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VALIDATION OF VACCINE CANDIDATE FOR H1N1 INFLUENZA BY USING BIOINFORMATICS TOOLS

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Abstract- The need for validation studies towards the immunogenic potential of vaccine developed for a specific viral strain that can combat infections from the other strains becomes very important due to the frequent variations in the viral genes and antigens. To achieve this objective, a novel tool is developed which validates a given vaccine against infections caused by new strains of pathogens due to occurrence of mutations. The tool requires a set of inputs, which include mutated sequence (query sequence), vaccine candidate (for which good immunogenicity is established and is currently used as vaccine) and a chosen receptor from a given list. The tool takes the sequences (query sequence and vaccine candidate) as data and then identifies the epitope regions in both these sequences. The epitope regions thus identified (of both the mutated and vaccine candidate) are then docked with that of the chosen receptor. The energy values corresponding to the mutated epitope -receptor complex are then compared with the energy values of the vaccine candidate epitope- receptor complex. Based on the determined energy values, the tool predicts the potential of the vaccine candidate in case of mutated viral infections; If the energy of the vaccine-receptor complex is lower than or equal to that of the mutated-receptor complex, then the vaccine is evaluated as suitable; If the energy value of the vaccine complex is higher than the mutated complex energy value, then the candidate is ruled void. Based on the results, the vaccine candidate is recommended for the mutated viral infections .

Keywords- Vaccine candidate, Mutation, Receptors, Energy Value

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Introduction

Designing a vaccine for a disease is one of the crucial tasks that involve millions and billions of dollars, several decades and yet there is no guarantee of successful results. Several pharmaceutical companies are investing their money and time in such activities. Computational biology could be of great help in these activities by proving a library of plausible candidates that might actually show some positive responses [3]. The pandemic of 1918 was caused by an H1N1 influenza A virus [Fig-1] and [Fig-2], which is a negative strand RNA virus. The world has recently overcome the first influenza pandemic of the 21st century caused by a novel H1N1 virus (pH1N1) which is a triple reassortant comprising genes derived from avian, human, and swine influenza viruses and antigenically quite different from seasonal H1N1 strains. Although the case fatality rates have decreased in many developed countries, the situation is still alarming in many developing countries including India where considerable numbers of new cases are appearing everyday. There is still a high morbidity and mortality of susceptible adult as well as young population without having underlying health issues due to the influenza infection [13]. With the potential for sudden outbreaks, rapid spread, and high incidence of complications, the prevalence of influenza infections has caused tremendous loss of human life and material resources [5,9]. Thus, it is important to develop new approaches towards preventing seasonal infections as well as potential pandemics of influenza. Based on their internal protein antigens, different influenza viruses can be divided into 3 types: A, B, or C. The surface antigens [Fig-3], hemagglutinin (HA) and neuraminidase (NA) are also used to identify different subtypes. At present, the prevalent human influenza viruses are the type A H3/H1 and type B viruses. However, in recent years, multiple subtypes (H5/H7/H9) of the avian influenza virus (AIV) have been able to cross the species barrier to infect humans [6,8]. As of 2009, the known SIV strains include influenza C and subtypes of influenza A known as H1N1, H1N2, H3N1, H3N2 and H2N3. Around the world, the highly pathogenic avian influenza virus subtype H5N1 has caused infectious outbreaks in various human populations [14]. Influenza vaccines based on the conventional subtypes of each species have been unable to effectively prevent this rising trend. Creating vaccines

