INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-3, Issue-2, April-June-2012 Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

Received: 11th Feb-2012 *Revised: 15th Feb-2012* *Accepted: 19th Feb-2012*

Research Article

IDENTIFICATION OF T-CELL EPITOPES IN STRUCTURAL PROTEINS OF TICK BORNE ENCEPHALITIS VIRUS FOR VACCINE DEVELOPMENT

Dharmendra Kumar Chaudhary[1,2], Indra Mani[1,3], Vijai Singh[1,4*]

[1]National Bureau of Fish Genetic Resources, Canal Ring Road, PO- Dilkusha, Lucknow, 226002, India

[2]Department of Biotechnology, H.N.B. Garhwal University, Srinagar Garhwal. [3]Department of Biochemistry, Faculty of Science, Banaras Hindu University, Varanasi, 221005, India

[4]Programme d'Epigenomique, Institute of Systems and Synthetic Biology, Universite d'Evry Val d'Essonne- Genopole- CNRS, 5 rue Henri Desbrueres, 91030 Evry, France

E-mail: vijaisingh15@gmail.com

ABSTRACT: Tickborne encephalitis (TBE) is a human viral infectious disease caused by tickborne encephalitis virus (TBEV). It is transmitted by the bite of an infected tick and also initiated the swelling of brain (encephalitis) and spinal cord. There is a pressing need to develop potent and sufficient amount of drugs and vaccines for control of TBE. We have selected the structural proteins such as anchored core protein C, core protein C, premembrane, matrix and envelope proteins of TBEV for identification of T-cell epitopes using immunoinformatics tools. These epitopes were showed the highest binding affinity with major histocompatibility complex (MHC) class I and II molecules. These finding may be used as an immunodiagnostic agent and also development of peptide based novel vaccines.

Keywords: Tick-borne encephalitis virus; epitopes; MHC; immunodiagnostic; vaccine.

INTRODUCTION

Tick-borne encephalitis virus (TBEV) is a human-pathogenic virus and endemic in many European countries, the Asian parts of Russia, China and Japan (Monath and Heinz, 1996; Takashima, et al., 1997; Hou, et al., 1997). It is also reported an extremely dangerous human pathogen, causing about 10,000 to 14,000 human cases of tick-borne encephalitis (TBE) across Europe and Asia annually (http://www4.tbe-info.com/epidemiology/). Approximately 11,000 of these cases reported in only Russia, and the other 3,000 occur in Western Europe. There is an important requirement of vaccines for controlling the infection of TBEV. The genome size of TBEV is an approximately 11 kb which is single-stranded positive-sense RNA. It is encoded the single chain polypeptide which expressed as five smaller structural and seven non-structural proteins (Chambers, et al., 1990).

TBEV belongs to genus Flavivirus of the family *Flaviviridae* (Heinz, et al., 2000). There are some reports available for isolation and characterization of TBEV from different part of world. There is a major study on molecular epidemiology of TBE virus have focused on the envelope glycoprotein (E). Glycoprotein plays an important role in receptor binding and fusion of host cell membrane (Rice, et al., 1996); and consequently induces neutralizing antibodies and provides protective immunity (Heinz and Roehrig, 1980). Three-dimensional (3-D) structure of glycoprotein has been determined by X-ray crystallography provides better understanding of virus- host interaction (Rey, et al., 1995). The non- structural proteins of TBEV also play an essential role in replication and complex formation. The helicase is a major non structural protein encoded by NS3 gene helps in the termination of viral replication (Neddermann, et al., 1999; Leyssen, et al., 2000).

There are different antigenic closely related TBEV strains circulated across Europe, Siberia and the Far East. The existences of two different pathogenic strains have been reported as central European encephalitis virus and Russian spring summer encephalitis virus (Chumakov, et al., 1944). However, there is another antigenic different strains have also been confirmed by serological assay (Clarke, 1960). A third- subtype of TBEV had been previously called west Siberian virus based on clinical signs in humans, geographical location and antigenic analysis (Pogodina, et al., 1981).

On the basis of existing reports, we require the expressed and purified protein for further development of immunodiagnostic reagent and vaccine production. Therefore, handing of pathogenic TBEV is needed to have high level biosafety camber for culturing and propagation. Nonetheless, there is limited report available on bioinformatics analysis of viruses for accelerating the laboratory practices. We would need bioinformatics tools for identification of antigenic regions (epitopes) in the sequences of protein. The aim of present study is to identify and map of specific epitopes from five different structural proteins of TBEV.

