Design and Antigenic Epitopes Prediction of a New Trial Recombinant Multiepitopic Rotaviral Vaccine: *In Silico* Analyses

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Abstract

Rotavirus is the major etiologic factor of severe diarrheal disease. Natural infection provides protection against subsequent rotavirus infection and diarrhea. This research presents a new vaccine designed based on computational models. In this study, three types of epitopes are considered—linear, conformational, and combinational—in a proposed model protein. Several studies on rotavirus vaccines have shown that VP6 and VP4 proteins are good candidates for vaccine production. In the present study, a fusion protein was designed as a new generation of rotavirus vaccines by bioinformatics analyses. This model-based study using ABCpred, BCPREDS, Bcepred, and Ellipro web servers showed that the peptide presented in this article has the necessary properties to act as a vaccine. Prediction of linear B-cell epitopes of peptides is helpful to investigate whether these peptides are able to activate humoral immunity.

Introduction

DIARRHEA IS THE SECOND LEADING CAUSE of death around the world for children younger than 5 years of age (6). Rotavirus is the major etiologic factor of severe diarrheal disease, often associated with fever, nausea, and dehydration in infants and young children (13,34). It has been estimated that rotaviral diarrhea may be responsible for 527,000 child deaths every year, the majority of which (>85%) occur in developing countries (35). Rotavirus is a double-stranded RNA (dsRNA) virus in the Reoviridae family. The retrovirus genome consists of 11 segments of dsRNA and encodes six structural proteins (VP1–4, VP6, VP7) and six nonstructural proteins (NSP1–NSP6). The capsid contains three layers of protein (core, inner, and outer capsid) (12). For the most part, each gene segment codes for a single protein. During coinfection with more than one rotavirus strain in a patient, gene segments from the parental viruses may reassort, which may be a mechanism of viral genome diversity (38). The word rotavirus is derived from the Latin word *rota*, meaning wheel, which is characteristic of the morphology of this virus (16). Diarrhea caused by the virus usually occurs in children aged 6–36 months.

Retroviruses have three antigenic indexes, based on group, subgroup, and serotype. The group is determined by the VP6 protein and is divided into seven groups (A–G). Groups A, B, and C have been found in both humans and

animals, and groups D, E, and F have been found only in animals (7). Rotaviral group A is a common cause of rotavirus diarrhea in humans, and it is the first-choice candidate for vaccine development. Serotype is determined by the VP4 and VP7 proteins. These two proteins are the main target for neutralizing antibodies. Rotaviruses are divided to G genotypes (glycoprotein VP7) and P (protease sensitive, VP4) based on the antigenic structure of the outer capsule; there are 24 G genotypes and 33 P genotypes. Of the genotypes described to date, 12 G and 15 P genotypes are known to be involved in human infections (9,16,32,38). Genotypes P [8] G1, p [4] G2, P [8] G3, and P [8] G4 are common rotavirus strains in human infections in many countries (21,37). Despite high diversity among the subgroups, VP6 protein is stringently conserved and very immunogenic among all group A rotaviruses. However, capsid proteins VP7 and VP4 have very high diversity among strains (17,23).

Studies indicate that natural infection provides protection against subsequent rotavirus infection and diarrhea (36). On August 31, 1998, the first rotavirus vaccine (RotaShield) was licensed by Witt *et al.* from the American Food and Drug Administration, and finally entered the market. The tetravalent rotavirus vaccine (RotaShield) based on attenuated virus was produced and was given orally three times a month to 2-, 4-, and 6-month-old infants. However, because of the correlation with intussusception and other intestinal

FIG. 1. Tertiary structure of fusion peptide. The results were prepared by Pyre2 and displayed with Hex. This peptide includes 28.4% α -helix, 10.5% β -sheet, 4.2% turn, and 66.5% coil.

diseases, as well as poor affectivity in children with poor health, RotaShield was taken off the market (43). RotaTaq and Rotarix are two live oral vaccines already available worldwide. They have been licensed in more than 85 countries and are part of the routine vaccination programs in many countries, including America, Brazil, Panama, Venezuela, Australia, and Belgium (12,22). RotaTaq is a live attenuated pentavalent vaccine isolated from five distinct human-bovine reassortant virus strains, which together express the human rotavirus G1, G2, G3, G4, and P antigens (11,52). In July 2004, a new rotavirus vaccine (51) composed of the live rotavirus strain RIX4414 was licensed in Mexico by GlaxoSmithKline. Rotarix is a monovalent live attenuated vaccine with G1P genotype (51). Yet, there is no effective vaccine program in many developing countries. Global vaccination could prevent 43% of the 527,000 rotaviral deaths in children. The World Health Organization (14,44,49) has included rotavirus vaccination in the global vaccination program (39). This study proposes a new generation vaccine against rotavirus designed using *in silico* analyses. In this study, three types of epitopes are considered—linear, conformational, and combinational—in a synthetic protein.

