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Tracking the elusive cytotoxic T cell response in pigs

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Quantitative and qualitative assessment of antigen-specific cytotoxic T cell (CTL) responses in pigs is not a straightforward process. Through the years we have developed a series of reagents, tools and protocols to characterize peptide-specific CTL responses in pigs.

The most common recombinant **SLA heavy chains were produced** and peptide binding motifs were determined by assays measuring the **affinity and stability of the peptide-SLA complex** (pSLA) interaction. These results have been used to **train neural networks** to predict the binding of any pSLA (http://www.cbs.dtu.dk/services/). Recombinant SLA molecules complexed with verified binding peptides can be assembled to **SLA multimers for staining of peptide-specific CTLs**, and measured by flow cytometry, as we have shown with **FMDV and influenza**. This, however, requires **SLA-matched pigs** for which we have developed two methods: a sequence-based, **high-resolution SLA genotyping** method by standard PCR for specific detection of eight in-house SLA molecules; and a **next-generation sequencing** method for parallel detection of up to 50 samples of barcoded cDNA PCR products spanning exon 2 and 3. The latter for a wider characterization of expressed alleles in candidate pigs.

The *in vivo* generation of CTL responses to antigens following peptide immunizations is thought to require **cross-presentation in appropriate dendritic cells (DC)**. In mice this was linked to targeting of $CD103^{+}$DCs recruited after intraperitoneal immunizations. We have therefore developed a protocol for **intraperitoneal delivery of peptides** formulated in poly(I:C)/MMG-decorated liposomes (**CAF09**) to investigate the influence of peptide dose on the generation of CTL vs. antibody responses. Finally, the induced CTL killing was assessed by an ***in vivo* cytotoxicity assay**, where purified autologous PBMCs, fluorescently labeled and pulsed with target peptides, were reinjected into the donor. The *in vivo* killing of peptide-pulsed cells was measured by flow cytometry relative to non-pulsed PBMCs at different time points after cell transfer.