MATERIAL AND METHODS

We have used different bioinformatics tools for analysis of genome and protein of Tick-borne encephalitis virus. The structural protein sequences of Tick-borne encephalitis virus were retrieved from NCBI GenBank (http://www.ncbi.nlm.nih.gov/genbank/). The genome sequences were verified using BLAST (Altschul, et al., 1997) while the open reading frames were identified from whole genome using Generunner, ORF finder and DNAstar softwares. The expected molecular weight and isoelectric point (pI) value were also verified using Generunner and ExPaSy (http://www.expasy.org/). We used Propred (http://www.imtech.res.in/raghava/propred/) and Propred1 (http://www.imtech.res.in/raghava/propred1/) immunoinformatics tools which are available for prediction of epitopes in the protein primary sequences. Significant efforts have been made in last few years as several groups devoted their research toward the development of procedures and algorithms that allow more effective and accurate prediction of MHC binding affinity. These tools cover maximum number of human leukocyte antigen (HLA) comparison to other epitopes prediction tools. We have considered the parameters during epitopes prediction such as 4% threshold with maximum binding score to HLA molecules (Singh and Raghava, 2001; 2003).

RESULTS AND DISCUSSION

The present study, five structural proteins of Tick borne encephalitis virus were used for the physicochemical analysis such as molecular weight, isoelectric point (pI value) and antigenic nature. Envelope protein showed highest molecular weight 53.5 kDa and the lowest molecular weight was 8.3 kDa of Matrix protein. Isoelectric point of proteins was ranged between 5.73 to 12.76. The physicochemical properties of 5 structural proteins were given (Table 1). The pI value of protein as indicated the stability of protein in that particular pI.

Table 1. Physicochemical properties of different structural proteins of Tick borne encephalitis virus

Protein	Accession number	Molecular weight (Da)	pI value
Anchored core protein C	NP_775500	12.416	12.53
Core protein C	NP_775499	10.628	12.76
PreM protein	NP_775501	18.544	5.73
Matrix protein	NP_775502	08.302	8.99
Envelope protein	NP_775503	53.509	7.94

In this study, identification of epitopes in anchored core protein C, Core protein C, PreM protein, Matrix protein and Envelope protein of Tick-borne encephalitis virus were investigated. Total 21 epitopes were predicted for class I MHC and 33 epitopes for class II MHC molecules in these proteins.

The predicted epitopes having the MHC alleles for structural proteins from Tick-borne encephalitis virus were showed in Table 2. Evaluation of synthetic peptides as vaccine candidate for flavivirus has been investigated. The prediction of epitopes using computational tools and synthetic peptides from E glycoprotein of Murray Valley encephalitis (MVE) and DEN 2 viruses have been earlier reported and their immunogenicity has been evaluated in mice (Gao, et al., 1990). In another recent report, the identification of T-cell epitopes from secretory and cell surface proteins of *M. tuberculosis* H37Rv strain. Total 69 promiscuous nanomer T-cell epitopes have been identified for MHC Class II and 47 promiscuous epitope has been identified for MHC class I molecules (Somvanshi, et al., 2008a). T-cell analysis of synthetic peptides to other viruses have been correlated and association with T- and B-cell responses (Hu, et al., 1999). There is a novel approach for vaccine design which is essential for discoveries in immunology research; immunoinformatics tools have potential applications to accelerate the wet laboratory research into design of vaccines and diagnostic tests by exploiting genome sequences.

Table 2. The predicted epitopes in the structural proteins of TBEV.

