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Immunogenic peptides from phage display libraries with potential of protecting mice against the Pseudorabies virus

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ABSTRACT

Phage display selection of combinatorial peptide libraries has demonstrated its almost unlimited potential in identifying binding ligands for many targets. The method shows promise for selection of immunogenic peptides against pathogens by antibodies. We have undertaken a study designed to select such mimics for one of the representatives of *Herpesviridae*, the Pseudorabies virus (PrV), infecting pigs and causing severe neurological complications known as Aujeszky's disease. By screening a 12mer linear and a 7mer cysteine-constrained libraries with immunoglobulins of a rabbit immunized with the virus, a family of 10 antigenic and immunogenic peptides was derived sharing a sequence motif K(**L**/P/V)**GDP**(**R**/K/L). Groups of six C57BL/6 mice were immunized with bacteriophages expressing peptides with this motif sequences. Some of the mice were found to be positive in seroneutralization assay; in a challenge setting, all but two immunized mice survived, albeit presenting some disease symptoms. We discuss the perspectives and limits of generating peptide leads by library screening with immune polyclonal antiserum for designing pure epitope-based vaccines to PrV in the future.

1. Introduction

Phage display of random peptides has developed into a high-throughput screening technology for the discovery of peptides useful in molecular recognition studies (Smith and Scott, 1993; Adda et al., 2002) and as leads for drug design (Gazarian, 2005). Among the many applications, selection of peptides recognized in random libraries by antibodies as mimics of their epitopes, *mimotopes* (reviewed in Adda et al., 2002; Gazarian, 2005) is an attractive strategy with promises of generating mimotope-based pure epitope vaccine options (Demangel et al., 1996; Sette and Fikes, 2003; Knittelfelder et al., 2009). Examples of protective mimotopes were reported for viruses (Roccasecca et al., 2001; Houimel and Dellagi, 2009) and other pathogens including parasites (Wu et al., 2006; Villa-Mancera et al., 2008). In recent years mimotopes have become attractive tools for designing anti-cancer therapeutics (Kozbor, 2010). Although monoclonal antibodies are ideal selectors of mimotopes (Smith and Scott, 1993; Adda et al., 2002; Gazarian et al., 2003), selection with polyclonal antibodies is often preferred because these antibodies are readily available and reflect the current state of the immunogenic epitopes. On the other hand, selection with host polyclonal antiserum may be inefficient and complicated by the pathogen's developed

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measures to conceal or to silence the most dangerous epitopes or weaken the immune response against them. Hence, the search of immunogenic peptides with vaccinepotential by means of polyclonal anti-pathogen serum is a special task requiring investigation of selecting capacities of serum antibodies raised to pathogen protective epitopes.

The present study describes experiments on the use of rabbit serum polyclonal antibodies raised against the Pseudorabies virus (PrV), a representative of *Herpesviridae*, for discovery and selection of peptides in two random libraries and evaluating their immunogenic potential.

PrV (also known as Aujeszky disease virus, ADV) infects neural cells of pigs causing severe complications (Nauwynck, 1997; Pomeranz et al., 2005) with lethal outcome for piglets (Mettenleiter, 2000). The proteomics of the virus entry, assembly, virulence and interaction with the host cell has been extensively studied by Mettenleiter and co-workers during last decades (see reviews: Mettenleiter, 2000, 2006; Mettenleiter et al., 2009). Like other herpes viruses, PrV expresses a large contingent of proteins required for infection, homing within specific condition in nervous cells, reproduction, and assembly and exporting. Special functions are developed for utilization of cell synthetic machinery and mechanisms for evading immune response; in this relationships with the host the virus profoundly affects cell biochemistry, modifying transcription of numerous genes re-directing them towards their proteins (Lin et al., 2010), and modulates the immune response, suppressing the interferon-mediated innate immune response (Brukman and Enquist, 2006), interfering with MHC class 1 antigen presentation to avoid CTL responses (Ambagala et al., 2005; Deruelle et al., 2009). Despite this sophisticated viral ability to survive, vaccines introduced in the 1990s were highly effective and helped eradicate the virus in many countries (Stegeman, 1995; Pensaert and Morrison, 2000). The vaccines use the whole inactivated or live attenuated virus as immunogen which also includes undesired immunomodulators apart from protective immunogens. In more recent years, vaccine design and testing has focused on simpler and molecularly better defined options, recombinant individual glycoproteins (Grabowska et al., 2009) or viral DNA (Ben-Porat et al., 1986; Marchioli et al., 1987; Van Rooij et al., 2006). In our previous (Villa-Mancera et al., 2008) and present study we used rabbit polyclonal antiserum to parasite *Fasciola hepatica* and PrV, respectively, to select mimics of protective epitopes. The ability of the virus to develop rapidly the disease with lethal outcomes in mice permits their use as adequate model for testing the immunogens' protective potential (Marchioli et al., 1987). We describe the screening of two random phage-peptide libraries with IgG from a rabbit immunized with culture medium containing PrV. The selected peptides presented the consensus **K**(**L**/P/V)**GDP**(**R**/K/L) sequence motif and induced antiviral immunity in mice. Although seroneutralization was observed in only one group of mice when challenged with the virus, all but two mice survived, despite presenting disease symptoms.

