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Short communication

Pre-screening of crude peptides in a serological bead-based suspension array

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ABSTRACT

Most serological assays detect antibody responses in biological samples through affinity of serum antibodies for antigens provided in the assay. Certain antigens, however, may be difficult to produce and/or may contain unwanted epitopes. In these cases, a practical alternative may be the use of peptides as representatives for specific epitopes. Peptides can be obtained after purification in large quantities for a modest price, but screening of a large set of peptides during development may be relatively expensive. To cut costs of screening peptides for a new serological assay, the concept was investigated of using cheap non-purified (crude) peptides instead of purified peptides.

Peptides were selected that represent three well-described linear epitopes of viral proteins: VP2 of canine parvovirus (CPV), gp41 of human immunodeficiency virus (HIV) and E2 of classical swine fever virus (CSFV). Crude and purified biotinylated peptides with either a short or long spacer between the biotin and the epitope were used to test their capability to bind antibodies in a bead-based suspension array.

The results show that, in a bead-based suspension array, crude peptides can function as antigen for specific monoclonal antibodies, and that the acquired signals are less than with purified peptides. CSFV-derived crude peptides were also able to detect specific antibodies in swine serum, indicating the applicability of crude peptides for pre-screening large numbers of different peptides during the development of serological peptide-based assays.

Using proteinaceous antigens in serological assays may sometimes be impractical due to production issues and/or to the presence of non-specific epitopes that exist in addition to specific epitopes. Peptides are a practical alternative as they can be synthesized with relative little effort and will only comprise a limited number of epitopes. For assays that aim to detect more than one target, recent developments have enabled assay designs with panels of antigens that can be used to probe for multiple serum antibodies. These include the use of peptides as antigens and suspension arrays as assay platform (Christopher-Hennings et al., 2013; Cretich et al., 2006). Various peptide-based suspension arrays have been described that demonstrate the viability of this approach for multiplex serology (Blomberg et al., 2012; Drummond et al., 2008; Perkins et al., 2006). A similar approach was chosen when setting up a bead-based suspension array to detect antibodies against the E2 protein of pestivirus classical swine fever virus (CSFV) in swine serum (Van der Wal et al., 2016). Very recently, a pestivirus suspension array was reported, using recombinant pestivirus proteins (CSFV E2 and E^{rns}, bovine viral diarrhea virus (BVDV) E^{rns}) to detect antibodies in swine sera (Xia et al., 2015). For an unambiguous differentiation however, use of 'isolated' peptide epitopes might be better to circumvent cross-reactions of antibodies against the immunodominant E2 and E^{rns} proteins, which are shared by all pestiviral species (Loeffen et al., 2009).

Although large quantities of purified peptides can be obtained for a modest price, during assay development considerable numbers of different peptides may need screening. During the development of the aforementioned pestivirus serological assay the need arose for an inexpensive method of screening peptides for their suitability in a bead-based suspension array. Crude peptides may facilitate this, given that they can be synthesized for a low price on a small scale and need no purification prior to use. After initial testing in the preferred assay format (suspension array), only a subset of peptides would need large scale synthesis and purification to enable further testing and completion of the envisioned assay.

Abbreviations: AEEA, amino ethoxy ethoxy acetic acid; MFI, median fluorescence intensity; PBST, phosphate buffered saline with Tween; PE, phycoerythrin; VNT, virus neutralization test

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Table 1
Peptides. Epitopes for CPV VP2, HIV gp41, and CSFV E2, were synthesized as biotinylated peptides in a crude format (4 μmol scale, no purification) or on a large scale (1 mg) followed by purification with pHPLC (Pepscan, Lelystad, The Netherlands). For VP2 and gp41 peptides, biotins were separated from the epitope by one (sp1) or five (sp5) [2-(2-aminoethoxy) ethoxy] acetic acid (AEEA) spacers. The length of one AEEA spacer comprises maximal 9.6 Å. For E2 peptides, biotins were separated from the epitope by five AEEA spacers; for one peptide set, a stretch of ten amino acids that precede the epitope in the E2 protein, was incorporated (italics). The type of the peptides is indicated by the last character in their designations (P, purified; C, crude). Purity was determined by UV spectrophotometry.