which can provide long-term protection against more than one subtype of influenza has become a hot topic in vaccine development. However, due to the rapidly changing influenza virus or the phenomena of "antigenic shift" and "antigenic drift", developing a vaccine that can protect against all possible circulating viruses is extremely challenging. Immunogenic epitopes in an antigen is determined by the major histocompatibility complex (MHC) class I for cytotoxic T cell lymhocytes (CTL) and MHC class II for T helper (Th) cells. These polymorphic MHC molecules present short peptides that are processed after an exogenous antigen (such as a viral protein) is taken up by antigen presenting cells (APC) such as macrophages and dendritic cells. These APC then "present" the peptide to the immune cells that recognize the MHC/peptide complex via the T cell receptor (TCR) or B cell receptor (BCR). Theoretically, given any set of MHC II restricted peptides presented to the Th cells, the optimal sequence would be those that could also stimulate B cells to produce antibodies since activation of antigen-specific. The cells also promote antibody production. By understanding the specific epitopes from pathogens that can stimulate optimal immune responses, we will better understand how to tailor vaccines to a specific population and/or pathogen. Each year, a new vaccine must be prepared that will be effective against the expected type of influenza virus. Thus there is a need to evaluate vaccine before being used on a population for the current ongoing infections. Hence we felt a need to develop a tool that can predict the immunogenic potential of a vaccine. Here we have attempted to develop a tool to validate an existing H1N1 vaccine with respect to the mutated strains on basis of standard parameters. The sequencing of genomes pathogens offers immense opportunities to aid in the development of new therapeutics and vaccine candidates through Bioinformatics tools and resources [12].

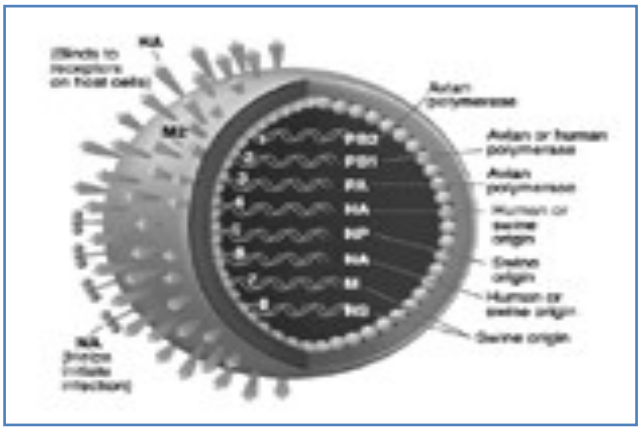

Fig. 1- The Structure of an H1N1 virion

The influenza virion (as the infectious particle is called) is roughly spherical. It is an enveloped virus i.e., the outer layer is a lipid membrane which is derived from the host cell in which the virus multiplies. Inserted into the lipid membrane are 'spikes', which are proteins - actually glycoproteins, because they consist of protein linked to sugars - known as HA (Hemagglutinin) and NA (Neuraminidase). These are the proteins that determine the type of influenza virus (A, B, or C) and the subtype (A/H1N1, for example). The HA and NA are important proteins in the immune response against the virus; antibodies (proteins made by us to combat infection) against these spikes may protect against an infection. The NA protein is the target of the antiviral drugs Relenza and Tamiflu [11]. Also embedded in the lipid membrane is the M2 protein, which is the target of the antiviral.

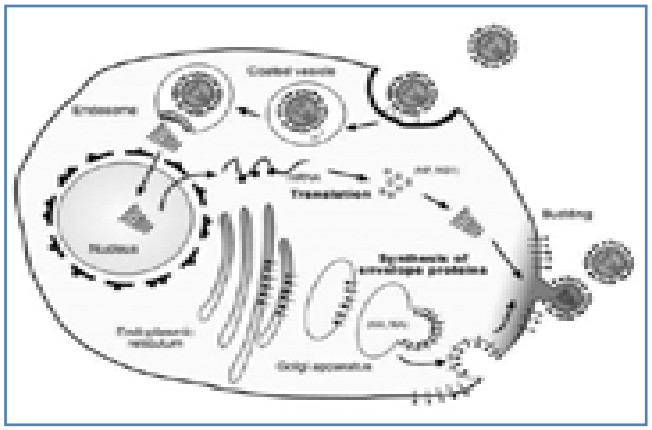

Fig. 2- Steps in the replication cycle of influenza A virus: a) binding b) entry of the virus c) fusion with endosomal membrane d) release of viral RNA e) replication within the nucleus and f) synthesis of structural and envelope proteins, budding and release of virions capable of infecting neighboring cells.

