Virus Research 163 (2012) 40–50

Contents lists available at SciVerse ScienceDirect

Virus Research

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

Induction of virus-specific neutralizing immune response against West Nile and Japanese encephalitis viruses by chimeric peptides representing T-helper and B-cell epitopes

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ARTICLE INFO

Article history:
Received 30 May 2011
Received in revised form 8 August 2011
Accepted 15 August 2011
Available online 27 August 2011

Keywords:
West Nile virus
Japanese encephalitis virus
Chimeric peptides
B-cell epitopes
T-helper epitopes
Peptide vaccine
Immunofluorescence assay
Peptide ELISA

ABSTRACT

West Nile virus (WNV) and Japanese encephalitis virus (JEV), the members of JEV serocomplex group are pathogens of global health concern. The co-circulation of these viruses poses challenges in effective diagnostics due to antigenic similarity between the E-protein of these viruses. The present study aimed to design chimeric peptides and study the immune response against the same. B-cell epitopes were predicted on structural proteins of WNV and JEV based on bioinformatics tools. The peptides representing to these B-cell epitopes were synthesized and subjected to ELISA. Two peptides, one each from WNV (named WE147) and JEV (named JE40) E-protein, showed virus-specific and strong reactivity to the immune mice sera and human clinical samples. The chimeric peptides for WNV and JEV were constructed by synthesizing the B-cell epitope of WNV (WE147) or JEV (JE40) with T-helper epitope (JM17) separated by diglycine spacer in between. The immune response generated against these chimeric peptides was found to be specific to the respective B-cell epitopes. The anti-peptide sera showed virus-specific reactivity in ELISA and in immunofluorescence assay with no cross-reactivity. Also, the anti-peptide sera could neutralize JE and WN viruses in an *in vitro* virus neutralization assay. The B-cell epitopes identified in the present study may be used as diagnostic markers for differentiating between WN and JE virus infections. The present study can form a basis for future design of vaccines.

1. Introduction

Amongst the mosquito-borne diseases Japanese encephalitis virus (JEV) and West Nile virus (WNV) are endemic in many parts of Southeast Asia including India. These viruses belong to genus *Flavivirus* of the family *Flaviviridae*, and serologically placed in the JEV serocomplex group. In India, JEV was first diagnosed in 1955 (Work and Shah, 1956) and many epidemics have also been reported in different parts of the country (Kabilan et al., 2004; Parida et al., 2006; Phukan et al., 2004; Roy et al., 2006; Sapkal et al., 2007). Presently, JEV is responsible for approximate 30,000–50,000 cases with a case-fatality rate of 30% annually in Asia, affecting mainly children below 15 years of age (Diagana et al., 2007; Misra and Kalita, 2010). In addition to JEV, West Nile virus (WNV) which, causes similar illness, has also become prevalent in newer areas of India (Khan et al., 2011b; Paramasivan et al., 2003) and isolated from various sources including human encephalitis cases and mosquitoes (George et al., 1984; Kedarnath et al., 1984; Paul et al., 1970; Umrigar and Pavri, 1977). WNV was first isolated in Uganda (Smithburn et al., 1940) and epidemics have been reported in several countries thereafter (Brown et al., 2008; Chowers et al., 2005; Hubalek and Halouzka, 1999; Lanciotti et al., 1999; Petersen and Roehrig, 2001; Savage et al., 1999; Tsai et al., 1998). WNV activity has been reported in central, western and southern India and recently WN virus has been reported in northeastern regions of India where JEV is endemic (Khan et al., 2011b) indicating active virus circulation. In northeastern India, Assam in the most populated state; it contains ~19 million (approximate 50% of the total) inhabitants of the northeastern India and co-infection with WNV and JEV associated with acute encephalitis syndrome has been reported recently (Khan et al., 2011a). Presence of neutralizing antibodies to both JEV and WNV has also been observed in patient and survey sera from Andhra Pradesh state and Dibrugarh regions of India (NIV unpublished data). Moreover, co-infection with other flaviviruses has earlier been reported from India (Katyal et al., 2000). Infections with more than one flavivirus makes the situation challenging because the antigenic similarity of WNV with JEV

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doi:10.1016/j.virusres.2011.08.008

and coupled with the co-circulation of these viruses makes differential diagnosis very difficult. This necessitates an urgent need for the development of rapid diagnostic and protective strategies to effectively control the growing threats to public health.

Vaccination remains the most effective way of controlling these viral diseases. Currently, live-attenuated as well as inactivated vaccines are available against JEV for human use (Marfin and Gubler, 2005). Although various WNV vaccines are at different developmental stages (Dauphin and Zientara, 2007; Gershoni-Yahalom et al., 2010; Iyer et al., 2009; Lieberman et al., 2009), but there are no specific treatment or licensed vaccine against WNV available for human use (http://www.cdc.gov/ncidod/dvbid/westnile/qa/wnv_vaccine.htm, accessed 30/05/2011). Various approaches have been used to improve the immunogenicity and protective efficacy of vaccines (Davidson et al., 2005; Gould and Fikrig, 2004; Kanesa-thasan et al., 2000; Minke et al., 2004a,b; Ng et al., 2003) including peptide based vaccine (Gershoni-Yahalom et al., 2010; Kim et al., 2010). Recent reports focused on using E-protein based strategies which allow protection against infections caused by flaviviruses (Chu et al., 2007; Fischer et al., 2010; Lieberman et al., 2009; Martina et al., 2008; Spohn et al., 2010; Watts et al., 2007). Virus-specific neutralizing antibodies are essential for the control of viral infection and a potential vaccine candidate should have the ability to induce virus-neutralizing antibodies, and appropriate T-helper and cytotoxic cell responses.

