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IN SILICO STUDY OF MHC, CD8 AND CTL BINDING REGIONS PRESENT IN *STAPHYLOCOCCUS AUREUS* AS IMMUNOGENS

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ABSTRACT
Staphylococcus aureus vaccine is used to treat *S. aureus* infection that may not respond to conventional antibiotics therapy. It is believed to elicit cell mediated immune response. The study was carried out with the aid of computational tools and programs like NCBI, ClustalW, Hex 4.2, CASTp. We have predicted high conservation regions between *S. aureus* surface proteins and MHC I (Major Histocompatibility Complex) which were used to derive stable structures with MHC I by using Hex as a tool. Analysis of binding pockets by CASTp. Evaluation of stable docked complexes was done by docking with CD8. This complex was docked with the T-cell receptor. Docked structures were selected by minimum energy values. Sequence alignment of MHC 1 and staphylococcal antigens showed that a few antigens had highest similarity with MHC 1. The CASTp results showed the extracellular adhesion protein and collagen binding surface protein could be good elicitors of immunological response. Evaluation of some of the antigens by docking with CD8+ and MHC alpha domain confirm that not only do the proteins bind to MHC 1 but also bind to CD8+ molecules on the CTL (cytotoxic T lymphocyte) cell via the T-cell receptor. By understanding the MHC binding regions which are specific to the antigens present in ASL we can eluciate the immunogenic ability of ASL (autologous *Staphylococcus* lysate). The problem in subunit vaccine design of searching for antigenic regions in an antigen that can stimulate T cells and T cell epitopes has been overcome by using insilico methods that integrates prediction of peptide MHC I class binding, CD8+ and MHC I binding and identification of peptides that can stimulate CTLs.

KEYWORDS: *Staphylococcus aureus, S. aureus* vaccine, cell mediated immunity, insilico methods, MHC binding regions.

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INTRODUCTION

Staphylococcus aureus is a gram positive cocci which acts as an opportunistic and adaptable pathogen with the ability to infect, invade, persist and replicate in any human tissue including skin, bone, visceral organs, or vasculature.[1] An estimated 30% individuals are colonized by *S. aureus* usually in the anterior nares which serves as a major reservoir from where the organism spread to other parts of the body.[2] Owing to a diverse arsenal of potential virulence factors like surface proteins, invasins, protein A, capsule coating, clotting factor, hemolysins, leukotoxin and leukocidin *S. aureus* can cause infections ranging from localized skin infection to bacteremia and septic shock.[3-5]

The virulence factors are highly antigenic, when present intact in *S. aureus* their antigenicity is masked due to the expression of certain cell bound coagulase and clumping factors which helps *S. aureus* to evade the host defense and cause infections.[3] Usually opsonin-dependent phagocytosis plays a major role in protection against staphylococcal infections and defects in opsonin generation by phagocytes are associated with increasing susceptibility to infection.[6] Moreover internalization and survival of *S. aureus* in neutrophils[7, 8] is another reason why antibiotic treatment fails to eliminate it.[9,10] Amongst the many attempts to treat staphylococcal infection autologous Staphylococcus lysate (ASL) therapy is found to be very effective and elicits cell mediated immunity.[11,12]

The vaccine in this case is a suspension of killed *S. aureus*, wherein the staphylococcal strain is isolated from the nares of an infected person and re-injected to the

same person in the form of autogenous vaccine which elicits immune response. However the immunological mechanism associated with the efficacy of this preparation is not well understood .[13,14]

It is known that opsonin independent phagocytosis plays a critical role in the host defense,[6] which reflects the fact that humoral immunity does not play an important role in conferring immunity towards staphylococcal infection. This suggests that ASL triggers cell mediated immune(CMI) response.The efficiency of T cell activation depends on the concentration of an antigenic peptide and affinity of T cell receptor (TCR) towards the Antigen-Major histocompatibility complex (MHC) which is facilitated by inflammatory stimuli, co-stimulatory signals. CD 8^+ native T cells develop into effector cytotoxic T lymphocyte (CTL) after interaction with antigen presenting cells (APC).[15]

For a vaccine to trigger cell mediated immune response, it is required that the antigenic components of the vaccine bind to MHC 1 present on all nucleated cells.The cell bearing the MHC 1 bound to the antigen acts as an antigen presenting cell (APC)[16] which can now be recognized by the TCR in association with CD 8+ molecule on the CTL, thus triggering the CMI response against the pathogen. Hence if the antigens in the ASL have a strong affinity towards MHC I and TCR it can act as a reliable immunogenic elicitor. Each of these stages of the APC-T cell interaction may be a target for intervention for the modification of the immune response. [17]

The new paradigm in vaccine design is emerging, following essential discoveries in immunology and development of new MHC 1 binding peptides prediction tools. Vaccine designing has now assumed a new form following essential discoveries in immunology. The study of MHC 1 molecules attracts a lot of interest because MHC 1 responds to almost all antigens and the length of the interacting peptides is variable. MHC molecules have been well characterized in terms of their role in immune reactions.[18-20] They bind to some of the peptide fragments generated after proteolytic cleavage of antigen.[21] This in turn generates immune response against the parent antigen. Transporter associated with antigen processing (TAP) is associated with MHC 1 antigen processing.Using the energy provided by ATP, TAP a heterodimeric transporter translocates the peptides across the membrane.[22] The subset of this transported peptide will bind to MHC 1 molecules and stabilize them.These MHC peptide complexes will be translocated on the surface of antigen presenting cells (APCs). This theme is implemented in designing subunit and synthetic peptide vaccines.[23]