Adamantanes - Amantadine and Rimantadine

Beneath the lipid membrane is a viral protein called M1, or matrix protein. This protein, which forms a shell, gives strength and rigidity to the lipid envelope. Within the interior of the virion are the viral RNA's – 8 of them for influenza A viruses. These are the genetic material of the virus; they code for one protein each. Each 'RNA segment', as they are called, consists of RNA joined with several proteins shown in the diagram: PB1, PB2, PA, NP. These RNA segments are the genes of influenza virus. The interior of the virion also contains another protein called NEP. Symptoms of Swine Flu in humans include chills, fever, sore throat, muscle pains, severe headache, coughing, weakness and general discomfort. CDC recommends influenza vaccination as the first and most important step in protecting against the flu [2]. All influenza viruses (all orthomyxoviruses) have RNA as their genetic material. When RNA is replicated it tends to have more errors than when DNA is replicated. These extra errors provide extra mutations upon which selection may act. That means RNA viruses (not just influenza viruses but all RNA viruses) have a high mutation rate and can evolve quickly - faster than a DNA virus or even a DNA human! Over time these mutations accumulate and eventually the virus evolves into a new strain. This progressive accumulation of individual mutations is called antigenic drift, because the shape of the antigen (the viral protein) slowly drifts into a different shape with each generation of virus. Eventually they drift so much that the original antibody can no longer bind to it. All viruses show antigenic drift, but RNA viruses mutate faster so they drift faster. Antigenic drift is responsible for many of the localized outbreaks of different strains of influenza, especially influenza B. The RNA genome of an influenza virus is divided into eight different segments numbered one through eight, with number one being the smallest segment. Each segment functions as an individual gene coding for one of the virus proteins. Segment number four contains the gene for hemagglutinin (HA) and segment six encodes the gene for neuraminidase (NA).

Importantly, type A - but not B or C - undergo a kind of gene swapping or genetic re-assortment to give it its proper name. If a cell is simultaneously infected by two different strains of type A influenza, the offspring virions may contain mixtures of each parents' genes. This really complicates things and makes it very easy for influenza A to quickly evolve new combinations of HA and NA genes. We know of 15 different kinds of HA and 9 different kinds of NA genes in type A influenza. All these different kinds have evolved by antigenic drift as described earlier. Any one virion can contain only one HA and one NA. For example we might have influenza A strain designated H1N1. Along comes another virus with different kinds of HA and NA genes, let's say it is H3N7.

Interaction of Epitopes with Receptors

By definition, antibodies bind to specific antigens. In the case of an antibody which fights viruses, the antigen is a specific viral protein. Antibodies bind the HA, the important receptor-binding protein of the influenza virus, blocking it so it can't infect other cells. Another group of antibodies bind to the NA of the virion and may prevent the spread of further infection. Development of an epitope-based vaccine for influenza may also be a useful strategy to overcoming the challenge of inducing a specific immune response against this constantly evolving virus. CTL epitopes mediate cytolytic effects on infected cells and induce inflammatory factors during viral clearance, while B cell epitopes can induce protective antibody-mediated humoral immune responses. The epitopes can activate CD4+ T cells to carry out important immune regulatory functions, and the identification of specific epitopes derived from influenza virus has significantly advanced the development of peptide-based vaccines [1,4,10,16]. Improved understanding of the molecular basis of antigen recognition and human leukocyte antigen (HLA) binding motifs has allowed the development of rationally designed vaccines based on motifs predicted to bind to human class I or class II MHC.

