Veterinary Microbiology 175 (2015) 132–138

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short Communication

Fine mapping and conservation analysis of linear B-cell epitopes of peste des petits ruminants virus nucleoprotein

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ARTICLE INFO

Article history:
Received 1 May 2014
Received in revised form 8 October 2014
Accepted 14 October 2014

Keywords:
Peste des petits ruminants virus
Nucleoprotein
Linear epitope
Minimal motif
Mapping

ABSTRACT

Nucleoprotein (NP) is the most abundant and highly immunogenic protein of morbillivirus, and is presently the basis of most diagnostic assays for peste des petits ruminants virus (PPRV). In this study, fine epitope mapping and conservation analysis of linear B-cell epitopes on the PPRV NP has been undertaken using biosynthetic peptides. Nineteen linear B-cell epitopes were identified and their corresponding minimal motifs were located on the NP of PPRV China/Tibet/Geg/07-30. Conservation analysis indicated that ten of the 19 minimal motifs were conserved among 46 PPRV strains. Peptides containing the minimal motifs were recognized using anti-PPRV serum from a goat immunized with PPRV vaccine strain Nigeria 75/1. Identified epitopes and their motifs improve our understanding of the antigenic characteristics of PPRV NP and provide a basis for the development of epitope-based diagnostic assays.

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1. Introduction

Peste des petits ruminants virus (PPRV), a *Morbillivirus*, causes an acute, highly contagious disease of domestic and wild small ruminants characterized by respiratory and gastrointestinal pathology (Munir, 2013). PPRVs have been classified into four lineages based on variable nucleotide sequences of the nucleoprotein (NP) gene. Western and Central African PPRVs cluster into Lineages I and II, Eastern African and PPRVs found in the southern part of the Middle East cluster into Lineage III, while Asian PPRVs cluster mainly into Lineage IV (Munir et al., 2013). Among the six structural proteins of morbilliviruses, the NP is the most abundant and highly immunogenic in spite of its internal location, and forms the basis of most diagnostic assays for PPRV (Libeau et al., 1995). Growing interest in the diagnostic applications of PPRV NP has focused attention on identifying more antigenic epitopes. B cell epitopes (BCEs) are categorized either as linear (composed of continuous aa) or conformational (discontinuous aa). In nature, the majority of BCEs are discontinuous but, due to difficulties in the mapping and design of such epitopes, more research has centered on linear BCEs (Han et al., 2013). Epitope mapping on PPRV NP has been reported using monoclonal antibodies/deletion mutants and computer based prediction algorithms/indirect ELISA (Choi et al., 2005b; Dechamma et al., 2006). Hitherto, however,

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http://dx.doi.org/10.1016/j.vetmic.2014.10.012
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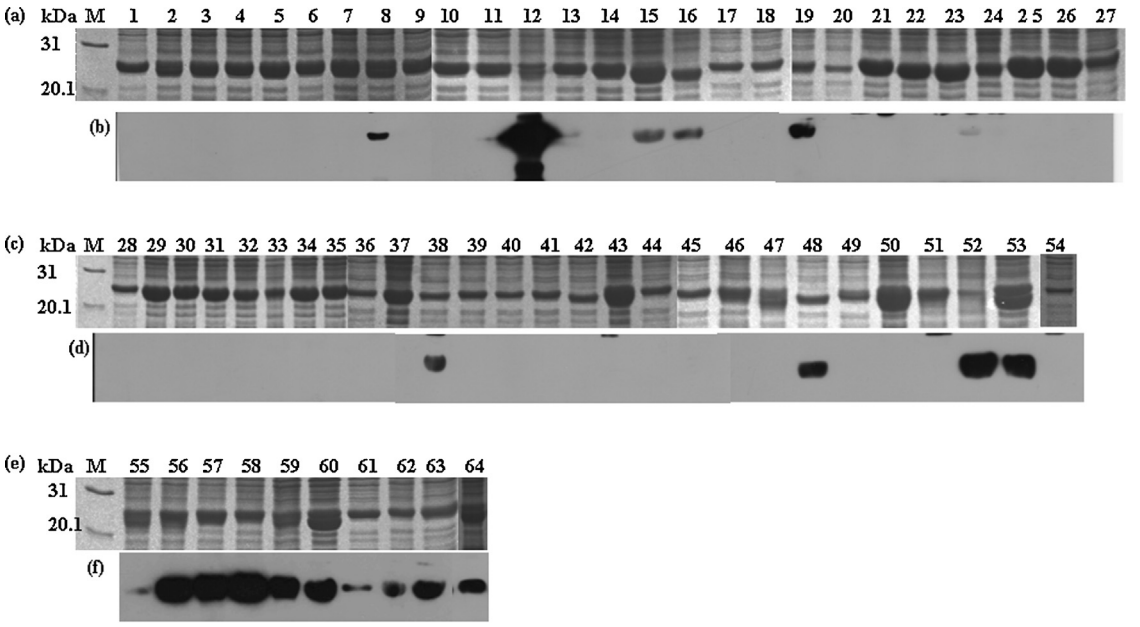


