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Immunoinformatics: Screening of Potential T Cell Antigenic Determinants in Proteome of H1N1 Swine Influenza Virus for Virus Epitope Vaccine Design

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Abstract

Influenza (H1N1) is a highly contagious respiratory pathogen that continues to evolve and threaten both veterinary and human public health. Presently existing vaccines against influenza (H1N1) are based on the generation of secondary response in form of neutralizing antibody primarily directed against surface proteins– hemagglutinin and neuraminidase. In this work, Propred and Propred I immunoinformatics tools have been used to predict the T cell epitopes from seven putative protein viz. Hemagglutinin ,neuraminidase, polymerase PA ,nucleocapsid protein, matrix protein, polymerase PB1, polymerase PB2 of influenza virus A/Minnesota/2009 (H1N1). Total of 15 epitopes were predicted for HLA class I and 14 epitopes for HLA class II molecules. These epitopes are found top scoring peptides and showed binding with maximum number of HLA alleles. The threshold percent taken in this analysis was 4% to select high affinity peptides by Propred and Propred I. The predicted epitopes may be served as a useful diagnostic reagent for evaluating T-cell responses in the context of natural infection and also might be helpful for designing of either a DNA vaccine or a subunit vaccine against H1N1influenza.

Keywords: Neuraminidase; Hemagglutinin; H1N1; Influenza A virus; T cell epitopes

Introduction

The H1N1 viral strain implicated in the 2009 flu pandemic among humans often is called “swine flu” (New York Times, 2009). The 2009 H1N1 virus is not zoonotic swine flu, as it is not transmitted from pigs to humans, but from person to person (Trifonov et al., 2009). Clinical features of H1N1 swine flu are body aches, especially joints and throat, extreme coldness and fever, fatigue, headache, irritated watering and reddened eyes. In children, gastrointestinal symptoms such as diarrhea and abdominal pain may occur (CDC, 2009).

Swine influenza A viruses are RNA viruses with a segmented genome that is comprised of eight negative-sense, single-stranded RNA segments, belong to the viral family of *Orthomyxoviridae*. These eight segments encode eleven proteins (Brockwell-Staats et al., 2009). The polymerase complex includes the PB2, PB1 and PA proteins as well as the nucleoprotein (NP). There are two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA) (Brockwell-Staats et al., 2009). Swine flu viruses are causing a huge amount of death to both human and swine. The World Health Organization (WHO) figures show that worldwide more than 209 countries and overseas territories or communities have reported laboratory confirmed cases of pandemic influenza H1N1 2009, including at least 15174 deaths (WHO, 2010). Protection against influenza infection is conferred by neutralizing antibody for the two surface proteins, namely the hemagglutinin (HA) and the neuraminidase(NA) (Luke and Subbarao, 2006). It has been difficult to develop a vaccine for H1N1 influenza A virus that provides long lasting immunity. This is due to the antigenic drift of the virus where the circulating strain in an infectious cycle is different from the previously circulating strain (Thomas et al., 2006; Boni et al., 2006). Current inactivated vaccines provide essential protection when the vaccine antigen and the circulating viruses share high degree of similarity in the structural protein. Since new influenza virus antigenic variants emerge frequently from accumulation of point mutations in the structural protein, influenza vaccine antigens need to be updated frequently.

The preparation of a vaccine against H1N1 influenza needs some basic considerations about the working of the H1N1 influenza vaccine. Hemagglutinin protein is responsible for attachment of the virus to the sialic acid α-2,3 or α-2,6 galactose sugar receptor on the human host cell surface (Wan and Perez, 2006). It is reasonable to presume that influenza vaccines do not generate antibodies against the receptor binding region of the protein as this region is not subjected to much antigenic drift which would seriously compromise the infectivity of the virus. In fact, mutation in this region has resulted in change in sugar specificity leading to change of host specificity, and loosing infectivity for the original host species (Wan and Perez, 2006). Antibodies directed against this region therefore are likely to provide protection against the influenza strains.

T cell immunity has been implicated in rapid clearance of influenza virus (Thomas et al., 2006). This means that the individual with good T cell response would suffer from milder form of the disease, get cured sooner, which is reflected in decreased fatality and less spread of the virus in population. Therefore, a vaccine generating robust T cell immunity against influenza needs serious attention. Good T cell immunity along with antibody response focused on receptor binding region of the hemagglutinin protein and enzymatic active site of neuraminidase, would meet the needs of a vaccine. Especially the internal proteins contain many conserved peptides which are potential T cell antigens and hence need serious consideration as T cell focused vaccine candidates.

