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ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

In-silico prediction of VP2-specific B- and T-cell epitopes as potential vaccine targets against the globally distributed human bocavirus 1

OBJECTIVE **We aimed at predicting B- and T-cell epitopes in the capsid protein VP2 which has been identified as a potential vaccine candidate against human bocavirus 1 (HBoV1).** METHOD **The VP2 sequence in FASTA format was obtained via the UniProt resource and the Phyre2 Protein Fold Recognition Server was used for 3D modeling. VP2 antigenicity was calculated by Vaxijen version 2.0. B-cell epitopes were predicted with the IEDB ElliPro tool. MHC class I and II epitopes were identified with the Vaxitop method of Vaxign. The MHC I epitopes were tested with IEDB's immunogenicity tool and MHC II binders with the IFNepitope server. Antigens' risk of allergenicity, toxicity, and autoimmunity triggering were tested via the AllerTOP version 2.0, Toxin Pred, and Peptide Match tools, respectively. Physicochemical properties were determined via Expasy ProtParam.** RESULTS **Immunoinformatic analysis resulted in the identification of VP2-specific B- and T-cell epitopes fulfilling prerequisites for vaccine design.** CONCLUSIONS **Our findings confirm the antigenicity of VP2 and indicate candidate B- and T-cell linear sequences suitable for in vitro validation.**

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ΑΡΧΕΙΑ ΕΛΛΗΝΙΚΗΣ ΙΑΤΡΙΚΗΣ 2026, 43(2):258–262

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In-silico πρόβλεψη των ειδικών για τη VP2 Β- και Τ-κυτταρικών επιτόπων ως δυνητικών στόχων εμβολίου κατά του παγκοσμίως διαδεδομένου ανθρώπινου μποκαϊού 1

Περίληψη στο τέλος του άρθρου

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Human bocavirus 1 (HBoV1) was identified as a new species of the genus *Bocaparvovirus* (subfamily *Parvovirinae*, family *Parvoviridae*) in 2005.[1] The virus has emerged in human populations, with an increasing number of cases reported worldwide.[2] HBoV1 is responsible for mild to life-threatening respiratory infections, mainly in children under the age of five years,[2,3] yet in recent years there have been increasing reports of fatal cases in adults.[4–6]

The linear single-stranded DNA genome of HBoV1 encodes the non-structural NS1-4 proteins and the structural capsid proteins VP1-3.[3] Research evidence has highlighted the importance of elucidating the role of the Bocavirus capsid proteins in developing effective antiviral therapies and vaccines that are currently unavailable.[3]

In mice, VP2 virus-like particles (VLPs) were shown to have good immunogenicity with induction of strong humoral and cellular immune responses, thus representing good candidate proteins for HBoV vaccine.[7] In a 2018 study Kalyanaraman[8] investigated potential vaccine candidates on the VP2 protein of HBoV1 using *in-silico* prediction tools. In this study, we focused on the same protein to identify novel epitopes fulfilling prerequisites for vaccine design.

MATERIAL AND METHOD

VP2 sequence retrieval

The VP2 sequence was downloaded in FASTA format from the Universal Protein Resource database UniProt (entry H9C5X6). UniProt provides quality protein sequence data that has been annotated to the highest possible standard.[9] The protein structure prediction server Phyre2 was used to build the VP2 three-dimensional (3D) model.[10]

VP2 antigenicity prediction

VP2 antigenicity was identified by using the VaxiJen version 2.0 server which employs an alignment-free approach for antigen prediction based on the physicochemical properties of proteins.[11] This investigation resulted in the identification of H9C5X6 as a probable antigen with a prediction probability equal to 0.4821 (the predefined threshold for the virus model was 0.4).

B- and T-cell epitopes prediction

Linear and discontinuous B-cell epitopes were retrieved by querying the ElliPro tool of the Immune Epitope Database Analysis Resource (IEDB),[12] which predicts antibody epitopes based on a protein antigen's 3D structure. The Vaxitop module of the Vaxign system[13] was used to predict the epitope binding to MHC class I and MHC class II alleles. Vaxitop is a very specific and sensitive method for epitope prediction, which is based on the principle of reverse vaccinology.