2. Materials and methods

2.1. Virus preparation, rabbit immunization, serum and IgG preparation

PrV (Shope strain) was grown in MDBK cells for 72 h at 37 °C, the virus-containing medium was centrifuged 20 min at 15,000 × *g*, the PrV in the supernatant was titered by end point assessing cytopathic effect (Reed and Muench, 1938). Two New Zealand rabbits (each weighing approximately 3 kg) were injected with 250 μl PrV-culture supernatant containing 2.5×10^5 $TCID_{50}$/ml (tissue culture infection dose, 50%) of the virus; this preparation (designated "crude PrV preparation") was inactivated 1 h at 55 °C, mixed with one volume of incomplete Freund adjuvant and injected three times with two-week intervals in two sites of the quadriceps muscle. Rabbit serum was prepared using blood drown from ear vein and its IgG fraction was purified as described (Palacios-Rodriguez et al., 2007).

For partial purification, the PrV was precipitated (Silhavy et al., 1984) from this crude preparation by adding PEG (Polyethylene glycol 8000, Sigma P 2139) to a final concentration of 10%, incubation 1 h on ice, centrifugation at 4000 × *g* for 20 min and suspension of pellet in PBS.

2.2. Pig anti-PrV sera

A 7 weeks old landrace pig was intramuscularly immunized with an inactive PrV vaccine (Boerhinger Ingelheim. Ingelvac Aujeszky KV Bartha strain), then, heat inactivated crude PrV preparation of the Shope strain (1×10^6 $TCID_{50}$/ml) was mixed with a volume of incomplete Freund's adjuvant and 500 μl portions were injected in two different sites of quadriceps muscle at 2, 4 and 6 post-vaccination weeks. Immune serum was prepared two weeks after the last inoculation.

Serum of two naturally PrV-infected pigs with *in vitro* virus neutralization ability was from the Department of Pigs of the Faculty of Veterinary Medicine and Zootechny of the Mexican National University (UNAM). All sera were assayed for the anti-PrV reactivity using HerdChek*PRVgI Antibody test kit (IDEXX Laboratories. Westbrook, Maine, USA).

2.3. Recombinant glycoprotein E (RgE) and rabbit anti-RgE serum

The gE-encoding gene was PCR-amplified from the Shope PrV strain DNA using nucleotide sequence of complete PrV genome (Genebank accession No. NC_006151) for designing primers: forward (G GAT CCG ATG CGG CCC TTT CTG CTG CGC), and reverse (Cg G GAT CCG ATT AGC GGG GCG GGA CAT CAA). The PCR product was sequenced to confirm the identity of the gE. The RgE was produced in the baculovirus expression system Bac to Bac (Invitrogen Life technologies, Carlsbad, CA). The Sf9 insect cells grown in monolayer were infected with the recombinant baculovirus containing the gene controlled by the polyhedrin promoter and cultured following the manufacturer's protocol (Guide to Baculovirus Expression Vector Systems and Insect Cell Culture Techniques, Invitrogen Life technologies, Carlsbad, CA). A rabbit was inoculated three times with the RgE-containing