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S. No	Putative Proteins	Accession No.	Expected M.W(kDa)	pI Value
1	Hemagglutinin	ADD21431	31.78	8.42
2	Neuraminidase	ACQ76351	51.60	6.11
3	Polymerase PA	ACU13105	82.65	5.42
4	Polymerase PB1	ACQ76349	86.35	9.30
5	Polymerase PB2	ACQ76350	85.86	9.49
6	Matrix Protein	ACU44211	27.82	9.40
7	Nucleocapsid Protein	ACR38842	55.99	9.38

Table 1: Physicochemical properties of different putative proteins of influenza H1N1.

In this communication, we have computationally analyzed the proteome of H1N1 influenza (A/Minnesota/2009) [NCBI] to identify putative epitopes for the formulation of a vaccine for T cell immunity. This vaccine should cover the HLA haplotype of the target population, be effective against influenza A strains and generate good immune memory response. A variety of computational tools are now available for prediction of T cell epitopes (Korber et al., 2006). We have analyzed overlapping nonameric peptides of all the proteins of H1N1 influenza virus for binding to human HLA class I molecules by PROPRED I algorithm, and for binding to class II alleles by PROPRED algorithm to select peptides for develop a robust T cell vaccine. Synthethic peptides can use as vaccines to induce either humoral or cell mediated immunity requires an understanding of the nature of T cell and B cell epitopes has been reported (Singh and Raghava, 2001; Bhasin et al., 2003; Singh and Raghava, 2003).

Materials and Methods

Hemagglutinin (ADD21431), neuraminidase (ACQ76351), polymerase PA (ACU13105), Polymerase PB1(ACQ76349), Polymerase PB2(ACQ76350), Nucleocapsid protein (ACR38842), matrix protein (ACU44211) are proteins of H1N1 2009 Minnesota strain have been used for this imminoinformatic analysis. The complete sequences of H1N1 2009 Minnesota strain are available in the NCBI protein database(http://www.ncbi.nlm.nih.gov/). Physicochemical analysis such as molecular weight and isoelectric point (pI) were also analyzed using ExPasy (http://www.expasy.org/)

All the seven structural proteins were analyzed for potential T cell epitope using immunoinformatic tools. Propred and propred I were used to analyse binding of all over lapping peptides to all HLA class I and class II alleles. This tools helped to identify those antigenic determinants peptides in all seven proteins which binds to several HLA molecules with good binding affinity.

All these peptides were predicted on the 4% threshold value with highest binding score with HLA class I and HLA class II molecule. The propred algorithms which predict binding of nonameric peptides to HLA alleles. Those nonameric peptides have highest binding affinity and maximum coverage of HLA alleles were selected.

Result and Discussion

In order to produce library of H1N1 peptides to determine the antigenic determinants for vaccine design. We analyzed the whole proteome of H1N1 2009 Minnesota strain by propred and propred I immunoinformatic tools. In this study all seven proteins of H1N1 were also analyzed for physicochemical analysis such as molecular weight and isoelectric point. The polymerase PB1 protein and Matrix protein has highest molecular weight (86.35 kDa) and lowest molecular weight (27.82 kDa) respectively. Isoelectric points (pI) of