Materials and Methods

Based on epidemiological studies and previous findings (9,16,32,38), serotype A rotaviruses were selected as a good model for antigenic epitope prediction study. The sequence of VP4 and VP6 proteins (GenBank accession number GQ225829.1 and GQ496209.1, respectively) belonging to rotavirus common strains were retrieved from the NCBI database (www.ncbi.nlm.nih.gov/). Using homology analyses by NCBI protein–protein BLAST server, conserved motifs of these proteins were selected for construction of a model peptide:

NH³⁺-ANYVETARNTIDYFVDNVCMDVMVRESQRNGI
APNIFPYSKTSWLKEMQYNRDIIRFKIDFKTLKNLNDN
ACTEYINNGLPPIQNTRNVVP-COOH

Physical and chemical properties of the peptide were analyzed with ProtParam (www.expasy.ch/tools/protparam.html). The secondary structure was predicted by phyre2 (www.sbg.bio.ic.ac.uk/phyre2/) (24), PHD (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.Pal?age=/NPSA/npsa_phd.html) (45), PSIPRED (<http://bioinf.cs.ucl.ac.uk/psipred>)

TABLE 1. B-CELL LINEAR MOTIF PREDICTION OF MODEL PEPTIDE PRESENTED IN THIS STUDY

Motif	Sequence	Start position	Length	GRAVY score	Mw (Da)	pI	Software
1*	DEMVRESQRNG	24	11	−1.964	1,320.4	4.68	1,2,3,4
2	IDYFVDFVDNCVC	11	13	0.95	1,551.7	3.42	1,2,3
3*	KNLNDNACT	69	9	−1.167	992.0	5.83	1,2,3
4*	NDNACTEY	72	8	−1.462	928.9	3.67	1,2
5*	PPIQNTRNVVP	85	11	−0.691	1,234.4	10.18	1,2,3,4
6*	FPYSKTSWLKEMQ	40	13	−0.954	1,644.9	8.50	1,2,3
7*	NYVETARNTID	2	11	−0.973	1,295.3	4.37	1,4
8	VINNGLPPI	79	9	0.711	936.1	5.49	2
9*	DIIRRFKIDFKTLK	56	15	−0.067	1,906.3	10.28	1,2,3
10*	QRNGIAPNIFPYSKTS	31	16	−0.781	1,793.0	9.99	2
11*	TSWLKEMQYNRDIIR	46	16	−0.713	2,066.4	8.28	2
12*	IDFKTLKNLNDNACTE	63	16	−0.688	1,839.0	4.56	1,2

Antigenic motifs were confirmed by different programs; hydrophilic motifs are marked with an asterisk. Servers used for prediction are shown as number described as follows: Bcepred, 1; ABCpred, 2; BCPred, 3; and Ellipro, 4. For each peptide, sequence, position, molecular weight, isoelectric pH, and GRAVY score are indicated. The GRAVY index indicated the hydrophobicity of the peptide and was calculated as the sum of the hydropathy values of the composing amino acids divided by the number of residues in the sequence. Peptides with a negative GRAVY index are hydrophilic, whereas peptides with a positive GRAVY index are hydrophobic.

GRAVY, grand average of hydropathy.

TABLE 2. CONFORMATIONAL B-CELL EPTOPEs WERE PREDICTED BY THE ELLIPro SERVER

Number	Residues	Length	Score
1	_:E29, _:S30, _:Q31, _:R32, _:N33, _:G34	6	0.809
2	_:K62, _:I63, _:D64, _:F65, _:K66, _:T67, _:L68	7	0.765
3	_:A1, _:Y3, _:V4, _:E5, _:T6, _:A7, _:R8, _:N9, _:T10, _:I11, _:D12, _:F14, _:V15, _:D16	14	0.762
4	_:M26, _:V27, _:R28, _:S46	4	0.616
5	_:K69, _:N70, _:L71, _:N72, _:D73, _:A75, _:P86, _:I87, _:Q88, _:N89, _:R91, _:V93, _:V94, _:P95	14	0.598

ElliPro associates each predicted epitope with a score, defined as a PI (Protrusion Index) value averaged over epitope residues. For each residue, a PI value is defined as percentage of the protein atoms enclosed in the ellipsoid, which approximates the protein surface, at which the residue first becomes lying outside the ellipsoid; for example, all residues that are outside the 90% ellipsoid will have PI=9 (or 0.9 in ElliPro).

