Development of a rapid in vitro protein refolding assay which discriminates between peptide-bound and peptide-free forms of recombinant porcine major histocompatibility class I complex (SLA-I)

The extracellular domains of swine leukocyte antigen class I (SLA-I, major histocompatibility complex protein class 1) were cloned and sequenced for two haplotypes (114 and H7) which do not share any alleles based on serological typing, and which are the most important in Danish farmed pigs. The extracellular domain of SLA-I was connected to porcine beta2 microglobulin by glycine-rich linkers. The engineered single-chain proteins, consisting of fused SLA-I and beta2 microglobulin, were overexpressed as inclusion bodies in Escherichia coli. Also, variants were made of the single-chain proteins, by linking them through glycine-rich linkers to peptides representing T-cell epitopes from classical swine fever virus (CSFV) and foot-and-mouth disease virus (FMDV). An in vitro refold assay was developed, using a monoclonal anti-SLA antibody (PT85A) to gauge refolding. The single best-defined, SLA-I restricted porcine CD8(+) T-cell epitope currently known is a 9-residue peptide from the polyprotein of CSFV (J. Gen. Virol, 76 (1995) 3039). Based on results with the CSFV epitope and two porcine haplotypes (H4 and H7), the in vitro refold assay appeared able to discriminate between peptide-free and peptide-occupied forms of SLA-I. It remains to be seen whether the rapid and technically very simple in vitro refold assay described here will prove generally applicable for the screening of virus-derived peptides for SLA-I binding.

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