Hemagglutinin	FTTANADTL	11-19	HLA-A1, A2, A*0201, A*0205, A*1101, A3, A*3101, A*3302, A20, B*2702, B*2705, B*5101, B*5102, B*5301, B*5401, B*51, B60, B61,B7, B8
	STDTVDTVL	28-36	HLA-A1, A*1101, A3, A*3101, A68.1, A2.1, B*2702 B*2705, B*3701, B*3801, B*3901 B*3902,B*4403,B*5103, B*5201, B*5801, B60
Neuraminidase	TIGMANLIL	15-23	A1, A*0201, A*0205 A*1101, A24, A3, A*3101, A*3302, A68.1, B*3801, B*3901, B*3902, B62, B7, B8
	AIYSKDNSV	97-105	A*0205, A*1101, A3, A*3101, A*3302, A68.1, A2.1, B*2705, B*5101, B*5102, B*5103, B*5201, B*5401, B62, B*0702, B8
	GQSVVSVKL	76- 84	A2, A*0201, A*0205, A*1101, A24, A3, A*3101, A20 Cattle, A2.1, B*2702, B*2705, B*3701, B*3801, B*3902, B40, B*5102, B*5201, B*5401, B60, B62, B7
Polymerase PA	KLLLIVQAL	663-671	A2, A*0201, A*0205, A*1101, A24, A3, A*3101, A2.1, B14, B*2702, B*2705, B*3501, B*5102, B*3901,B62, B*0702
	FLLMDALKL	281-289	A2, A*0201, A*0205 A*1101, A3, A*3101, A2.1,B*5102, B*5301, B*5401, B*51, B62
Polymerase PB1	FVANFSMEL	500-509	A2, A*0201, A*0205, A*1101,A3,A68.1, A2.1, B*5301, B*5401, B*51, B7
	STVLGVSIL	415-423	A2, A*3101, A68.1, B14, B*3901, B40, B*4403, B*5201, B*5801,B60, B62
Polymerase PB2	VLTGNLQTL	343-351	A2, A*0201, A*0205, A3, A2.1,B*3801, B*3901 B62
	VVFPNEVGA	165-173	A1, A2, A*0201, A*0205, A*1101, A*3101, A3, A68.1, B*5301, B*5401, B*51
Matrix protein	GILGFVFTL	57-65	A2, A*0201, A*0205, A*1101, A24, A3, A*3101, A2.1, B14, B*2705, B*5101, B*5102, B*5103, B*5401, B62, B7
	NNMDKAVKL	90-98	A*0201, A*0205, A24,B*2705, B*3501, B*3801, B*3901, B*4403, B*5102,B60,B7,B8
Nucleocapsid protein	CLPACVYGL	274-282	A2, A*0201, A*0205,A24, A3, A*3101, A2.1, B14,B*3901, B*3902,B7
	FQGRGVFEL	457-465	A*0201, A*0205,A*3101, A2.1,B*2702, B*2705,B*3902, B*5301, B*5401, B*51,B60, B62

Table 2: Selected peptides of H1N1 proteome and their HLA class allele coverage (The given peptides are predicted to bind to the depicted HLA class I molecules, PROPRED I~4%).

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these proteins were ranged between 5.42-9.49 (Table 1). pI value of any protein indicates the stability of proteins in that particular isoelectric points. For predictions of potential T cell antigenic determinants in proteome of H1N1, we took seven putative proteins sequences of H1N1 2009 strain was divided into all possible nonamers. Each peptide undergone for binding analysis with all HLA alleles. Those peptide shown highest binding score and also coverage of maximum number of HLA alleles were selected as potent immunodominant epitopes for vaccine design. Those peptides which have higher affinity for HLA molecules are more likely to be recognized by TCR of specific T cells. Binding specificity of peptides to HLA Class I and Class II molecule by propred I and propred were analyzed at 4% threshold value respectively (Table 2 and Table 3). A total of 15 and 14 peptides were predicted as potent antigenic determinants, presented by HLA class I and II supertypes respectively. Out of these 29 peptides 'FTTANADTL' (Hemagglutinin), 'GQSVVSVKL' (Neuraminidase) peptides showed maximum binding with HLA class I molecules and for HLA class II molecules maximum binding peptides are 'VVLLYTFTT' (Hemagglutinin), 'MRAIGTHPS' (Matrix protein) and 'LRILVRGNS' (Polymerase PB2).

Highly immunogenic, cross-conserved epitopes can be designed by carefully overlapping conserved and immunogenic 9-mer sequences found in the influenza strains of interest (De Groot et al., 2009). Computational analysis of proteome of H5N1 avian influenza virus to define T cell epitopes with vaccine potential was evaluated for vaccine potential (Parida et al., 2007). Comparative Sequence Analysis on Different Strains of Swine Influenza Virus Sub-type H1N1 for Neuraminidase and Hemagglutinin was also done (Sharma et al., 2010). Inactivated influenza vaccines elicit neutralizing antibody responses that provide reasonable protection against the homologous H1N1, H3N2, and B viruses (Palese, 2006). However, antibody-mediated selection drives changes (known as *antigenic drift*) in the viral hemagglutinin and neuraminidase surface glycoproteins, which in turn dictate the frequent production of a new vaccine (Peter, 2008).

