

P014 Peptides complexed to HSP70 Generate Efficient Human CTL Responses

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Microbial heat shock proteins are implicated in the induction of the innate and adaptive arms of the immune response. We set out to determine whether peptides complexed to HSP70 generate efficient CTL responses. Human dendritic cells (DC) pulsed with peptide-loaded microbial HSP70 complexes generate potent antigen specific cytotoxic lymphocyte (CTL) responses, that are dependent on an HSP70-stimulated calcium signalling cascade. Using fluorescence anisotropy we have calculated the peptide binding affinity of mycobacterial HSP70 ($K_D = 14\mu\text{M}$) and show that 1-200pM HSP70-bound peptide is sufficient to generate a peptide specific CTL response that is 10 000 times more efficient than peptide alone. Through the generation of mycobacterial HSP70 truncations we find that the minimal 136 amino acid, mycobacterial HSP70 peptide binding domain is sufficient to generate CTL responses. The design of an HSP70 mutant, in which the peptide binding site of HSP70 is filled with a bulky hydrophobic residue, leads to a decrease in the peptide binding affinity. This mutant HSP70 retains stimulatory capacity but is unable to generate DC and has for the first time separated antigen delivery from immunostimulation of DC.