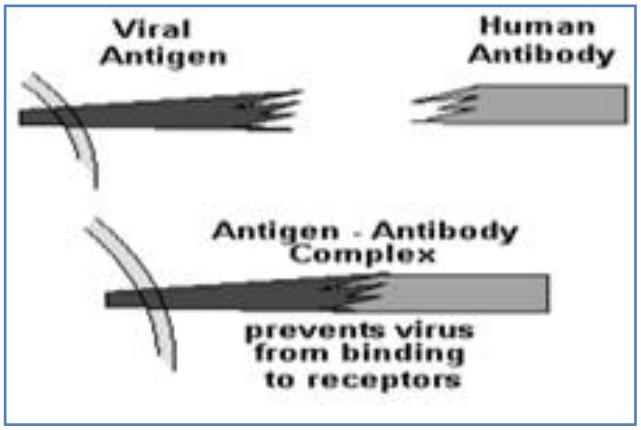

Fig. 3- Antigen-antibody reaction

Therefore, identification of the corresponding functional influenza epitopes will have important theoretical and practical value in studies on immunity against virus infection and on vaccine development. Detailed information on the patterning of the epitopes and other motifs of importance from the viewpoint of reverse vaccinology has been obtained on the most probable protein candidates for vaccine investigation from three major malarial species by the developers of Malvac, a malarial vaccines' database [12]. If a different strain of influenza gets into the lungs, the old antibodies will not bind it correctly because the shape of a virus' receptor-binding proteins is not the same from one strain to another. So, that NEW strain will go about establishing a new infection with all those horrible symptoms. Immune system will eventually create a new group of antibodies to fight the new strain. Once, recovered patient will be protected from that new strain, but not the next new strain. And so it goes on throughout our lives. By the time patient is very old he/she will have antibodies to several different influenza strains, each antibody corresponding to a previous infection and thus protecting us from re-infection with that strain. Unfortunately, as they get very old the immune system tends to "forget" some of the older strains and also has difficulty fighting off new ones. That's one of the reasons why influenza is particularly serious among the very old.

T Cell Receptors and T Cell Epitopes

Protection by current human influenza vaccines is achieved by use of inactivated or attenuated forms of the corresponding pathogen and appears to require the function of neutralizing antibodies against the external HA and NA glycoproteins. However, these glycoproteins mutate rapidly through antigenic drift and current vaccines become ineffective as mutational differences accumulate in the circulating strains. In order to overcome the antigenic variability of influenza external glycoproteins, new vaccine strategies are increasingly directed at conserved regions of the viral proteins for production of T cell epitope-based vaccines. The goal is to identify conserved sequences that function as epitopes recognized by human leukocyte antigen (HLA) molecules for presentation to $CD8^+$ and $CD4^+$ T cells that provide immunity against all influenza A virus subtypes and obviate the need for yearly vaccine update. Several animal model studies taking this approach have reported Tcell responses that reduce morbidity and promote recovery in mouse models of influenza challenge. Both CD8+ and CD4+ T cell responses are required; CD8+ T cells to kill infected cells and CD4+ T cells for the development of an effective immune response and immune memory. A complication of cellular immunity is that T cell responses are dependent upon antigen presentation by highly polymorphic HLA molecules that vary greatly among human populations. However, the limited population coverage of some HLA alleles may be alleviated by focusing on T cell epitopes recognized by HLA super types that bind largely overlapping peptide repertoires on the basis of the specificity for the main anchor positions of the presented peptides [14].

H1N1 Vaccines

In 2009 the global community was struck by one of the worst epidemics in history. A disease so viral its spread is virtually impossible to contain and manage. The Swine Influenza epidemic of 2009 has now achieved a pandemic status. The first time something like this has happened in the last four decades.

A major concern about the ongoing swine-origin H1N1 influenza virus (S-OIV) outbreak is that the virus may be so different from seasonal H1N1 that little immune protection exists in the human population. In this study, we examined the molecular basis for pre-existing immunity against S-OIV, namely the recognition of viral immune epitopes by T cells or B cells/antibodies that have been previously primed by circulating influenza strains. Using data from the Immune Epitope Database, we found that only 31% (8/26) of B-

cell epitopes present in recently circulating H1N1 strains are conserved in the S-OIV, with only 17% (1/6) conserved in the hemagglutinin (HA) and neuraminidase (NA) surface proteins. In contrast, 69% (54/78) of the epitopes recognized by CD8_T cells are completely invariant. We further demonstrate experimentally that some memory T-cell immunity against S-OIV is present in the adult population and that such memory is of similar magnitude as the pre-existing memory against seasonal H1N1 influenza. Because protection from infection is antibody mediated, a new vaccine based on the specific S-OIV HA and NA proteins is likely to be required to prevent infection. However, T cells are known to blunt disease severity. Therefore, the conservation of a large fraction of T-cell epitopes suggests that the severity of an S-OIV infection, as far as it is determined by susceptibility of the virus to immune attack, would not differ much from that of seasonal flu. These results are consistent with reports about disease incidence, severity and mortality rates associated with human S-OIV.