Protein name	T-cell epitopes	Amino acid position	No. of MHC Class II binding alleles	T-cell epitopes	Amino acid position	No. of MHC Class I binding alleles
Anchored core protein C	MVKKAILKG	01	17	TVSALMVGL	81	10
	MRMMGILWH	39	47	KAFWNSVPL	60	15
	LRKIKRTVS	74	14	LWHAVAGTA	46	04
	WLLVTTLLG	103	14			
	VQMPNGLVL	30	05			
	MVGLQKRGK	85	18			
Core protein C	LRKIKRTVS	74	13	TVSALMVGL	81	10
	MRMMGILWH	39	49	KAFWNSVPL	60	14
	VQMPNGLVL	30	05	LWHAVAGTA	46	04
	MVKKAILKG	01	17			
	MVGLQKRGK	85	20			
PreM protein	VVLLCLAPV	157	21	LAMVTVVWL	137	08
	LRTHLTRVE	117	13	RVAVLVVLL	153	05
	MVTVVWLTL	138	05	EGWVWKNKL	126	04
	WVWKNKLLA	127	25	VENGTCVIL	30	05
Matrix protein	VVLLCLAPV	64	21	LAMVTVVWL	44	04
	MVTVVWLTL	45	05	RVAVLVVLL	60	05
	LRTHLTRVE	24	11	EGWVWKNKL	33	04
	WVWKNKLLA	34	25			
Envelope protein	LVLAMTLGV	485	37	GLFGKGSIV	106	04
	VVMEVTFSG	325	15	FLPKLLLGV	454	06
	LELDKTVEH	199	09	FLSSIGKAV	429	06
	VKMDVYNLG	249	05	FLLAGGLVL	480	09
	IVACVKAAC	112	16	REYCLHAKL	57	05
	YVGELSHQW	383	03	CPTMGPATL	74	05
	LNMRNPTMS	468	03	LAQTVILEL	194	03
	MLTTPNPTI	355	04	EPHTGDYVA	144	03
	WNNAERLVE	234	05			
	FLPKLLLGV	453	06			
	VLLKALAGV	262	03			
	VFQKTKKGI	401	05			
	VGFLPKLLL	451	11			
	YCLHAKLSD	58	04			

In silico analysis is combined with *in vitro* screening methods to identify the peptides that are immunogenic in nature. The chemically synthesized domains of FMDV (Food-mouth disease virus) VP1 have been tested as peptide vaccine. The peptide corresponding to amino acid 141-160, 151-160 and 200-213 which are located near C- terminal end of VP1 and 9-24, 17-32 and 25-41, and N-terminal end of VP1 (Bittle, et al., 1982). The prediction of T-cell epitopes in highly virulence surface proteins such as hemagglutinin and neuraminidase from Influenza A virus H5N1 has been earlier reported (Somvanshi, et al, 2008b). Recently, the T-cell epitopes have been identified in thermostable hemolysin protein of *Aeromonas hydrophila* that can use in immunodiagnostic reagent (Singh, et al., 2011).

CONCLUSION

Immunoinformatics is a newer strategy for identification and mapping of epitopes in the protein sequences of Tick-borne encephalitis virus exclusive of the virus culture. The predicted TBEV nanomer epitopes for T-cell is recognized against MHC Class II and MHC class I may be useful for development of sensitive, rapid and cost effective diagnosis. Further, these epitopes of TBEV may be served as vaccines candidates for prevention of disease.

RERERENCES

Altschul, S.F., et al. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25: 3389-3402.

Bittle, J.L., Houghten, R.A., Alexexander, H., Shinnick, T.M., Sutcliffe, J.G., Lerner, R.A., Rowlands, D.J. and Brown, F. (1982). Protection against food and mouth disease by immunization with a chemically synthesized peptide predicted from the viral nucleotide sequences. Nature 298: 30-33.

Chambers, T.J., Hahn, C.S., Galler, R. and Rice, C.M. (1990). Flavivirus genome organization, expression, and replication. Annu. Rev. Microbiol. 44: 649–688.

Chumakov, M.P., Zyaitlenok, N.A. and Vorob'eva, M.S. (1944). The studies of virus encephalitis. Report II. The geographical distribution and epidemiological characteristics of tick-borne encephalitis in European part of Soviet Union, Siberia and Kazachstan. Neuropathol. Psychiatry 13: 20–23.

Clarke, D. H. (1960). Antigenic analysis of certain group B arthropod-borne viruses by antibody absorption. J. Exp. Med. 111: 25–36.

Clarke, D. H. (1964). Further studies on antigenic relationships among the viruses of the group B tick-borne complex. Bulletin of the World Health Organization 31: 45-56.