Subsequently, predicted MHC I binding peptides were tested for the ability to induce immunogenicity with the class I immunogenicity tool of IEDB.[12] Predicted MHC II epitopes were further tested for their potency to induce IFN-gamma (IFN-γ) release from CD4+ T cells using the predict module of the IFNepitope server and the hybrid approach algorithm (motif- and support vector machine-based models).[14] The antigenicity of the predicted B- and T-cell epitopes was re-examined via VaxiJen and the peptides identified as potential antigens were individually tested for the risk of allergenicity and toxicity, as well as the possibility of inducing autoimmunity via the AllerTOP version 2.0, Toxin Pred and Peptide Match tools, respectively.[15–17] Important physicochemical properties (estimated half-life and instability index) of the predicted sequences were also determined with the Expasy ProtParam tool.[18] Default parameters were used and where appropriate, homo sapiens was selected as the host species.

RESULTS

B-cell epitopes

The PDB file of the VP2 model was entered in the ElliPro tool which identified twelve linear and seven discontinuous B-cell epitopes. Out of the twelve predicted linear peptides, only four were found to fulfill the requirements for vaccine design and each one was characterized as probable (a) antigen (antigenicity score >0.4), (b) non-allergen, and (c) non-toxic. Also, no match for these peptides was identified in the peptide search database and all were classified as stable based on the calculated instability index.

YIPPGLMFNPKVPTRRVQYIRQNGSTAASTGRIQPYSKPTSWM: The antigenicity score was 0.5978 and the ElliPro score was 0.839. The estimated half-life was 2.8 hours in mammalian reticulocytes (*in vitro*), ten minutes in yeast (*in vivo*), and two minutes in *Escherichia coli* (*in vivo*). The instability index was 34.22 (stable).

YNLQIKQILSNGADTTYNNDLTAG: The antigenicity score was 0.6581 and the ElliPro score was 0.753. The estimated half-life was 2.8 hours in mammalian reticulocytes (*in vitro*), ten minutes in yeast (*in vivo*), and two minutes in *Escherichia coli* (*in vivo*). The instability index was 17.92 (stable).

GVGISTGGWVGGSHFSDKY: The antigenicity score was 0.6141 and the ElliPro score was 0.700. The estimated half-life was 30 hours in mammalian reticulocytes (*in vitro*), greater than 20 hours in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was -3.31 (stable).

CTNPEGTHINTGAAGFG: The antigenicity score was 0.7372 and the ElliPro score was 0.698. The estimated half-life was 1.2 hours in mammalian reticulocytes (*in vitro*), greater than 20 hours in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was 34.15 (stable).

MHC I and MHC II epitopes

The VaxiTop method identified 53 and 37 unique MHC I and MHC II alleles, respectively. Based on specific criteria (>0.2 immunogenicity score for MHC I binding epitopes; >0.4 antigenicity score for B-cell linear peptides and MHC class I binders; >0.8 antigenicity score for MHC class II binders) 17 T-cell epitopes were found. However, the subsequent computation of physicochemical parameters indicated that ten of the predicted sequences were stable.

MHC class I binding epitopes

QVSCEIVWEV: The antigenicity score was 0.7710 and the immunogenicity score was 0.40345. The estimated half-life was 0.8 hours in mammalian reticulocytes (*in vitro*), ten minutes in yeast (*in vivo*), and ten hours in *Escherichia coli* (*in vivo*). The instability index was 44.83 (unstable).

MPFFLLENS: The antigenicity score was 0.5877 and the

immunogenicity score was 0.21528. The estimated half-life was 30 hours in mammalian reticulocytes (*in vitro*), greater than 20 hours in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was 79.11 (unstable).

RCVTTPWTY: The antigenicity score was 0.5300 and the immunogenicity score was 0.28944. The estimated half-life was one hour in mammalian reticulocytes (*in vitro*), two minutes in yeast (*in vivo*), and two minutes in *Escherichia coli* (*in vivo*). The instability index was -28.82 (stable).