The development of a swine influenza vaccine is a long and tedious process which can only be undertaken by only a handful of pharmaceutical companies. The vaccine development process is quite intricate; they utilize various bits of several different flu viruses to construct a vaccine which specifically stimulates the body's own immune system against the Swine Influenza Virus. The reason for using bits of the virus is because it would be the only way to generate sufficient quantities of the vaccine (the bits of viruses are actually grown in hen eggs). After the viruses are extracted from the hen eggs, they are broken down into smaller pieces which ensure the protein coat of the virus is exposed so that it can induce an auto-immune response in humans. Antibodies are then formed in the blood as a direct response to the external protein particles. These are the particles that best resemble those of the swine flu virus. Therefore the vaccine actually loads the immune system full of antibodies which will attack the swine flu virus should a person contract it. But as it has already been stated that developing a vaccine is not an easy task due to the mutations occurring in the prevailing strains of the virus, the original vaccine would cease to initiate response, especially if the mutation has occurred in the epitope regions, which would then cause pathogenicity in different ways.

This is where Bioinformatics play a major role. With the help of Bioinformatics we can come up with vaccines in just mere weeks as opposed to the six, seven long years as with the traditional biotechnology. The better understanding and basic knowledge of influenza virus including their high variability and potential for antigenic drift, has prompted the development of new and more efficacious vaccines. We can now use a Bioinformatics tool to validate the vaccine candidate with respect to the mutated strains on the basis of certain parameters. Thus the most suitable vaccine from amongst the many vaccine candidates for H1N1virus can be found out with the help of a bioinformatics tool. Based upon the growing number of bioinformatics tools and antigen sequences available in public databases for identifying pathogen peptides, the in silico prediction of T-cell epitopes can greatly reduce the list of candidate epitopes. Such a shortlist is then the starting point for molecular experiments that can validate the vaccine targets based on the biological function of the selected antigen sequences.

MUTVAC [7] is our attempt to simplify the process of vaccine selection. MUTVAC is a Bioinformatics tool [Fig-4] written in for the validation of vaccine candidates based on immunological and molecular kinetics parameters. The tool is coded in Java which happens to be a very dynamic, user friendly and flexible object oriented programming language. Credit is to be given to the programming language for the design, GUI or the look and feel of the tool.

Methodology

A wide literature survey was carried out on all strains of influenza virus and the genome sequence of the Human Influenza Virus (India) were obtained by the help of NCBI in FASTA format. Through literature surveys, the important proteins of the virus were recognized and the gene sequences were obtained and converted to the protein counterparts.

Open Reading Frames

Using the consensus sequences obtained from the database of NCBI, ORF prediction was done using ORF finder from NCBI. The ORF Finder (Open Reading Frame Finder) is a graphical analysis tool which finds all open reading frames of a selectable minimum size in a user's sequence or in a sequence already in the database. This tool identifies all open reading frames using the standard or alternative genetic codes. The deduced amino acid sequence can be saved in various formats and searched against the sequence database using the WWW BLAST server. The ORF Finder should be helpful in preparing complete and accurate sequence submissions. It is also packaged with the Sequin sequence submission software. The sequence obtained is in the FASTA format was blasted using ORF finder. Basic Local Alignment Search Tool or BLAST, is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of different proteins or the nucleotides of DNA sequences. A BLAST search enables a researcher to compare a query sequence with a library or database of sequences, and identify library sequences that resemble the query sequence above a certain threshold. For example, following the discovery of a previously unknown gene in the mouse, a scientist will typically perform a BLAST search of the human genome to see if humans carry a similar gene; BLAST will identify sequences in the human genome that resemble the mouse gene based on similarity of sequence.

