Biomedical Data Mining

Extended Abstract

In silico identified immunogenic Ebola nucleoprotein peptides elicit immune response

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Abstract

Immunoinformatics has dropped significantly to discovering strong antibody competitors against different microorganisms. In the momentum study, a blend of various T (CD4+ and CD8+) and B cell epitope expectation devices was applied to discover peptides containing numerous epitopes against Ebola nucleoprotein (NP) and the introduction of peptides to human leukocyte antigen (HLA) atoms was examined by forecast, docking and populace inclusion apparatuses. Further, ELISA was done to gauge IFN-γ in peptide animated fringe blood mononuclear cells disengaged from the blood of sound volunteers. Six peptides containing various T and B cell epitopes were acquired after expectation examines and dispensing with the peptides at risk to create immune system and unfavorably susceptible reaction. All peptides showed 100% conservancy in Zaire Ebola infection. Forecast devices, Auto dock Vina and CABS-dock results affirmed the capacity of anticipated peptides to tie with assorted HLA alleles. Populace inclusion investigation anticipated high inclusion (> 85%) for anticipated insusceptible reaction in four landmasses (Africa, America, Asia and Europe). Peptide animated cells showed upgraded IFN-γ emission when contrasted with unstimulated cells. Thusly, the recognized NP peptides can be considered as potential engineered antibody competitors against Ebola virus.

Introduction

Ebolavirus (EBOV) is a Filoviridae member responsible for Ebola virus disease (EVD) which prompts ungoverned viral replication and multi-organ failure. The virus is known to multiply in various cell types (hepatocytes, macrophages, endothelial and epithelial cells) and speedily makes its way into the vital organs of the host. Most extreme instances of EVD occur because of individual to individual transmission. Roughly, 30,000 instances of Ebola have been accounted for till date since 1976 with North Kivu territory being the site of most recent episode in 2018.

EBOV is a single non-segmented negative-stranded RNA virus with an unusual, variable-length, filamentous morphology. It consists of seven proteins viz. nucleoprotein (NP), polymerase cofactors (VP35 and VP40), glycoprotein (GP), transcription activators (VP30 and VP24) and RNA-dependent RNA polymerase (L). Like its family members, EBOV RNA is incapable of existing in naked form. Nucleoprotein (NP) serves as scaffold for assembly of filovirus nucleocapsid (NC) which includes VP35, V30, VP24 and L. NP interacts with VP35 and VP30 which in turn interact with polymerase and help in assembling viral replication complex. NC plays role in viral RNA synthesis during proliferation cycle. NP is fundamental in viral RNA union and infection get together as ssRNA restricting is likely reliant on oligomerization and legitimate direction of NP. NP additionally shields the infection from have natural safe reactions and gives protection from have ribonucleases. At the point when NP-explicit CTLs were given to gullible mice tested with a deadly EBOV portion, they assisted with inciting assurance against EBOV demonstrating the job of cell-intervened invulnerability against NP. In other late examination, investigation of T cell reaction was done for seven proteins of EBOV in 30 people who made due after EBOV contamination and it was seen that the greatest survivors (96%) reacted against the NP protein when contrasted with different proteins. Consequently, NP is an exceptionally basic protein and, subsequently,

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introduces itself as a rewarding antibody configuration target..

Vaccine development against EBOV is as yet being developed stage and different preliminaries incorporate viral vector-based vaccines, protein-based vaccines and subunit vaccines. In recent years, there has been a noteworthy advancement in peptide-based vaccines which are fragments of protein antigen sequences assembled into a single molecule capable of inducing an immune response. Immunoinformatics tools have shown success in elucidating potent peptide vaccine candidates against influenza virus, hepatitis C, West Nile virus and EBOV. The immunogenic peptides got utilizing this methodology were approved in vitro framework for flu and in vivo framework for Brucella abortus and EBOV.

In the current examination, peptides containing various epitopes against EBOV NP were chosen dependent on various epitope forecast instruments and analyzed for their preservation among the EBOV species and other Filoviridae individuals. These peptides were searched for restricting potential to assorted HLA atoms dependent on various forecast instruments, mooring and populace inclusion investigation. Further, in vitro approval of immunogenic reaction of three potential peptides was done by estimating IFN-γ discharged by peptide-animated fringe blood mononuclear cells (PBMC) disconnected from solid blood tests.