Target	Designation	Composition	Purity (%)
CPV VP2	CPV1-P	biotin-sp1-GGQPAVRNE	99.5
	CPV1-C	biotin-sp1-GGQPAVRNE	58.8
	CPV2-P	biotin-sp5-GGQPAVRNE	100.0
	CPV2-C	biotin-sp5-GGQPAVRNE	59.2
HIV gp41	HIV1-P	biotin-sp1-AQELLELDKWASLA	100.0
	HIV1-C	biotin-sp1-AQELLELDKWASLA	48.4
	HIV2-P	biotin-sp5-AQELLELDKWASLA	100.0
	HIV2-C	biotin-sp5-AQELLELDKWASLA	43.0
CSFV E2	CSFV3-P	biotin-sp5-TAVSPTTLRTEVV	90.2
	CSFV3-C	biotin-sp5-TAVSPTTLRTEVV	43.6
	CSFV2-P	biotin-sp5-*PIGWTGVIES*TAVSPTTLRTEVV	90.3
	CSFV2-C	biotin-sp5-*PIGWTGVIES*TAVSPTTLRTEVV	42.1

To test this concept, peptides were selected that represent three well-described linear epitopes of viral proteins for which antibodies are available, *i.e.* canine parvovirus (CPV) VP2 (antibody 3C9) (Langeveld et al., 1993), human immunodeficiency virus (HIV) gp41 (antibody 2F5) (Zwick et al., 2005), and E2 of CSFV (antibody WH303) (Edwards and Sands, 1990; Lin et al., 2000). Crude and pure peptides (Table 1) representing these epitopes were immobilized on beads and tested for their capability to bind specific antibodies in a suspension array. To investigate whether this principle is feasible for serological assays, immobilized crude and pure CSFV-derived peptides were also tested for their capability to bind specific antibodies in sera from swine infected with CSFV.

Pure and crude biotinylated peptides (Table 1) were coupled to avidin conjugated paramagnetic beads (low capacity, Radix BioSolutions, Georgetown, TX, USA), using peptides at different concentrations (40, 400, 4000 nM), essentially as described before (Angeloni et al., 2014), applying a magnet to handle the paramagnetic beads. After coupling, remaining free avidin molecules were blocked with 4000 nM biotin in phosphate buffered saline (pH7.2) with 0.05% Tween 20 (Merck) (PBST) for 30 min in the dark at room temperature on a plate shaker (approx. 600 rpm). To serve as control, additional bead sets were prepared in absence of peptides (0 nM).

First, the resulting bead sets were tested in suspension arrays for their ability to bind the corresponding specific monoclonal antibodies, diluted in PBST from 1 mg/ml stock solutions (1:10.000 CPV 3C9 (Ingenasa, Madrid, Spain); 1:1000 HIV 2F5 (Polymun Scientific Immunbiologische Forschung); 1:500 CSFV WH303 (AHVLA Scientific, Weybridge, United Kingdom)). Beads were washed prior to the assay, in between the respective incubations, and prior to analysis with a Luminex 200, essentially as described before (Van der Wal et al., 2013). Captured CPV and CSFV antibodies were detected with phycoerythrin-conjugated goat-antimouse (1:1000 in PBST) (Santa Cruz Biotechnology, Heidelberg, Germany), captured HIV antibodies were detected using a recombinant immunoglobulin binding protein A/G (ProtAG) labelled with phycoerythrin (PE) (1:1000 in PBST). ProtAG-PE was made by conjugating 10 μg Protein A/G (Pierce, Thermo Fisher Scientific) with PE, using the Lightning Link R-Phycoerythrin conjugation kit (Innova BioSciences, Cambridge, United Kingdom). Results were expressed as median fluorescence intensity (MFI),

Acquisition of fluorescent signals on beads carrying CPV VP2-derived peptides (3300–9500 MFI; Fig. 1A) showed that both crude and purified peptides were capable of binding antibody 3C9. Beads carrying purified peptides resulted in higher signals than beads carrying the crude peptides, and of the purified peptides, use of the variant with a five [2-(2-aminoethoxy) ethoxy] acetic acid (AEEA) spacer resulted in slightly higher signals than use of the peptide with one AEEA spacer. In all cases, MFIs were low if no peptide was coupled to the bead (empty bead) or if no antibody was present (PBST) (Fig. 1A), which indicated that the observed signals are the consequence of an interaction between immobilized peptides and the added antibodies.

