Title:	A Combinatorial in-silico, in-vitro and in-vivo Approach to Quantitatively Study Peptide Induced MHC Stability
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Abstract:	Here, we describe a combinatorial approach in reverse vaccinology to identify immunogenic class I major histocompatibility complex (MHC) displayed epitopes derived from a morbillivirus named pestes des petits ruminants (PPRV). The protocol describes an in silico prediction of immunogenic epitopes using an IEDB tool. The predicted peptides were further analysed by molecular docking with mouse class I MHC (H-2Kb), to assess their binding affinity, and their immunogenicity was validated, using acellular and cellular assays. Finally, an enumeration of the expanded PPRV-specific CD8+ T cells in infected or immunized mice against the immunogenic peptides was performed ex vivo. Synthetic peptide derivatives from different structural and non-structural proteins of PPRV were used to measure the extent of stabilized H2-Kb, using an ELISA based acellular assay and TAP deficient RMA/s cells. Fluorescently labelled H2-Kb-tetramers were generated by displacing a UV photocleavable conditional ligand with the PPRV-peptides. The resulting reagents were used to identify and enumerate virus-specific CD8+ T cells in immunized or PPRV-infected mice. The combinatorial approach described here could be used to identify immunogenic epitopes of any pathogen, autoantigens, as well as cancer antigens. Graphic abstract: Figure 1.General schematic to identify immunogenic peptides and their stabilization on MHC I molecule.
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