Mycobacterium tuberculosis related T-cell epitope peptide-based vaccine candidates

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The most challenging factor in preventing tuberculosis (TB) is the ability to provide immunity against the multiple stages of the pathogen and provide cross-protection within the subtypes. Immunization with a multivalent subunit vaccine, which combines multiple antigens derived from different stages of the pathogen's life cycle, hold promise for overcoming the major obstacles. The use of minimal epitope sequences which can trigger the desired immune response is a safe and reliable approach however, the low immunogenicity of relatively small peptides needs to be addressed.

In this study, promiscuous T-cell epitopes from different proteins expressed by *Mycobacterium tuberculosis* (Rv1886c, Rv0341, Rv3873) were selected based on previously reported antigenic properties. To induce a more potent immune response, epitope peptides were conjugated to a Tuftsin carrier in branched chain arrangement. The trivalent conjugate showed higher tendency to fold and increased internalization to professional antigen presenting cells. Cellular uptake was further improved by the incorporation of a palmitoyl group to the conjugate. Immunization of CB6F1 mice with the conjugates resulted in significantly higher T-cell proliferation with prominent expression of IFN-γ, IL-2 and IL-10 cytokines, compare to free epitopes. To enhance bioavailability and vaccine efficacy the multi-epitope conjugate was encapsulated to poly(D,L-lactic-*co*-glycolic acid) (PLGA) nanoparticles. As adjuvant, trehalose-6,6-dibehenate (TDB) was used, which is a synthetic analogue of trehalose-6,6-dimycolate, the most studied immunostimulatory cell wall component of *Mycobacterium tuberculosis*. Characterization (DLS, SEM analysis) of PLGA constructs revealed that the size of the nanoparticles was between 100-120 nm and the encapsulation efficacy of the multiepitope conjugate was 80%. The injection site clearance, which was followed by MRI, was significantly slower in the case of PLGA encapsulated conjugate compared to the free conjugate. Vaccine efficacy of the compounds was evaluated in a murine

model of tuberculosis. In histologic sections prepared from the organs of un-vaccinated control animals, rod-shaped acid-fast bacteria were observed within small groups of epitheloid macrophages. This indicates, that the used infection method was successful in modelling experimental tuberculosis. When mice were immunized with the nanoencapsulated constructs, significantly lower number of bacteria were enumerated compare to the un-vaccinated control group. Finally, the results highlight the importance of appropriate formulation of epitope peptides which allow developing epitope-based vaccine candidates against tuberculosis.