From Viral genome to specific peptide epitopes - Methods for identifying porcine T cell epitopes based on in silico predictions, in vitro identification and ex vivo verification

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Title (26 words):
From Viral genome to specific peptide epitopes - Methods for identifying porcine T cell epitopes based on *in silico* predictions, *in vitro* identification and *ex vivo* verification.

Abstract (250 words):
The affinity for and stability of peptides bound by major histocompatibility complex (MHC) class I molecules are instrumental factors in presentation of viral epitopes to cytotoxic T lymphocytes (CTLs). In swine, such peptide presentations by swine leukocyte antigens (SLA) are crucial for swine immunity during viral infections and disease. Here we combine the ability of complete nonamer peptide based binding matrices for three different SLA proteins to predict good candidates for peptide-SLA (pSLA) binding with that of an online available algorithm, NetMHCpan. Further we analyze the correlation between high affinity and high stability peptides bound by the highly expressed SLA molecules, SLA-1*0401, SLA-2*0401, and SLA-3*0401, using a luminescence oxygen channeling (LOCI) and a scintillation proximity assay, respectively. With this procedure, high affinity and highly stable SLA peptide epitopes can be identified within a given viral genome, along with the elimination of hundreds, or even thousands, of peptide sequences, which are not likely to be bound. Applying these methods can save enormous amounts of time and costs of epitope discovery studies and MHC binding analysis not only in swine but in almost any species of interest. Finally, peptide candidates of interest were verified as actual T cell epitopes using peptide-SLA complexes assembled into fluorescent tetramers to stain influenza-specific CTLs derived from vaccinated animals. From 20 such animals 16 had the correct SLA allele match and 7 of these qualified as potential candidates for tetramer staining. From the 7 animals 3 responded with a positive tetramer staining of 1% or higher.