

Vaccines against tuberculosis: identification and characterization of immune parameters involved in immunological protection

Tuberculosis, caused by the intracellular pathogen *Mycobacterium tuberculosis*, is an increasing health issue, and a more effective vaccine is urgently needed. Here we focused on the mycolyl transferase antigen 85A (Ag85A, Rv3804c) of *M. tuberculosis* for the development of a new vaccine. A fusion protein consisting of Ag85A and the outer membrane lipoprotein I (OprI) from *Pseudomonas aeruginosa*, a documented TLR2/TLR4 trigger, was used in systemic and intranasal vaccination protocols. Despite increased type 1 immune responses, boosting with OprI-Ag85A did not increase protective efficacy against *M. tuberculosis* of either plasmid DNA or BCG in this experimental setting. In an attempt to increase cross-priming induced by Ag85A-encoding DNA through apoptosis, 3 sequences encoding caspase fragments were inserted in the vaccine. These fragments consisted of caspase-2 prodomain (C2P), followed or not by wild type (csp(wt)) or catalytically inactive mutated caspase-3 (csp(mut)). Vaccination with pro-apoptotic DNA increased antigen specific IFN- γ production and triggered more efficiently IL-2 and IFN- γ producing memory cells in spleen and lungs after *M. tuberculosis* challenge. Only vaccination of mice with Ag85A-DNA co-expressing csp(wt) increased protection after infection with *M. tuberculosis* as compared to vaccination with Ag85A-DNA, while vaccination with plasmid co-expressing csp(mut) was not protective, possibly due to the induction of IL-6, IL-10 and IL-17A production. Ag85A-DNA priming followed by BCG boosting can enhance BCG mediated protection, and this can be further enhanced by co-expression of C2P in the priming plasmid. At 16 weeks of *M. tuberculosis* infection, BCG vaccination increased expression of the transcription complex HAF-1a (consisting of IRF1, IRF8 and PU.1) in lung macrophages compared to control vaccinated and TB infected mice. Decreased expression of the pro-inflammatory chemokines CCL5 and CCL8 and of the type 2 cytokine receptor IL-4R α in TB infected mice correlated with increased protection. Unraveling of the signal transduction revealed that the transcription factor STAT3 lies downstream of these molecules, and further analysis of its target genes may clarify the protective mechanism.