Consensus Sequence

To Obtain a Consensus Sequence, Data mining of Indian Swine flu Genome set for Influenza A in humans for different proteins and nucleotides was done, using NLM/NCBI H1N1 flu resources. Literature survey for the different proteins in Influenza A was done.

Antigen Prediction

During the course of identifying the antigens, many tools were available. To conclude on a tool required for our purpose, an extensive search of available tools was done and a benchmark was created. Based on which, the EpiToolkit was used for the purpose of our study.

MUTVAC Tool

- A provision of two text input fields is made for the acquisition of the mutated sequence and the vaccine candidate sequence. These are to be provided in the FASTA format.

- A drop down box is provided, whereby the user is given the choice to select a receptor from a list of five most probable ones involved in protection of H1N1.
- Once inputs are obtained,

⇒ The epitope regions of the mutated sequence are identified and stored after which the most suitable one will be selected.

⇒ Similarly the epitope regions of the vaccine candidate are identified and stored and the most suitable one will be selected.

- Now, we will have essentially two different categories of epitopes,

⇒ The Mutated epitope

⇒ The Vaccine Candidate epitope.

- Following this, a docking procedure will be performed.

The Selected Mutated Epitope with the receptor.

The Selected vaccine candidate Epitope with the receptor.

- The docked structure of the vaccine candidate epitope and the receptor is associated with a certain energy value.
- The docked structure of the mutated sequence epitope and the receptor is associated with a certain energy value.
- If the energy value of the vaccine candidate epitope docked with the receptor is lower than or equal to that of the mutated sequence epitope with the receptor, then the vaccine candidate is considered to be a good vaccine for the mutated strain.
- If the energy value of the vaccine candidate epitope docked with the receptor is higher to that of the mutated sequence epitope with the receptor, then the vaccine candidate is considered to be void or incompatible for that strain.

Working Principle of MUTVAC Tool

- NCBI database is incorporated to our tool.

A direct access is provide in our home page which links to the home page of NCBI for the ease of selection of sequences.

⇒ User could choose a vaccine candidate sequence of his/her choice from the database and feed the input in the “vaccine candidate” input box.

⇒ Similarly user could also feed a mutated sequence of his/her choice to the “mutated sequence” input box.

⇒ This can be done again by choosing one from the NCBI database or the user can also give one of his/her own choice.

⇒ Any one receptor is chosen as per the user from amongst the 5 receptors provided in the dropdown box.

⇒ An EPITOPE FINDING TOOL is incorporated in our tool.

⇒ The mutated sequence is fed to the epitope finding tool.

⇒ The epitope regions in the mutated sequence are predicted and stored.

⇒ Similarly, the vaccine candidate is given as input to the epitope finding tool.

⇒ The epitope regions in the vaccine candidate is predicted and stored.

This is done in the following manner: Example:

1. Let us consider the mutated sequence to be variable M.
2. Let us consider the vaccine candidate to be variable V
3. Let us consider the chosen receptor to be variable R.
4. Let us assume that M be "ABCDEFGHIJ” which is provided to the epitope prediction tool.
5. The tool then identifies the epitope regions of this sequence and give an output like "CD" or "GH" which is assigned temporary variables as M1 and M2 respectively.
6. Similarly for "V", we get V1 and V2. NOTE: The number of epitopes will vary with each sequence.

⇒ A DOCKING TOOL is incorporated into our tool.

⇒ The epitope regions of the vaccine candidate and the chosen receptor are given as inputs to the DOCKING TOOL.

Example:

V1 and V2 are docked with R.

⇒ The epitope region which forms the best fit with the receptor (i.e gives the lowest energy value) is considered.

Example:

Let us assume V1 makes the best fit (that is V1 docked with R gives the least energy value).

This energy value is considered as variable X.

⇒ The epitope regions of the mutated sequence and the chosen receptor are given as inputs to the DOCKING TOOL.

