-18, significantly higher level of IFN-γ was produced in the vaccine group compared to the placebo group one day before challenge with Mhs. In contrast, significantly higher level of IFN-γ was measured in the placebo group compared to the vaccine group at day 6 after challenge. The latter could be due to increased systemic infection in the placebo group. Cell-mediated immune response was further characterised by four colour flow cytometry analysis of peripheral blood mononuclear cells (PBMCs) before Mhs challenge (day -1) and at days 6 and 9 after challenge. IFN-γ producing cells were found to be CD4 and especially CD4CD8 double positive T-cells simultaneously expressing CD25. Interestingly, the proportion of CD4CD8 double positive T-cells within the total population of CD4 positive cells increased in the vaccine group after challenge, indicating that generation of specific T-cell memory had occurred.