DLTAGVHIF: The antigenicity score was 0.6212 and the immunogenicity score was 0.22889. The estimated half-life was 1.1 hours in mammalian reticulocytes (*in vitro*), three minutes in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was 57.71 (unstable).

VSCEIVWEV: The antigenicity score was 0.6121 and the immunogenicity score was 0.49715. The estimated half-life was 100 hours in mammalian reticulocytes (*in vitro*), greater than 20 hours in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was 57.08 (unstable).

IPIENELADL: The antigenicity score was 0.9721 and the immunogenicity score was 0.27069. The estimated half-life was 20 hours in mammalian reticulocytes (*in vitro*), 30 minutes in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was 41.57 (unstable).

ESTEFTFNF: The antigenicity score was 1.5252 and the immunogenicity score was 0.35891. The estimated half-life was 1 hour in mammalian reticulocytes (*in vitro*), 30 minutes in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was 48.70 (unstable).

VTTPWTYFNF: The antigenicity score was 1.1013 and the immunogenicity score was 0.34648. The estimated half-life was 100 hours in mammalian reticulocytes (*in vitro*), greater than 20 hours in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was -32.43 (stable).

VTTPWTYFN: The antigenicity score was 0.4220 and the immunogenicity score was 0.31896. The estimated half-life was 100 hours in mammalian reticulocytes (*in vitro*), greater than 20 hours in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was -20.44 (stable).

CEWVNNERA: The antigenicity score was 0.7831 and the immunogenicity score was 0.21579. The estimated half-life was 1.2 hours in mammalian reticulocytes (*in vitro*), greater than 20 hours in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was -17.24 (stable).

CEWVNNERAY: The antigenicity score was 0.5981 and the immunogenicity score was 0.26163. The estimated half-life was 1.2 hours in mammalian reticulocytes (*in vitro*), greater than 20 hours in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was -14.52 (stable).

TTPWTYFNFN: The antigenicity score was 1.2462 and the immunogenicity score was 0.42663. The estimated half-life was 7.2 hours in mammalian reticulocytes (*in vitro*), greater than 20 hours in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was -23.94 (stable).

MHC class II binding epitopes

GPGLLSAQR: The antigenicity score was 1.1470 and there was a positive IFN-γ induction. The estimated half-life was 30 hours in mammalian reticulocytes (*in vitro*), greater than 20 hours in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was 8.89 (stable).

TGAYIQPTS: The antigenicity score was 0.9760 and there was a positive IFN-γ induction. The estimated half-life was 7.2 hours in mammalian reticulocytes (*in vitro*), greater than 20 hours in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was 11.42 (stable).

ERAYIPPGL: The antigenicity score was 1.2301 and there was a positive IFN-γ induction. The estimated half-life was one hour in mammalian reticulocytes (*in vitro*), 30 minutes in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was 27.09 (stable).

NFDCEWVNN: The antigenicity score was 1.6979 and there was a positive IFN-γ induction. Its estimated half-life was 1.4 hours in mammalian reticulocytes (*in vitro*), three minutes in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was -20.23 (stable).

TALGMSLGG: The antigenicity score was 0.9480 and there was a positive IFN-γ induction. The estimated half-life was 7.2 hours in mammalian reticulocytes (*in vitro*), greater than 20 hours in yeast (*in vivo*), and greater than ten hours in *Escherichia coli* (*in vivo*). The instability index was 71.42 (unstable).

DISCUSSION

Viral respiratory disease is a leading cause of morbidity and mortality globally.[19] HBoV1 has been identified as one of the most detected respiratory viruses in young children with respiratory tract infections[2] and research evidence suggests that it can function as a co-infectious agent, but also in isolation as a respiratory pathogen causing serious mono-infections both in young children and adults.[20] Expanding on previous research on the immunogenic capsid protein VP2[7,8] in this study, we applied an *in-silico* immunology approach to identify novel epitopes for peptide vaccine design against HBoV1.