Gao, X.-M., Liew, F.Y. and Tite, J.P. (1990). A dominant Th epitope in influenza nucleoprotein: analysis of the fine specificity and functional repertoire of T-cells. J. Immunol. 144: 2730-2737.

Heinz, F.X. and Roehrig, J.T. (1980). Flaviviruses. In *Immunochemistry of Viruses*, vol. II, pp. 289-305. Edited by M. H. V. van Regenmortel and A. R. Neurath. Amsterdam and NewYork: Elsevier.

Heinz, F.X. et al. (2000). Family *Flaviviridae*,. *In* M. H. V. van Regenmortel, C. M. Fauquet, D. H. L. Bishop, E. Carstens, M. K. Estes, S. Lemon, J. Maniloff, M. A. Mayo, D. McGeogch, C. R. Pringle, and R. B. Wickner (ed.), Virus taxonomy: classification and nomenclature of viruses. Seventh report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego, California. 859–878.

Hou, Z., Heinz, F. X., Ecker, M., Zi, D., Liu, R. and Yu Y. (1997). The nucleotide and deduced amino acid sequence of protein E of HLJ-1 strain of tick-borne encephalitis virus from northeastern China. Chinese J. Virol. 13: 47-53.

Hu, G.J., Wang, R.Y.H., Han, D.S., Alter, H.J. and Shih, J.W.K. (1999) Characterization of the humoral and cellular immune responses against hepatitis C virus core induced by DNA-based immunization. Vaccine 17: 3160-3170.

Leyssen, P., de Clercq, E. and Neyts, J. (2000). Perspectives for the treatment of infections with *Flaviviridae*. Clin. Microbiol. Rev. 13: 67–82.

Monath T.P., Heinz, F.X. Flaviviruses. Edited by Fields, B.N., Knipe, D.M. and Howley, P. M. (1996). Philadelphia & New York: Lippincott-Raven Publishers. In Fields Virology, 3rd edn, 961-1034.
Neddermann, P., Clementi, A. and De R. (1999). Francesco Hyperphosphorylation of the hepatitis C virus NS5A protein requires an active NS3 protease NS4A NS4B and NS5A encoded on the same polyprotein. J. Virol. 73: 9984–91.

Pogodina, V.V., Bochkova, N.G. and Koreshkova, G.V. (1981). Strain properties of the Aina/1448 serotype of the tick-borne encephalitis virus. Vopr. Virusol. 6: 741–746.

Rey, F.A., Heinz, F.X., Mandl, C., Kunz, C. and Harrison, S.C. (1995). The envelope glycoprotein from tick-borne encephalitis virus at 2 Å resolution. Nature 375: 291-298.

Rice, C. M. (1996). Flaviviridae : the viruses and their replication. In *Fields* Shapoval, A.N. (1961). *In* S. N. Astakhov (ed.), Tick-borne encephalitis (encephalomyelitis). Medgiz, Leningrad,Russia.

Singh, H. and Raghava, G.P.S.(2001). ProPred: prediction of HLA-DR binding sites. Bioinformatics 17: 1236-1237.

Singh, H. and Raghava, G.P.S. (2003). ProPred1: prediction of promiscuous MHC class-I binding sites. Bioinformatics 19: 1009-1014.

Singh, V., Mani, I., Chaudhary, D.K. and Somvanshi, P. (2011). Molecular detection and cloning of thermostable hemolysin gene from *Aeromonas hydrophila*. Molecular Biology 45: 551–560.

Somvanshi, P., Singh, V. and Seth, P.K. (2008a). *In silico* prediction of epitopes in virulence proteins of *Mycobacterium tuberculosis* H37Rv for diagnostic and subunit vaccine design. J. Proteomics & Bioinformatics 1: 143-153.

Somvanshi, P., Singh V. and Seth P.K. (2008b). Prediction of epitopes in hemagglutinin and neuraminidase proteins of Influenza A virus H5N1 strain: A clue for diagnostic and vaccine development. OMICS: J. Integrative Biology 12: 61-69.

Takashima, I., Morita, K., Chiba, M., Hayasaka, D., Sato, T., Tekezawa, C., Igarashi, A., Kariwa, H., Yoshimatsu, K., Arikawa, J. and Hashimoto, N. (1997). A case of tick-borne encephalitis in Japan and isolation of the virus. J. Clin. Microbiol. 35: 1843-1947.