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Identification of Immune-dominant Epitopes within Bovine Rotavirus VP6 Protein by Synthetic Peptide Approach

Naveen Kumar[1], Yashpal Singh Malik[2]*, Satish Kumar[3], Arvind Kumar[3], Kuldeep Sharma[4], Subhankar Sircar[2] and Kuldeep Dhama[5]

[1]National Institute of High Security Animal Diseases (NIHSAD), Bhopal, India.
[2]Biological Standardization Division, Indian Veterinary Research Insitute (IVRI), Izatnagar, India.
[3]Division of Animal Biotechnology, Indian Veterinary Research Insitute (IVRI), Izatnagar, India.
[4]National Institute of Research in Tribal Health, Jabalpur, Madhya Pradesh, India.
[5]Division of Veterinary Immunology, Indian Veterinary Research Insitute (IVRI), Izatnagar, India.

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Bovine group A Rotavirus (RVA) is considered the major cause of diarrhea in neonatal calves. Use of peptides in diagnostics offers several advantages. In this study, we predicted regions within VP6 protein of bovine RVA showing high antigenic index using Bioinformatics tools and correspondingly synthesized linear peptides over Wang resin. Reactivity of individual peptides with anti-RVA polyclonal sera identified two immune-reactive epitopes, spanning 112-134aa and 125-149aa regions within the VP6 protein. Multiple antigenic peptides constructed from these regions could serve as a potential tool for the development of diagnostics and prophylactics.

Keywords: Diarrhea; Bovine Rotaviruses; Immuno-dominant epitopes; Peptides.

Acute diarrhea has been recognized as the second leading cause of death due to infections among children under five years of age worldwide (Walker *et al*., 2012). Gastroenteritis, which is caused by an array of infectious agents, including Rotavirus, Coronavirus, Adenovirus, Calicivirus, Picobirnavirus, enteropathogenic *Escherichia coli*, Salmonella and Cryptosporidium continues to pose threat to newborns globally (Lanata *et al*., 2013). Among the enteric viruses, rotaviruses (RVs) are the major contributor leading to acute diarrhea in both animals and human worldwide (Matthijnssens, 2012; Papp *et al*., 2013).

Rotavirus, member of the *Reoviridae* family has a triple layered protein capsid of 100 nm in diameter, surrounding a genome composed of 11 segments of double-stranded RNA and encodes six structural (VP1, VP2, VP3, VP4, VP6 and VP7) and six non-structural proteins (NS1, NS2, NS3, NS4, NS5 and NS6) (Greenberg and Estes, 2009). The virus has been classified into nine serological species or groups (A-I) based on the antigenic properties of inner capsid protein (VP6) (Mihalov-Kovács *et al*., 2015); and among these all, rotavirus A (RVA) are more frequently reported from acute diarrhea episodes. The clinical manifestations of RVA infections are not sufficiently alone enough for the confirmatory diagnosis. Therefore, confirmatory diagnosis requires detection of RVA antigen in the faces of the new born. Many assays have been developed, but these assays suffered one or other limitations like time consuming, costly, cross reactivity, low sensitivity and specificity.

In such circumstances, synthetic peptide based

* To whom all correspondence should be addressed.
E-mail: malikyps@gmail.com

diagnostic assays are in demand because of their chemically defined nature, being safe, avoid risk of infection and accidental laboratory escape, non-infectious specific reagents and safe supply of diagnostic reagents to various destinations. Synthetic peptides mimicking specific epitopes of infectious viral proteins have been proved to be an alternative to viral antigens in disease diagnosis (Noh *et al*., 2014).

The perusal of literature reveals that till now, peptides have not been used for diagnostic assay development for RVA. Therefore, the aim of the study was to identify the immune-dominant epitopes within highly conserved VP6 protein of bovine RVA.

MATERIALS AND METHODS

Antigenic epitopes prediction

The immune-dominant epitopes were identified within the bovine RVA VP6 protein by Protean software implemented in DNA STAR using various predictive algorithms. The peptide sequences were selected and designed accordingly covering these immune-dominant epitopes. In this study, a total of six peptides within VP6 protein (6-29aa, 98-121aa, 112-134aa, 125-149aa, 161-175aa, and 368-393aa) were selected having high Jameson-Wolf antigenic index.