(31), and Jpred (www.compbio.dundee.ac.uk/www-jpred/) (10). The tertiary structure of the model peptide was predicted by phyre2 (www.sbg.bio.ic.ac.uk/phyre2/) and m4t servers (18). PDB structure was studied topologically using Hex 6.3 software.

Prediction of B-cell epitopes

In order to identify linear epitopes, the primary sequence of the peptide was submitted to ABCpred (www.imtech.res.in/raghava/abcpred/) (46), BCPREDS (<http://ailab.cs.iastate.edu/bcpreds/>) (15), Bcepred (www.imtech.res.in/raghava/bcepred/) (48), and ElliPro (http://tools.immuneepitope.org/tools/ElliPro/iedb_input) (40).

Prediction of conformational B-cell epitopes:

ElliPro (http://tools.immuneepitope.org/tools/ElliPro/iedb_input) (40) and Discotope 1.2 (www.cbs.dtu.dk/services/DiscoTope/) (26) were used to predict conformation of diagnosable epitopes for B lymphocytes.

Prediction of T-cell epitopes

To predict T-cell-specific epitopes, primary sequence of the model antigenic peptide was submitted to CTLPred (www.imtech.res.in/raghava/ctlpred/) (5) NetCTL 1.2 (www.cbs.dtu.dk/services/NetCTL/) (27), EpiJen (www.ddg-pharmfac.net/epijen/) (14), and TAPPred (www.imtech.res.in/raghava/tappred/) (4). nHLAPred (www.imtech.res.in/raghava/nhlaped/) (1) and ProPed I (www.imtech.res.in/raghava/propred1/) (49) were used for a comparative analysis of outputs, which can

identify MHC binding regions by various matrices. HLA-DR4Pred (www.imtech.res.in/raghava/hladr4pred/) (3) was used for investigation and identification of T-helper epitopes in the peptide.

Results

Epitopic motifs of a model were predicted. This peptide comprised 95 amino acid residues with 1,169.6 kD molecular weight. Based on the algorithm ProtParam, the peptide is stable and hydrophobic (GRAVY: -0.529) and its PI (isoelectric pH) is 5.30. As shown in Figure 1, the 2D structure of this peptide included 28.4% α -helix, 10.5% β -sheet, 4.2% turn, and 66.5% coil.

In addition to linear epitopes (Table 1), conformational B-cell epitopes were predicted with ElliPro, as shown in Table 2 and Figure 2. Analyses with Discotope 1.2 r showed more details of tertiary structure (Fig. 3).

Motifs E29, S30, Q31, R32, N33, G34 and K62, I63, D64, F65, K66, T67, L68 were identified as both linear and conformational B cell epitopes by the different programs.

Different programs were used to predict T-cell epitopes in the proposed peptide (Table 3). The various programs detected identical motifs.

Furthermore, MHC-I binding motifs were predicted and similar results were found in both programs. Interestingly, this motif (IDFKTLKNL) is identified by seven MHC_I in both programs, but the exact molecules vary. Five types of HLA (HLA-B*3701 HLA-B40, HLA-B60, HLA-B61, HLA-Cw*0602) were shared between both programs.

FIG. 2. Tertiary structure and position of conformational B-cell epitopes. These structures were predicted by ElliPro as shown in Table 2. (A) Motif 1 (_:E29, _:S30, _:Q31, _:R32, _:N33, _:G34) with a score of 0.809. (B) Motif 2 (_:K62, _:I63, _:D64, _:F65, _:K66, _:T67, _:L68) with a score of 0.765. (C) Motif 3 (_:A1, _:Y3, _:V4, _:E5, _:T6, _:A7, _:R8, _:N9, _:T10, _:I11, _:D12, _:F14, _:V15, _:D16) with a score of 0.762. (D) Motif 4 (_:M26, _:V27, _:R28, _:S46) with a score of 0.616. (E) Motif 5 (_:K69, _:N70, _:L71, _:N72, _:D73, _:A75, _:P86, _:I87, _:Q88, _:N89, _:R91, _:V93, _:V94, _:P95) with a score of 0.598. Scores were calculated as described in the legend to Table 2.

FIG. 3. Epitopic residues colored by binary code. Threshold=1.900. *Light*, predicted epitope residues. Residues colored by DiscoTope score >1.9 (0.17 sensitivity; 0.95 specificity). *Dark gray*, high score; *darker gray*, low score. DiscoTope predicts discontinuous B-cell epitopes from protein three-dimensional structures. The method utilizes calculation of surface accessibility (estimated in terms of contact numbers) and a novel epitope propensity amino acid score. The final scores are calculated by combining the propensity scores of residues in spatial proximity and the contact numbers. A specificity of 0.95 means that 5% of the nonpeptide residues were predicted as part of epitopes. A sensitivity of 0.17 means that 17% of the epitope residues were predicted as part of nonpeptides.

