Tuning CD8$^+$ T cell Multifunctionality with Peptide Self-assemblies

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Statement of Purpose: Mycobacterium tuberculosis (MTb) is an opportunistic pathogen that causes nearly 2 million deaths and over 8 million new or reactivated infections each year worldwide[1]. It is known that CD4$^+$ and CD8$^+$ T cells are required to mount an effective immune response and IFN-γ and TNF-α are key cytokines in fighting MTb infection[1]. While IFN-γ is crucial in the protective immune response to MTb, it is not sufficient on its own. Multifunctional CD8$^+$ T cells that produce IFN-γ/ TNF-α/IL-2 have been associated with lower risk of reactivation of latent infection and enhanced control of active infection[2]. BCG is the only currently approved vaccine against MTb and only provides transient protection in early childhood[1]. Thus, there is an urgent need to develop new vaccination strategies that can elicit antigen-specific multifunctional CD8$^+$ T cells against MTb. We have previously reported a self-assembling peptide nanofiber platform that can elicit high levels of IFN-γ producing CD8$^+$ T cells and protect against viral infections[3]. In this work, we investigated whether self-assembling peptide (KFE8) nanofibers displaying multiple MTb epitopes (ESAT6 and TB10.4) or toll-like receptor (TLR) agonists (MALP, a TLR-2 agonist) can be co-assembled to generate vaccines capable of eliciting higher levels of IFN-γ$^+$ CD8$^+$ T cells and multifunctional CD8$^+$ T cells that produce IFN-γ/TNF-α/IL-2. Our data indicates that co-assembled peptide nanofiber vaccines bearing TB10.4 and ESAT6 elicit significantly higher levels of IFN-γ$^+$ CD8$^+$ T cells compared to single epitopes and inclusion of MALP leads the production of multifunctional CD8$^+$ T cells in mouse models.

Methods: Peptides, ESAT6 (QQWNFAGI) and TB10.4 (IMYNYPAM) were coupled to KFE8 (FKFEFKFE) via a short proteolytic cleavage linker GGAAY. MALP was conjugated through a cysteine linker to the N-terminus of KFE8. All peptides were synthesized on a CS Bio 336 XT peptide synthesizer and purified to >95% by reverse phase HPLC. ESAT6-KFE8 and TB10.4-KFE8 were co-assembled (1:1) and MALP-KFE8 and TB10.4-KFE8 were co-assembled (1:5) by mixing dry powders, solubilized in water, and fibrillized in PBS. Co-assembled nanofibers were visualized using transmission electron microscopy (TEM). C57BL6 mice were immunized in the footpad with 100 nmol of co-assembled formulations, boosted on day 28, and sacrificed on day 32. The draining lymph nodes were excised and isolated lymphocytes were stimulated in vitro for 6h at 37°C, stained, and analyzed for cytokines IFN-γ, TNF-α, and IL-2 by flow cytometry.

Results: TEM images indicated that co-assembled formulations formed nanofibers in physiological buffers similar to individual peptides (data not shown). In mice vaccinated with co-assembled nanofibers of ESAT6 and

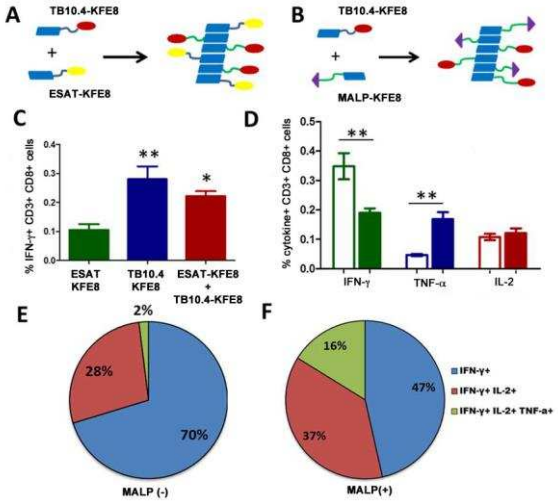

Figure 1. Schematic showing co-assembly of peptide nanofibers bearing different epitopes (A) or toll-like receptor agonists (B). IFN-γ$^+$ CD8$^+$ T cells in mice vaccinated with individual or co-assembled nanofibers of ESAT6 and TB10.4 (C). Cytokine production by CD8$^+$ T cells in mice vaccinated with TB10.4 nanofibers alone (open bars) or co-assembled with MALP nanofibers (closed bars) (D). Percentage of multifunctional CD8$^+$ T cells in mice vaccinated with nanofiber adjuvants alone (E) or in combination with a TLR-2 agonist (F). *p< 0.05 and **p< 0.01 by ANOVA using Tukey post-hoc test.

TB10.4, the lymphocytes were stimulated ex vivo with both antigens, and significantly higher levels of IFN-γ$^+$ CD8$^+$ T cells were observed compared to mice vaccinated with ESAT6 nanofibers alone (Fig 1C). No significant differences in IFN-γ$^+$ CD8$^+$ T cells were observed between mice vaccinated with TB10.4 nanofibers alone or co-assembled nanofibers of ESAT6 and TB10.4. Due to lack of commercially available tetramers for ESAT6 and TB10.4 we did not look at antigen-specific populations. Nonetheless the data validates the use of nanofiber platform for delivering multiple epitopes. Co-assembled nanofibers of TB10.4 and MALP elicited CD8$^+$ T cells that produced multiple cytokines notably TNF-α which, was not significantly expressed by vaccinating with TB10.4 nanofibers alone (Fig. 1D). An 8-fold (2% to 16%) increase in multifunctional CD8$^+$ T cells (IFN-γ/TNF-α/IL-2) was observed in mice vaccinated with co-assembled nanofibers of TB10.4 and MALP (Fig. 1E-1F).

Conclusions: In conclusion, self-assembling peptide nanofibers can be used for developing co-assembled MTb vaccines that can incorporate multiple epitopes/TLR-agonists for eliciting robust multifunctional CD8$^+$ T cells.

References: 1. Woodworth et al. Crit Rev Immunol 2006, 26:317-52, 2. Sutherland et al. J Immunol 2010, 184:6537-44, 3. Chesson et al. Vaccine 2014, 32:1174-80.