Example:

M1 and M2 are docked with R

⇒ The epitope region which forms the best fit with the receptor (i.e gives the lowest energy value) is considered.

Example:

Let us assume M1 makes the best fit(that is M1 docked with R gives the least energy value)

This energy value is considered as variable Y

⇒ If X <= Y then validation is positive i.e., the vaccine candidate holds good for the mutated sequence.

⇒ If X > Y then validation is negative i.e., the vaccine candidate does not hold good for the mutated sequence.

Results and Discussion

Open Reading Frames

- For further discovery of novel functional proteins, the ORF of the expressed genomic sequence were identified.
- The above research was carried out with NCBI ORF finder with Indian strains which resulted in 8 new ORFs.
- The longest segment among the 6 reading frame were shortlisted and blasted with human database.
- CY020460, CY020459, CY020458, CY020453, CY020456, CY020455, CY020454, CY020457.

Consensus Sequence

Based on the above statistics the important proteins were used to get the database of human influenza A of different population. The above obtained data was formatted and the accession numbers of the required proteins were uploaded in the Entrez Batch tool. We tried the same using nucleotides to get a better understanding of the variation of the different populations.

Hemagglutinin	92
Neuraminidase	56
Matrix 1 (M1)	13
M2 protein	18
Structural proteins	3
Surface glycoproteins	2
Antigen protein	2
Recombinant RSV fusion protein	1

Antigen Prediction

From the consensus sequences, the antigenic property sites were identified with a degree of immunogenicity. Peptides of 9, 10, 11 were identified and tabulated.

Some of them shortlisted are:

1. Matrix protein M3 - influenza A virus (strain A/FPV/ Weybridge [H7N7], remantadine-sensitive)

>gi|112616|pir||PN0085 matrix protein M3 - influenza A virus (strain A/FPV/ Weybridge [H7N7], remantadine-sensitive)

2. Matrix protein M3 - influenza A virus (strain A/FPV/ Weybridge [H7N7], remantadine-resistant)

>gi|112617|pir||PN0088 Matrix protein M3 - influenza A virus (strain A/FPV/ Weybridge [H7N7], remantadine-resistant)

Hemagglutinin [Influenza A virus (A/Hong Kong/CUHK33106/2002 (H3N2))]

3. >gi|194268835|gb|ACF36341.1| Hemagglutinin [Influenza A virus (A/Hong Kong/CUHK33106/2002(H3N2))]
4. >gi|194268763|gb|ACF36305.1| Hemagglutinin [Influenza A virus (A/Hong Kong/CUHK24167/2002(H3N2))]
5. >gi|193805010|gb|ACF22148.1| Hemagglutinin [Influenza A virus (A/Yucatan/844/2003(H3N2))]
6. >gi|194268831|gb|ACF36339.1| Hemagglutinin [Influenza A virus (A/Hong Kong/CUHK33047/2002(H3N2))]
7. >gi|194268861|gb|ACF36354.1| Hemagglutinin [Influenza A virus (A/Hong Kong/CUHK34175/2002(H3N2))]
8. >gi|194268863|gb|ACF36355.1| Hemagglutinin [Influenza A virus (A/Hong Kong/CUHK34193/2002(H3N2))]
9. >gi|194268757|gb|ACF36302.1| Hemagglutinin [Influenza A virus (A/Hong Kong/CUHK24044/2002(H3N2))]
10. >gi|194268837|gb|ACF36342.1| Hemagglutinin [Influenza A virus (A/Hong Kong/CUHK6383/2003(H3N2))]
11. >gi|194269005|gb|ACF36426.1| Hemagglutinin [Influenza A virus (A/Hong Kong/CUHK5723/2003(H3N2))]
12. >gi|194268603|gb|ACF36225.1| Hemagglutinin [Influenza A virus (A/Hong Kong/CUHK13339/2003(H3N2))]
13. Neuraminidase [Influenza A virus (A/duck/Eastern China/48/2002(H11N2))]

>gi|167859426|gb|ACA04672.1| Neuraminidase [Influenza A virus (A/duck/Eastern China/48/2002(H11N2))]

These were later used as vaccine candidates for the testing of the tool. Also because they play a major role as vaccine candidates, hence they were shortlisted.