Designing, synthesis and purification of linear peptides

The selected peptides were synthesized manually using solid phase peptide synthesis methodology employing Fmoc chemistry. All peptides were synthesized over Wang resin. After cleavage from resin support; they were precipitated by adding dry and chilled diethyl ether. The precipitated peptides were vacuum dried and the white powder thus obtained was stored under dry conditions till further use. Each peptide was purified by Reversed Phase High Performance Liquid Chromatography (RP-HPLC).

Reactivity of individual peptides with anti-RVA sera

The immune-reactivity of individual peptides with anti-RVA polyclonal sera was examined by standard protocol. Briefly, individual peptides were coated in duplicate in high binding polystyrene Maxisorp ELISA plate (Nunc, Denmark), followed by blocking with 2% BSA. Each peptide was made to react with anti-RVA polyclonal sera raised in rabbit. Reactivity of peptides with sera was detected by anti-Rabbit HRP conjugate and use of OPD substrate. Finally, optical density was recorded at 492nm wavelength in an ELISA reader (Biorad, Model 680).

RESULTS AND DISCUSSION

The proteins present on the outer surface of protein of any virion are responsible for production of neutralizing/non-neutralizing antibodies. The epitopes present in these proteins exist as conformational and linear epitopes. Linear epitopes, the majority one bind specifically to antibodies. Peptides imitating immuno-dominant epitopes present on the outer surface of the virion have been evidenced as a striking reagent for detection of virus specific antibodies/viral antigens because of their ease to synthesize and cost-effective in production (Marsden *et al*., 1992; Simmonds *et al*., 1993; Saravanan *et al*., 2004a,b). Smartly designed peptides covering the immuno-dominant epitopes of viral antigen have an advantage of eliminating the non-specific reactions due to cross-reactivity of serum antibodies with host cell antigens (Larzábal *et al*., 2010).

In order to identify these reactive epitopes, overlapping peptide library approach is generally used. To avoid the cumbersome approach of synthesizing an overlapping peptide library covering the whole protein in search of immuno-reactive epitopes, bioinformatics tools were used to predict the immuno-dominant regions using various algorithms employed in Protean software (DNA STAR). The VP6 protein is the most frequently target protein in diagnostic assays to detect RVA particles owing to its high conservative and immunogenic nature, therefore this protein was selected in this study (Tang *et al*., 1997). Therefore, locating the regions within VP6 protein of bovine RVA that are reactive with anti-RVA antibodies are very critical to make use of peptides in development of diagnostic assay.

In this study, we identified six regions with high antigenic index and correspondingly synthesis free linear peptides by solid phase peptide synthesis approach over Wang resin. Reactivity of two peptides covering 112-134aa and 125-149aa regions displayed, comparatively higher reactivity

than others. These two regions could be a potential target for development for diagnostic assay.

Owing to low molecular weight, the linear peptides could not produce high-titred sera. This limitation could be overcome by the use of Multiple Antigenic Peptide (MAP) (Tam and Zavala, 1989; Sadler and Tam, 2002). Using this method it had been possible to produce high titred anti-peptide antisera and also to produce highly immunogenic peptide vaccine for many pathogens. One major advantage of anti-peptide antibodies produced against MAPs is that they are targeted against single epitope and presumably could serve as homogenous antibodies similar to monoclonal antibodies. These anti-peptide antibodies have been used as a source of safe, non-infectious reagents in immunoassays for sero-diagnosis of Hepatitis C virus (Simmonds *et al*., 1993), Infectious Bronchitis virus (Jackwood and Hilt, 1995), Infectious Bursal Disease (IBD) virus (Saravanan *et al*., 2004a, b), Peste des Petits Ruminants (PPR) virus (Dechamma *et al*., 2006), herpes simplex virus-1 (Ran *et al*., 2014). Recently, the potential of MAPs as vaccine candidate has been experimentally proven in lab animal models for *Bacillus anthracis* (Oscherwitz *et al*., 2010), *Staphylococcus aureus* (Wang *et al*., 2015), Swine influenza viruses (Wen *et al*., 2015), and Influenza A viruses (Stepanova *et al*., 2015). Consequently, we will design and synthesize MAPs covering these reactive regions within bovine RVA VP6 protein and will evaluate their potential in the development of diagnostic assay and as vaccine candidates.

CONCLUSION

Linear peptides synthesized from 112-134aa and 125-149aa regions within VP6 protein of bovine RVA are highly specific and reserve the potential to be used in diagnostic assay with high sensitivity and specificity.

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