Similar observations were made for beads carrying the HIV gp41-derived peptides, tested with the corresponding antibody 2F5 (Fig. 1B). Beads with purified peptides resulted in higher signals than beads with crude peptides, and of purified peptides the variant with the five AEEA spacer resulted in higher signals than the variant with one AEEA spacer.

For the CSFV E2-derived peptides, two peptide sets with a long AEEA spacer (sp5) were tested, of which one set carried ten extra E2-derived amino acids between spacer and epitope. All beads carrying CSFV E2-derived peptides were capable of acquiring fluorescence when antibody WH303 was added (Fig. 1C). The signals were lower than with beads carrying the CPV- and HIV-derived peptides, even though monoclonal antibody WH303 has a high affinity for the TAVSPTTLR epitope (Edwards and Sands, 1990; Lin et al., 2000); the reason for this difference is not clear. Similar to the CPV- and HIV-derived peptides, beads carrying purified CSFV peptides resulted in higher signals (1900–2600 MFI) than beads with crude peptides (800–900 MFI). For purified peptides, use of the long variant resulted in higher signals (2100–2600 MFI) than use of the short variant (1900 MFI); for crude peptides there was no difference between the long and short variants.

Next, beads with pure and crude CSFV peptides were investigated in a serological suspension array for their ability to capture specific antibodies in swine serum. Beads were prepared with 4000 nM of the longest CSFV-derived peptide (CSFV2, *cf.* Table 1), or in absence of peptide, and blocked with biotin. Colour coded beads with peptide or without peptide (empty control beads), were used to investigate swine sera (*cf.* Table 2). To block non-specific binding of serum components to the avidin beads, diluted sera (1:50 in PBST) were pre-treated with 100 μg/ml neutravidin (Pierce, Thermo Fisher Scientific) in a 96-well plate for 30 min in the dark at room temperature on a plate shaker (Norton et al., 1996). Assays were performed as described above, using ProtAG-PE for detection of swine serum antibodies. Signal-to-noise (S/N) ratios were calculated by dividing fluorescent signals acquired with beads carrying peptide by fluorescent signals on empty control beads.

Bead sets carrying crude or purified CSFV peptides were able to acquire fluorescent signals for the CSFV sera that are positive in the virus neutralization test (VNT) (Table 2), whereas signals remained low with empty control beads (< 275 MFI; Fig. 2A), resulting in signal-to-noise ratios from 5 to 11 and from 3 to 10 for beads with resp. pure and crude peptides (Fig. 2B). For the two VNT-negative sera, one serum gave low signals with a signal-to-noise ratio around 1, whereas the other serum caused extremely high signals on all beads tested. The calculated signal-to-noise ratio of this high background serum however was also around 1 (Fig. 2B). These observations indicate that the calculated signal-to-noise ratios larger than 1 for all CSFV positive sera represent an interaction between the immobilized peptides and E2-specific serum antibodies. The acquired signals on beads carrying crude peptides were less than half of the signals acquired by beads carrying purified peptides (Fig. 2A), similar to what was observed for capture of monoclonal antibody WH303 (*cf.* Fig. 1C).

Immobilized crude peptides (purity 42–59%) are capable of capturing monoclonal antibodies in suspension arrays, as demonstrated with three peptide sequences derived from CPV, HIV, and CSFV. Likewise, the serology results show that crude peptides are able to capture antibodies from sera, as demonstrated with CSFV E2-derived peptides using swine sera. Purified peptides (purity 90–100%) were, however, superior over crude peptides in all experiments. The crude peptides were produced at a concentration of 4 μM, but differences in molarity