The receptors predicted were:

1. NK inhibitory receptors
2. KIR2DL1 and the LIR1
3. Alphabeta T-cell receptor (TCR)
4. Mannose receptor
5. Ly49 receptor

These receptors are used in the tool. The screenshot of the home page is as shown below:

It contains the layout of the tool, a brief description of the tool, the home page contents and an access to the query page.

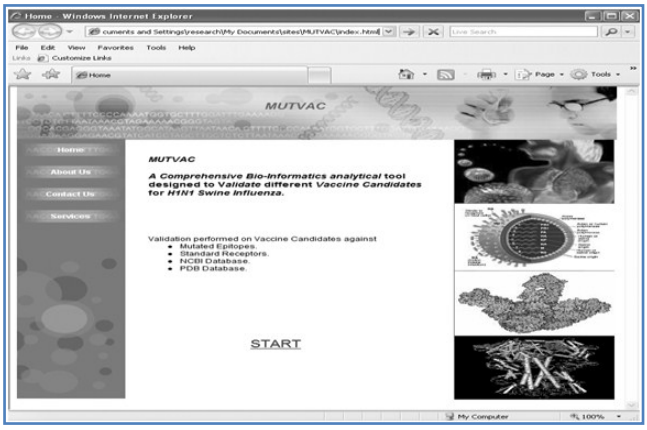

Fig. 4- MUTVAC: The screenshot of the home page

The basis of a good vaccine candidate validation in MUTVAC is performed on the basis of energy parameters. This is so because, if X <= Y (Energy values), it means that E docked with the chosen receptor proves to be a more stable structure and hence it can be said to be a good vaccine candidate for the mutated sequence.

Summary and Conclusion

MUTVAC is a one of a kind tool which provides the validation of vaccine candidates with respect to the mutated strains of H1N1. The tool provides the flexibility of validating the vaccine candidate against any mutated sequence as per the choice of the user. It also permits the user to choose from a list of standardized human receptors. The tool incorporates powerful modeling and docking tools which result in high degrees of accuracy and specificity. The parameters considered for validation is based on energy values which is by far the basis of all type of vaccine validation. Finally, the result includes the best vaccine candidate for the mutated strain along with the display of the other thirteen candidates in an ascending order of their preference for that particular mutated strain with respect to a particular receptor. Adding on, a graphical display of the result (vaccine complex versus mutated epitope complex) is also obtained which facilitates an analytical overview.

A remarkable feature of the tool which is worth noting is the user friendly environment it provides. The tool requires a net-bean environment for it to be used.

- The tool can be used in an online mode which facilitates rapid information bundling and searching and sorting from across the web.
- The tool encompasses a wide spread usage. The facility provided by the tool i.e. the Insilco validation of the vaccine candidate saves both time and work required which would otherwise have proven tedious if conducted Invitro.
- It incorporates various tools, and algorithm in one single package which is also available elsewhere but, that defines it, elsewhere in a lot of different sites.
- The various sectors which could be benefited by the tool include Research Labs, Academics, Pharmaceutical industries etc.

Acknowledgement

MUTVAC was developed by Reddy J., Niranjan V., Venkat D.S., Ajith V., Sharma C.Y. and ArunKumar B.R. at the Centre for Molecular and Computational Biology (CMCB), St. Joseph's Post Graduate and Research Centre, 36, Langford Road, Bangalore – 560027, India. The tool was unveiled in the 2nd International Conference on Intelligent Science and Technology on 18th and 19th March 2010 in Erachakulam, Tamil Nadu, India. The process of implementation of this tool is being carried out by Arjun, H.S a research fellow at CMCB under the supervision of Dr. Jayarama Reddy. The entire team is thankful to Dr. Daniel Fernandes, the Principal of St. Joseph's College, Bangalore for all the support provided during this process.

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