Methods

Conserved peptides identification

195 unique Ebola nucleoprotein sequences (739 amino acids) out of a total of 2407 entries (1976–May 2018) were downloaded from viprbrc and NCBI databases. These sequences comprised 187 (Zaire), five (Sudan), two (Bundibugyo) and one (Taï Forest) sequences belonging to various Ebola species pathogenic to humans. MUSCLE and AVANA tools were employed to identify peptide fragments showing at least 90% conservancy.

Prediction of T and B cell epitopes

T cell epitopes were predicted based on a consensus approach that includes three prediction tools (SYFPEITHI, NetCTL 1.2 and IEDB consensus) for CD8+ T cell epitopes (HLA class I) and three tools (MHC2Pred, Propred and IEDB consensus) for CD4+ T cell epitopes (HLA class II). The epitopes showing overlaps were further joined to obtain peptide fragments containing both CD4+ and CD8+ T cell epitopes.

Screening of peptides for autoimmune and allergic response

Peptides having seven out of nine consecutive amino acids identical to human proteome were eliminated using BLAST analysis. Allergenicity of the peptides was predicted using the online tool AlgPred, which is based on screening IgE epitopes in query protein sequence and Motif Alignment & Search Tool.

Conservancy analysis amongst Ebola species and other filoviridae members

The identified peptides were looked for conservancy in human-pathogenic Ebola species (Zaire, Sudan, Bundibugyo and Taï forest) sequences as well as in 18 unique out of a total of 79 Marburgvirus nucleoprotein sequences and the only available unique nucleoprotein sequence of Lloviuvirus obtained from viprbrc and NCBI databases.

Molecular docking

HLA–peptide interaction analysis was done with the help of CABS-dock which allows for flexibility of peptide and receptor backbone. High-resolution crystal structures of eighteen HLA class I and II alleles (nine each) bound to their respective native peptide were obtained from PDB. The HLA crystal structures without their native peptides (peptides already bound to the HLA) were obtained using Discovery Studio

Visualizer (version 4.1). RMSD values obtained by docking the native peptides to their respective HLA structures served as standard. Peptides showing RMSD > 5 or found to be interacting outside the binding groove were eliminated.

Population coverage analysis

IEDB population coverage analysis tool, based on peptide-HLA data and HLA genotypic frequency, plays an important role in a bid to develop a globally protective vaccine. The selected peptides and their HLA alleles obtained from prediction tools were used as input for this tool. For this analysis, four different geographical continents (Africa, America, Asia and Europe) were chosen. Africa, America and Asia comprised 13 different geographical regions and, therefore, the average of population coverage for these regions was considered. Analysis was also carried out by taking into account the whole world.

Statistical analysis

One-way Anova followed by Tukey's multiple comparison test using GraphPad Prism was carried out to analyze the docking data.

Measurement of IFN-γ secreted by peptide-stimulated peripheral blood mononuclear cells

P2, P3 and P5 were commercially synthesized by GL Biochem (Shanghai) Ltd. Healthy blood samples were obtained from Nitin Hospital, Patiala and Rajindra Hospital, Patiala (India) after informed consent from all volunteers. The study was approved by the institutional ethical committee. Peripheral blood mononuclear cells (PBMC) were isolated via ficoll density gradient method. Restimulation assay was carried out for measuring peptide-induced IFN-γ secretion with certain modifications to the previous report. In a 96-well cell culture plate, 2 × 105 cells were seeded per well in a total volume of 200 µL complete media (RPMI-1640 supplemented with 10% fetal bovine serum, 100 µg/mL streptomycin, 100 I.U./mL penicillin and 10 mM HEPES) and stimulated with each peptide (50 µg/mL). Unstimulated cells served as negative control while cells stimulated with 10 µg/mL of concanavalin A (ConA, Sigma-Aldrich) served as positive control. Restimulation was done on 3rd day with each peptide. On 5th day, IFN-γ secreted by unstimulated, peptide-stimulated and ConA-stimulated cells was measured by performing ELISA with the help of human IFN-γ mini Elisa development kit (Peprotech, USA). A microplate reader (Tecan Austria) was used to take absorbance at 405 nm with 630 nm as reference wavelength. All experiments were carried out in triplicates. IFN-γ production was expressed as fold change which is the ratio of absorbance of peptide-stimulated cells and unstimulated cells.