The viruses of JEV serocomplex group have a similar ecology, and their co-circulation poses challenges in effective diagnosis. Hence, it is necessary to identify epitopes that induce virus specific neutralizing response. It has been reported that for JEV and other closely related flaviviruses the virus-specific neutralizing antibody responses are generally against envelope glycoprotein (E-protein) (Brinton et al., 1998; Pierson and Diamond, 2008). However, the extensive similarity between the E-proteins of WNV and JEV often resulted in a cross-reactive immune response. This immunocrossreactivity can be eliminated by using only immunodominant regions of the viral protein as an immunogen. Identification of immunodominant regions/B-cell epitopes for several viruses has already been reported (Anandarao et al., 2005; Beasley and Barrett, 2002; Huang et al., 1996; Kutubuddin et al., 1993; Pothipunya et al., 1993; Wang et al., 2009). Also, synthetic peptides of virus-specific B-cell epitopes have progressively been used to generate protective immune response against viral infections (Moynihan et al., 2001; Power et al., 2001; Saini and Vrati, 2003). Earlier, author's laboratory has reported a putative neutralizing B-cell epitope on JEV (Dewasthaly et al., 2001).

Synthetic peptides representing B-cell epitopes are generally poor immunogens. To act as an effective immunogen a peptide should be able to induce both the B-cell and T-cell mediated immune responses (Milich, 1989). This can be achieved by incorporating B-cell and T-helper epitopes in the form of a single chimeric peptide. T-helper epitopes have also been identified for many viruses (Kumar et al., 2004; Leclerc et al., 1993; Mathews et al., 1991; Roehrig et al., 1994). Earlier studies from author' laboratory identified T-helper epitopes on JE, WN and DEN viruses (Kutubuddin et al., 1991). Chimeric peptides incorporating T-helper–B-cell epitopes have been shown to induce virus-specific neutralizing and protective immune response by many investigators (Dewasthaly et al., 2007; Kazanji et al., 2006; Partidos et al., 1991; Su and Caldwell, 1992). Also, generation/induction of an effective immune response requires co-presentation both the epitopes (Denton et al., 1994; Partidos et al., 1991; Roehrig et al., 1992; Singh et al., 2010).

Over the years several T-helper and B-cell epitopes have been identified for WNV (Beasley and Barrett, 2002; Kutubuddin et al., 1991; Larsen et al., 2010; McMurtrey et al., 2008) and recently a chimeric peptide has also been reported which incorporated a peptide from heat-shock protein 60 and a B-cell epitope from domain III of WNV E-protein (Gershoni-Yahalom et al., 2010). However, to the best of our knowledge, reports of chimeric T-helper–B-cell peptide for WNV do not exist so far.

In the present study, we have designed two chimeric peptides one each for WNV and JEV. In each case B-cell epitope from the E-proteins have been combined with the T-helper epitope from the M-protein using bioinformatics tools (*in silico* studies) and studied the immunogenicity in *in vivo* using laboratory experiments.

2. Materials and methods

2.1. Cell lines, virus strains and monoclonal antibodies

Porcine stable kidney (PS) cells, cultured in Eagle's minimum essential medium (MEM, Sigma, USA) in humidified 5% CO_2 environment at 37 °C were used for propagation of JEV strain 733913 (Rodrigues et al., 1975) and WNV strain B956, and for immunofluorescence and *in vitro* virus neutralization assay. The culture media were supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco, USA) and antibiotics (100 IU/ml of Penicillin and 0.1 mg/ml of Streptomycin). The virus pools were prepared in PS cells and stored at −80 °C until experimentation.

2.2. Prediction and selection of epitopes

2.2.1. B-cell epitopes

The B-cell epitopes were predicted on the structural proteins of WN and JE viruses using sequence-based antigenicity (Kolaskar and Tongaonkar, 1990) as implemented at the Immune epitope database and analysis resources (Zhang et al., 2008). Potential β-turns were also predicted using BetaTPred2 server (Kaur and Raghava, 2003). Sequence alignments of the flavivirus E-protein sequences were carried out using MEGA 4 (Tamura et al., 2007). The antigenic regions unique to the respective virus were identified based on the alignment.

2.2.2. T-helper epitopes

T-helper epitope reported earlier from author's laboratory (Dewasthaly et al., 2007) has been considered as a component in the chimeric peptide for the present study. We have selected the T-helper epitope from the JEV-WNV cross-reactive region of the M-protein (residue position 17–26; named JM17).

2.2.3. 3D structure predictions for peptides and E-proteins of WNV and JEV

The 3D structures of the B-cell and chimeric peptides were predicted using PEP-FOLD server (Maupetit et al., 2009) and the obtained structure was compared with the modeled 3D structures of the E-proteins of the respective virus.

The 3D structure of the E-protein of WNV and JEV was predicted by homology modeling using the online SWISS-MODEL server (Arnold et al., 2006). The crystal structures of the E-proteins of WNV (PDB ID: 2HG0) and JEV (PDB ID: 3P54) were chosen as the template for modeling of WNV and JEV E-proteins respectively. Energy minimization of the modeled structure was calculated using GROMOS96 force field (Scott et al., 1999; van Gunsteren and Berendsen, 1987) application in Swiss-PDB Viewer (SPDBV) (Guex and Peitsch, 1997).

Visualization of all the models and rendering of images were carried out in Discovery Studio v.2.0 (Accelyrs Inc., USA). The Ramachandran plots and the surface electrostatics of the predicted structures were studied using PROCHECK (Laskowski et al., 1993) and the NOC software (v3.01, Chen et al.) respectively.