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CD4+ T-cell lines used to evaluate a *Mycobacterium avium* subsp. *paratuberculosis* (MAP) peptide vaccine

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The aim of the study was to establish a protocol for generation of MAP-specific T-cell lines and to use these lines for evaluation of a peptide vaccine.

A protocol for culturing T-cell lines from peripheral blood of goats naturally infected with MAP was established. CD4+ T cells were positively selected using an anti CD4 mAb and Dynabeads. Sorted CD4+ cells were cultivated with purified protein derivative from MAP (PPDj) or *E. coli* sonicate, IL-2, and IL-15. After two cultivation cycles, T cells were tested for recall responses in a proliferative T-cell assay. T-cell line responses were in average 92 % for PPDj, and -3 % for *E. coli* sonicate. CD4+ T-cell lines stimulated with PPDj showed a 6 fold increase in IFN- γ production compared to controls. These results indicated that the T-cell lines were MAP-specific.

The protocol was subsequently used to evaluate MAP-specific peptides as vaccine antigens. T-cell lines were now generated by cultivating CD4+ cells with peptides instead of PPDj. Initially, both healthy and MAP-infected goats were vaccinated with 119 peptides defined by *in silico* analysis. Cellular responses to the peptides were not detected using standard IFN- γ plasma ELISA. However, testing of T-cell lines from the MAP-infected goats identified peptides that induced strong proliferative responses. The 23 peptides inducing the strongest responses were used in a second vaccination trail with healthy goat kids. Vaccinated kids developed strong IFN- γ and antibody responses, and these MAP-specific peptides show great potential for use in a subunit vaccine.

Generation of T-cell lines was a valuable tool for selecting MAP vaccine antigens, and the protocol can also be applied for identifying vaccine candidates for other diseases.