Results

Conserved peptides containing T and B cell epitopes and having no autoimmune and allergic properties

Four overlapping fragments (C1–C4) with ≥90% conservancy in 195 Ebola nucleoprotein sequences were obtained after multiple sequence alignment via MUSCLE and conservancy analysis via AVANA. Next, epitopes commonly predicted in the identified fragments by six epitope prediction tools (three each for HLA class I and II) were considered. Initially, 105 and 79 HLA class I (CD8+ T cell) and II (CD4+ T cell) binding epitopes were obtained, respectively. Twelve peptide fragments containing multiple CD8+ and CD4+ T cell epitopes were obtained by merging overlapping epitopes.

A total of 201 linear B cell epitopes were obtained after analyzing the four conserved fragments (C1–C4) via ABCpred. The predicted B cell epitopes were present only in eight identified fragments. These eight peptides were checked for autoimmune and allergic responses. Two peptide fragments (VGHMMVIFRLMRTNFLIKFLLIHQGMHMV and YAPFARLLNLSGV) exhibited similarity to intrinsic human proteins based on BLAST analysis and, hence, was eliminated. Algpred tool confirmed none of the peptides to be allergic in nature. Thus, six non-self and non-allergic peptide candidates possessing multiple B and T cell epitopes were selected.

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Conservation analysis of peptides amongst different Ebola virus species and related family members

Six identified fragments were investigated for their conservancy in different species of Ebola virus (Zaire, Sudan, Bundibugyo and Taï Forest) and other members (Marburgvirus and Lloviuvirus) of Filoviridae to judge the potential of these candidates to develop cross protective immunity. Interestingly, all selected peptides showed 100% conservancy amongst Zaire ebolavirus nucleoprotein sequences. P3 was found to be 100% conserved in all Ebola virus species and Lloviuvirus and there was a single amino acid variation in case of Marburgvirus. P5 was found to be 100% conserved in three Ebola virus species (Zaire, Bundibugyo and Taï Forest) while one amino acid variation at same position was observed in Sudan, Lloviu and Marburg viruses. Rest of the peptides were found with few variations in different Ebola virus species. In most cases, one variable sequence was observed but P2 (Sudan ebolavirus) and P4 (Marburgvirus) were found to be with two variable sequences. P1, P2 and P6 could not be located in NP sequence of Lloviu and Marburg viruses.

Discussion

Peptides as a choice for vaccine formulation is one of the recent developments and many peptide-based vaccines are in different stages of clinical trials. More than ten anti-cancer peptide vaccine candidates have made it to phase III trials. Phase II trials are being conducted for peptide vaccines against influenza and HPV-induced cancer. Relying solely on in vitro or in vivo analysis for immunogenic peptide identification is cumbersome and not feasible in all facilities around the world. Computational immunology offers advantages in downsizing the number of peptide candidate to be validated in vitro or in vivo. In the current study, six EBOV NP peptides containing multiple epitopes having potential to interact with an array of HLA molecules were identified. Numerous computational works have been done in identifying epitopes against different infectious organisms such as Leishmania, Mycobacterium tuberculosis and influenza virus. Immunoinformatically identified peptides have shown enhanced proliferation and IFN-γ production in peptide-induced peripheral blood mononuclear cells. Also, in vivo validation of computationally generated peptide vaccine candidates with mice as subject for Leishmania donovani, Moraxella catarrhalis and Brucella abortus showed promising results. Hence, computational identification of potential peptide vaccine candidates against different infections has picked pace in recent years. EBOV represents a serious concern for human health and, thus, with the application of various computational tools, six EBOV NP peptides containing multiple T and B cell epitopes were designed. Owing to the presence of different epitopes, these peptides may be capable of generating both humoral and cell-mediated immunity.

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