Fig. 1. SDS-PAGE and Western blotting analysis of 16mers of PPRV N protein expressed with GST tags. 16mers of PPRV N protein were expressed in *E. coli* strain BL21. After induction, cells were harvested by centrifugation and cell pellets were resolved by SDS-PAGE using 15% gels. Gels were either stained or processed for Western blots. Nitrocellulose membranes were first blocked with 5% (w/v) skimmed milk, incubated sequentially with RAN01 (dilution, 1:8000) and goat anti-rabbit IgG (1:100,000 dilution), and then visualized by enhanced chemiluminescence. (a, c, e) SDS-PAGE analysis of GST fusion expressed 16mers of N protein. (b, d, f) Western blot analysis of GST fusion expressed 16mers of N protein fragments. 1–64: GST fusion expressed 16mers for P1–P63 and 21mer for P64, respectively.

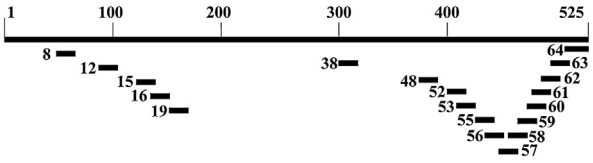
there are no reports of in-depth mapping and minimal motif identification of BCEs associated with PPRV NP.

An efficient method of epitope screening, which has been adopted for fine mapping of epitopes on SARS S protein (Hua et al., 2005), involves the use of biosynthetic peptides (Paterson et al., 1998; Xu et al., 2012). This strategy has now been used to identify 19 linear epitopes and their minimal motifs on the NP of PPRV. Data from this research increase our knowledge of BCE distribution on the PPRV NP and provide a more solid foundation for the design of PPRV diagnostics.

2. Materials and methods

2.1. Immunization of animals

Recombinant NP of PPRV China/Tibet/Geg/07-30 was expressed in *Escherichia coli* and purified using a Ni-chelated agarose column as described previously (Zhang et al., 2012). Five female New Zealand white rabbits (~2 kg body weight) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd (Shanghai, China) and maintained according to the standard laboratory animal care protocols approved by the Institutional Animal Care Committee of the SAAS. Three rabbits (#1–3) were injected intramuscularly with 0.5 mg of purified recombinant N protein emulsified in complete Freund's adjuvant (CFA) (Sigma Aldrich, San Francisco, USA) at multiple back sites. Three booster injections of 0.25 mg of the same antigen emulsified in incomplete Freund's adjuvant were administered at two-week intervals, and serum samples were collected from the immunized animals seven days after the third booster dose. The antibody titer of the combined serum from the three immunized animals (designated RAN01) was determined by indirect ELISA as previously reported (Zhang et al., 2012). Briefly, wells were coated with recombinant N protein and, after blocking with 5% skimmed milk, incubated sequentially with RAN01 and goat anti-rabbit IgG. Antigen-antibody reactions were detected using 3,3′,5,5′-tetramethylbenzidine and the intensity of the color reaction was determined by measuring the absorbance at 450 nm. Non-immune sera from the remaining two animals that received only CFA were used as negative controls.



8:57-72	12:89-104	15:113-128	16:121-136
19:145-160	38:297-312	48:377-392	52:409-424
53:417-432	55:433-448	56:441-456	57:449-464
58:457-472	59:465-480	60:473-488	61:481-496
62:489-504	63:497-512	64:505-525	

Fig. 2. Distribution of positive 16mers on PPRV N protein. The short segments were GST fusion expressed 16mers of the PPRV NP (except P64 which was a 21mer). Numbers assigned to the 16-mers are the same as in Fig. 1. Numbers shown above the long fragment denote the beginning and end amino acids of the NP of PPRV China/Tibet/Geg/07-30N protein sequence (ACQ44667.1).

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