Available online at www.sciencedirect.com
ScienceDirect
Veterinary Microbiology 118 (2006) 201–211
veterinary microbiology
www.elsevier.com/locate/vetmic

Identification of T-helper and linear B epitope in the hypervariable region of nucleocapsid protein of PPRV and its use in the development of specific antibodies to detect viral antigen

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Received 23 May 2006; received in revised form 19 July 2006; accepted 25 July 2006

Abstract

Peste des petits ruminants is a highly contagious viral disease of small ruminants making its diagnosis difficult from the similar symptoms of Rinderpest. Computer based prediction algorithms was applied to identify antigenic determinants on the nucleocapsid (N) protein of PPRV. Specificity and antigenicity of each peptide was evaluated by solid phase ELISA. Six specific peptide sequences were evaluated in multiple antigenic peptide (MAP) form and immune response was evaluated by supplementing universal T-helper epitope human IL-1β peptide (VQGEESNDK, amino acids 163–171). Out of the six peptides 19mer sequence corresponding to 454–472 region of N protein of PPRV was found to be highly immunogenic and specific to PPRV. Evaluation of overlapping peptides differing in length for this 452–472 region, showed minimum length of 14 amino acid residues were required for the stable affinity binding of antigen–antibody. The results of immunization and indirect ELISA indicated the presence of T-helper epitope at the N-terminal end and linear B epitope at the C-terminal region of 454–472 19mer of nucleocapsid peptide of PPRV-nucleocapsid protein. The antipeptide antibodies developed against this region showed specificity to PPRV antigen differentiating it from RPV when used in indirect ELISA and western blot analysis.

Keywords: Peste des petits ruminants; Antipeptide antibody; T-helper epitope; Diagnosis

1. Introduction

Peste des petits ruminants (PPR) is an acute and highly contagious viral disease causing high morbidity and mortality in small ruminants, such as goats and

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doi:10.1016/j.vetmic.2006.07.023

sheep (Taylor, 1984). The disease has accounted for significant economic losses to the livestock industry in many developing countries where Rinderpest has been present. There is a growing threat for the emergence of PPR in countries free of the disease, especially the neighboring areas where PPR is endemic. PPR is caused by an enveloped RNA virus, which belongs to the *Morbillivirus* genus in the family Paramyxoviridae. Other members of the genus *Morbillivirus* are Rinderpest virus, measles virus, canine distemper virus which are serologically related (Gibbs et al., 1979). Rinderpest virus can infect all artiodactyls, with cattle and buffaloes being the most susceptible species. Their diagnosis had ambiguity when they co-existed in country like India and the situation is more complicated if small ruminants are involved. Laboratory confirmation is done by conventional virus isolation and characterization or by more specific, rapid and sensitive techniques like enzyme linked immune sorbent assay (ELISA) using MAbs, nucleic acid hybridization using DNA probes, RT-PCR using specific primers. Currently, RP has been eradicated in India and the identification of a PPR-specific peptide which can be used for serological confirmation of infection should benefit serosurveillance studies on the prevalence of this disease in India. Therefore, always a search is on for more simple, rapid, economic and environment friendly diagnostic assays which can be performed at farm level.

Peptide based enzyme immuno assays (PEIAS) have been widely used for serodiagnosis of bacterial or viral infections in recent years. Because of the enhanced specificity over the whole lysate based assays, peptide based serology is often used in differential diagnosis of closely related agents such as HIV-1 and HIV-2 (Viscidi et al., 1991; Pau et al., 2000; Brattegaard et al., 1995). Synthetic peptides that mimic specific epitopes are used as antigens for the detection of serum antibodies directed against the proteins because they are relatively cheaper to produce than the whole protein. Moreover synthetic peptides offer the advantage of eliminating non-specific reactions resulting from cross-reactivity in the specimen with the host cell antigens. Cross-reactivity between PPRV and RPV has been a diagnostic challenge, particularly for serological monitoring in endemic areas, which raises the need of a diagnostic antigen conserved among PPRV but distinct from RPV. Among the structural proteins, N protein is antigenically the most conserved among morbilliviruses and is highly immunogenic in spite of its internal location (Libeau et al., 1995). Nucleocapsid (N) protein of PPRV is a major viral protein, which has been used as diagnostic antigen for various purposes, towards which antibodies are directed during infection (Diallo et al., 1987, 1994). In the present study we have attempted to identify the highly specific PPRV 'N' protein minimum epitope length required to elicit antibody response. Antipeptide antibody developed to the specific PPRV-N peptide was evaluated for its application in differentiating the PPRV antigen from that of RPV antigen.

Interleukin-1 has been shown to stimulate both the cellular and humoral immune responses to foreign antigens (Lin et al., 1995). Human IL-1β peptide (VQGEESNDK, 163–171 aa), which is crucial to binding to IL-1 receptor, was previously shown to be effective in the T cell-independent and T cell-dependent immune responses, so as to enhance both primary and secondary responses (Antoni et al., 1986; Beckers et al., 1993). So in the present study IL-1β 163–171 peptide was used as universal T-helper epitope to enhance the humoral immune response to peptides of PPR-N.

2. Materials and methods

2.1. Reagents and viruses

Protected amino acids, resins and reagents for peptide synthesis were obtained from Novabiochem (Switzerland) and other chemicals were procured from Sigma–Aldrich–Fluka (Spain). Peptide synthesis grade dichloromethane (DCM) and *N*,*N*′-dimethylformamide (DMF), and HPLC-grade acetonitrile (MeCN) were from Orpegen pharma.

Analytical HPLC was performed on semi-preparative reverse phase columns (7 mm × 50 mm, 5 μm particle size) C_{18} for free peptides and C_4 for multiple antigenic peptides. Preparative HPLC runs were done on C_8-reverse phase columns. Peptide hydrolysates (6N HCl, 120 °C and 2 h) were analyzed in a Beckman 6300 auto analyzer. MALDI-TOF mass spectra were recorded in a Voyager model DE-STR (Applied Bio Systems).

2.2. Immunologicals

PPR virus isolate 'pprv Sungri-96' Vero/57-62 (Sreenivasa et al., 2000) and goat polyclonal positive