Our results suggest that the predicted B- and T-cell linear sequences are antigenic, immunogenic, non-allergen, non-toxic, and impotent in triggering an autoimmune response. Regarding physicochemical features, the GVGISTGGWVGGSHFSDKY B-cell epitope, as well as the VTTPWTYFNF, VTTPWTYFN, and GPGLLSAQR T-cell peptides, were classified as stable also presenting a long half-life in three experimental models (mammalian reticulocytes *in vitro*, yeast *in vivo*, and *Escherichia coli in vivo*). These parameters are important for vaccine development as stable proteins in test tubes,[18] are less prone to losing their structural integrity and activity while prolonged *in vivo* half-life is indispensable when expressing constructs in vectors.

Interestingly, the CTNPEGTHINTGAAGFG, VTTPWTYFNF, VTTPWTYFN, CEWVNNERA, CEWVNNERAY, TTPWTYFNFN, TGAYIQPTS, and NFDCEWVNN residues identified as stable having an estimated half-life >10 hours in *Escherichia coli in vivo*, are also present in peptides that have been previously predicted by the study of Kalyanaraman.[8]

Reverse vaccinology, the *in-silico* screening for the most probable protective antigens in sequenced pathogens, is the first step toward vaccine development.[11] Since there is a lack of available HBoV1 vaccines, we have employed this method to identify B- and T-cell epitopes as potential vaccine targets against the virulent strain HBoV1. Our results confirm the antigenicity of VP2 and point to specific B- and T-cell linear sequences that fulfill prerequisites for vaccine design, thus being suitable for *in vitro* validation.

In conclusion, our findings lay the basis for the evidence-directed experimental investigation of specific B- and T-cell linear sequences for peptide vaccine design against HBoV1.

ΠΕΡΙΛΗΨΗ

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ΣΚΟΠΟΣ Η πρόβλεψη επιτόπων Β- και Τ-κυττάρων στην καψιδιακή πρωτεΐνη VP2, η οποία έχει ταυτοποιηθεί ως δυνητικός στόχος του εμβολιασμού κατά του HBoV1. **ΥΛΙΚΟ-ΜΕΘΟΔΟΣ** Η αλληλουχία της VP2 σε μορφή FASTA ελήφθη μέσω του UniProt και το Phyre2 χρησιμοποιήθηκε για την τρισδιάστατη μοντελοποίηση. Η αντιγονικότητα της VP2 υπολογίστηκε με το Vaxijen έκδοση 2.0. Οι επίτοποι των Β-κυττάρων προβλέφθηκαν με το εργαλείο IEDB ElliPro. Οι επίτοποι MHC τάξης I και II προσδιορίστηκαν με τη μέθοδο Vaxitop του Vaxign. Οι επίτοποι MHC I ελέγχθηκαν με το εργαλείο ανοσογονικότητας του IEDB και οι προσδέτες MHC II με τον διακομιστή IFNepitope. Τα αντιγόνα ελέγχθηκαν ξεχωριστά για τον κίνδυνο πρόκλησης αλλεργίας και τοξικότητας, καθώς και για την πιθανότητα πρόκλησης αυτοανοσίας μέσω των εργαλείων AllerTOP έκδοση 2.0, Toxin Pred και Peptide Match, αντίστοιχα. Οι φυσικοχημικές ιδιότητες προσδιορίστηκαν μέσω του Expasy ProtParam. **ΑΠΟΤΕΛΕΣΜΑΤΑ** Η ανοσοπληροφορική ανάλυση οδήγησε στην ταυτοποίηση ειδικών για τη VP2 Β- και Τ-κυτταρικών επιτόπων που πληρούν τις προϋποθέσεις για τον σχεδιασμό εμβολίου. **ΣΥΜΠΕΡΑΣΜΑΤΑ** Τα ευρήματά μας επιβεβαιώνουν την αντιγονικότητα της VP2 και υποδεικνύουν υποψήφιες γραμμικές αλληλουχίες Β- και Τ-κυττάρων κατάλληλες για *in vitro* επικύρωση.

Λέξεις ευρετηρίου: Αναπνευστική λοίμωξη, Ανθρώπινος μποκαϊός, Ανοσοπληροφορική, Επίτοποι, Καψιδιακή πρωτεΐνη

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