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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.