RFKIDFKTL was identified by HLA (HLA-B*0702, MHC-Kb, MHC-Kd, HLA-Cw*0401) in ProPed I and HLA-G in nHLAPred. Ten T-helper epitopes were identified by HLA-DR4Pred. The results are shown in Table 4.

Discussion

The fusion antigenic protein introduced here may be a starting point for designing effective rotaviral vaccines. Rotavirus infection results in high child mortality rates and is a significant economic burden in the developing world. Improvements in healthcare have not had a great impact on the incidence of rotavirus infection; vaccination is the best and most effective way to control this infection (39).

The role of humoral immunity against rotavirus has been demonstrated in multiple studies (33,50,53). Prediction of

TABLE 4. THE RESULTS OF THE HLA-DR4Pred PROGRAM

Peptide	Sequence	Start position	Score	Prediction
1	MDEMVRESQ	23	0.663	Binder
2	DFVDNVCMDE	16	0.555	Binder
3	IDYFVDFVD	11	0.490	Binder
4	FVDNVCMDE	17	0.485	Binder
5	TEYINNGLP	77	0.470	Binder
6	YFVDFVDNV	13	0.442	Binder
7	SKTSLWKEM	43	0.432	Binder
8	IDFKTLKLN	63	0.418	Binder
9	LNDNACTEY	71	0.410	Binder
10	VETARNTID	4	0.395	Binder

The HLA-DR4Pred is a support vector machine (SVM) and artificial neural network (ANN)-based HLA-DRB1*0401 (MHC class II alleles) binding peptides prediction method. The accuracy of the SVM- and ANN-based methods is ~86% and ~78%, respectively. The performance of the methods was tested through five-set cross-validation.

linear B-cell epitopes is helpful to investigate whether these peptides are able to activate humoral immunity. Bcpred (55), Bcepred (8), ABCpred (47), Ellipro (40,42,54), and DiscoTope 1.2 server (30) scored the peptide in this study high for humeral immunity.

NetCTL 1.2 (20), EpiJen (41), nHLAPred (28), CTLPred (44), TAPPred (29), and HLA-DR4Pred (25) confirmed the possibility of the peptide in this study stimulating cellular immunity as well.

One of the biggest complications in producing powerful vaccines against retroviruses is the variability of their genome. In developing countries, human rotavirus strains include different combinations of G/P types. This is due to rearrangement of different rotavirus strains, including animal-specific types, and this results in diversity in strains from area to area (58). This diversity results in variable efficacy of present vaccines in different countries (59). In this study, conserved regions of the rotavirus genome were considered as a vaccine target, as rapid genomic changes may have less impact on its effectiveness.

Another advantage of the model peptide presented in this research as a vaccine is the absence of motifs that may promote an autoimmune response. The current live vaccines have side effects such as bowel obstruction, restlessness,

TABLE 3. T-CELL MOTIFS WERE PREDICTED BY DIFFERENT PROGRAMS

Motif	Sequence	Start position	Length	GRAVY score	Mw (Da)	pI	Software
1*	YNETARNTIDY	4	11	-1.473	1,359.4	4.37	1,2
2	NTIDYFVDFV	8	10	0.600	1,232.3	3.56	1,2,4
3	YFVDFVDNVC	12	10	0.890	1,220.3	3.56	2,4
4*	QRNGIAPNIFPY	30	12	-0.525	1,389.5	8.75	1,2,3
5*	WKEMQYNRDI	47	10	-1.820	1,382.5	6.07	2,4
6*	RFKIDFKTL	59	9	-0.289	1,167.4	9.99	2,3
7*	TLKNLNDNA	66	9	-1.022	1,002.0	5.50	2,4
8*	CTEYINNGLPPI	75	12	-0.067	1,333.5	4.00	1,2
9*	GIPPIQNTRNVPG	85	14	-0.279	1,461.6	9.75	2,4

Antigenic motifs were confirmed by different programs; hydrophilic motifs are marked with an asterisk. Programs used for prediction are shown as number described as follows: Bcpred, 1; ABCpred, 2; BCPred, 3; and Ellipro, 4. For each peptide, sequence, position, molecular weight, isoelectric pH, and GRAVY score was indicated.

fever, and nausea in infants (60,61). Production, transportation, and storage of current vaccines are very expensive (62). In addition, this vaccine was designed for production in a prokaryotic expression system that is simpler and cheaper than a eukaryotic system. Practical studies are needed to validate the model peptide, but this type of vaccine could improve health in developing and underdeveloped countries.

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Author Disclosure Statement

No competing financial interests exist.

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