Conclusion

Immunoinformatics approaches are currently used for prediction of antigenic determinants in the proteins sequence of influenza virus (H1N1) without using their cultures. The prediction of influenza virus nanomer epitope for T cells is recognized against HLA class I and HLA class II. The predicted epitopes may be served as a useful diagnostic reagent for evaluating T-cell responses in the context of natural infection and also might be helpful for designing of either a DNA vaccine or a subunit vaccine against H1N1influenza.

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Protein	T cell epitopes	Amino acid position	Predicted binding to HLA Class II alleles
Hemagglutinin	LKGVAPLHL	60-68	DRB1_0101, 0102,0701,0703,1104,1106,1128,1305,1311,1321,1501,1506, DRB5_0101, DRB5_0105
	VVLLYTFTT	5-13	DRB1_0101, 0102,0301, 0305, 0306, 0307, 0308,0309, 0311 0401,0402,0404, 0405,0408,0410,0421,0423,0426,0801,0804,0806, 0813, 0817, 1101,1102, 1104,1106,1107,1114, 1120,1121, 1128, 1301,1302, 1304, 1305,1307, 1311,1321, 1322,1327,1328,1501,1502,1506
Neuraminidase	FVIREPFIS	114-122	DRB1_0305,0309,0801,0802,0804,0813, 0817, 1101,1102, 1104,1106, 1107, 1114, 1120,1121, 1128,1302,1304, 1305,1311,1321,1322,1323,1327,1328,1502,DRB5_ 0101, DRB5_0105
	VNISNTNFA	66-74	DRB1_0306, 0307, 0308,0311, 0401,0402, 0404,0405,0408,0410,0421,0423,0426 ,1102,1107,1121,1301, 1322,1327,1328
Polymerase PA	FLLMDALKL	281-289	DRB1_0101,0102,0405,0408,0421,0701,0703, 0817,1101,1114, 1128,1305,1307,1 321,1323,1501,1502,1506, DRB5_0101, DRB5_0105
	VVNFVSMEF	516-524	DRB1_0102, 0301,0402,0404,0405,0408,0410, 0423,0701, 0703,1102, 1104,1106, 1121,1128,1301,1305,1311, 1321, 1327, 1328,1501,1502,1506
Polymerase PB1	VRKMMTNSQ	280-288	DRB1_0306, 0307, 0308, 0311, 0401,0402, 0404,0405, 0408, 0410,0421, 0423,0426,0804, 0801, 0802,0806, 0813,1102,1104, 1106,1114, 1121,1301,1304,1307,1311, 1322,1323,1327,1328
	MITYITRNQ	315-323	DRB1_ 0402, 0801, 0802, 0804, 0806, 1102, 1114, 1121, 1301,1304, 1322,1323,1327,1328
Polymerase PB2	LRILVRGNS	639-647	DRB1_0301,0305,0306,0307,0308,0309,0311,0801,0802,0804,0806, 0813,0817, 1101,1102,1104,1106,1107, 1114,1121, 1128,1301,1305,1307,1311,1321, 1322,13 23,1327,1328,1501,1502,1506
	LRISSSFSF	316-324	DRB1_0101,0102,0301,0309,0421,0701,0703,1501,1502,1506, DRB5_0101, DRB5_0105
Matrix protein	FVFTLTVPS	61-69	DRB1_0101,0301,0308,0401,0402, 0404,0405,0408,0410,0421,0423,0426,0701 ,0703, 0802,0813,1101,1114,1120,1128,1302, 1305,1307,1321,1323
	MRAIGTHPS	215-223	DRB1_0102,0301,0305,0306, 0307, 0308, 0309,0311,0401,0402,0404,0405,0408 ,0410,0421, 0423,0426,0802,0804,0806, 0813,1101,1102,1104,1106,1107,1114,1121, 1128,1301,1305,1307,1311,1321, 1322,1323,1327,1328,DRB5_0101, DRB5_0105
Nucleocapsid protein	LVWMACHSA	327-335	DRB1_0101, 0102,0402, 0404,0423,1102, 1104,1106, 1114,1121,1311, 1322,1323, DRB5_0101, DRB5_0105
	IRMMESAKP	444-452	DRB1_ 0102,0401,0402, 0404,0405,0408,0410,0421,0423,0426,0802,0804, 1101,1102, 1104,1106,1114,1121,1301, 1307,1311,1322,1323,1327,1328

Table 3: Selected peptides of H1N1 proteome and their allele coverage (The given peptides are predicted to bind to the depicted HLA class II molecules, PROPRED~4%).

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