In this study in order to find the regions of high conservation between the *S. aureus* surface protein and MHC 1 a multiple sequence alignment was carried out by using ClustalW. The sequences of antigens with higher similarity was chosen for docking using Hex. The CASTp was used to indicate good binding pocket to indicate whether the antigens are good elicitors of immune response. Also the evaluation of stable docked structures by docking with CD8+ and MHC alpha domain was done to affirm that the antigens are able to bring about binding to CD8+ molecules on the CTL cell via the TCR. The docking of CD8+ and MHC alpha domain to TCR was also done to understand the antigens immunological value.

Thus using an *in silico* approach we hypothesize the possible mechanism behind the efficacy of ASL against staphylococcal infection by docking the selected staphylococcal surface protein with MHC 1 and the TCR and evaluating their relative stabilities using molecular dynamics and their binding energies using free energy simulations

MATERIALS AND METHODS

The study was carried out with the aid of computational tools and programs like NCBI, ClustalW, Hex 4.2, CASTp.

NCBI

The National Center for Biotechnology Information (NCBI) is a digital archive that houses genome sequencing data in gene bank and an index of biomedical research articles in PubMed. [24]

Hex 4.2

Hex is an interactive molecular graphics programe which employs spherical polar Fourier correlation to accelerate docking and superimposition calculations. It calculates and displays feasible docking models for pair of protein molecules or protein with ligand which in turn give an insight towards energy minimization values and further to binding affinity.[25]

CASTp

Computer atlas of surface topography of protein (CASTp) is a computational server which uses weighed delaunay triangulation and the alpha complex for shape measurement, and pocket algorithm to identify and measure surface accessible pockets and interior inaccessible cavity for protein and other molecules. [26]

ClustalW

ClustalW enables diagnosis of patterns to characterize protein and to detect or demonstrate homology between sequences of protein using dynamic programming. It builds up a multiple alignment progressively by a series of pairwise alignments. The clustalW alignment gives the consense between the query alignments and demonstrate

their evolutionary relationship.[27]

METHODOLOGY

Computer aided drug as well as vaccine designing is a specialized discipline that uses computational methods and is heavily dependent on bioinformatics tools, applications and databases.[28]

The potential antigenic surface proteins of *Staphylococcus aureus* were retrieved through NCBI search engine Entrez. The nucleotide sequences of all the antigenic proteins collagen binding domain of adhesion protein (1amx), extracellular adherence protein (1yn3), staphylocoagulase thrombin complex (1nu7), teichoic acid (2b71), Protein A domain B (1edk), Protein GB1 (2gi9),Clumping factor A precursor (1 n67), Extracellular fibrinogen binding complex (2noj), Collagen binding protein (1d20) and MHC 1 molecule were procured from the NCBI with the aid of their respective NCBI accession ID in FASTA format.

To find the regions of high conservation between the surface protein and MHC 1 a multiple sequence alignment was carried out by ClustalW. The sequences of antigen with higher similarity was chosen for docking.

The protein data bank (PDB) format of MHC 1 was loaded to Hex 4.2 and taken as a receptor, further each of the selected antigens was loaded as a ligand and docked. Docking allows the scientist to predict the strongest binders based on various scoring functions.The docking analysis was carried out using the following parameters.

Search mode	Full rotation
Corelation type	Shape only
Receptor range	180
Samples	492
Ligand range	180
Samples	642
Twist range	30
Sampling rate	128/min
Distance range	30
Grid dimension	0.6
Steric scan	16
Final search	12

The binding pockets of the various surface proteins were evaluated using CASTp with radius set to 1.4 A. Evaluation of the stable docked structures (MHC 1 and surface protein) was carried out by further docking with CD 8+ co-receptor to MHC alpha domain and the complex was docked with the T-cell receptor.

RESULT

The multiple sequence alignment of the MHC 1 with select surface antigen areas of the *Staphylococcus aureus* gave the alignment score as listed in **Table I**. Docking the MHC 1 with the selected surface proteins was carried out and the energy minimization values obtained for each complex are as listed in **Table II**. CASTp analysis was done to study the area and volume of binding pockets present in the docked complex which is listed in **Table III.** The energy values obtained upon docking MHC1 complex with CD8+ co-receptor using Hex are as given in **Table IV.** The energy values obtained when the protein-MHC 1-CD8+ complex were docked with the TCR are given in **Table V.**

DISCUSSION

Staphylococcus aureus is a frequent causative organism of hospital acquired and community acquired infections. The present scenario of emergence of antibiotic resistance among clinical isolates has renewed interest and the need towards the development of specific and effective vaccines against staphylococcus.[29,30]

Staphylococcus aureus has several factors which enable it to camouflage itself without being noticed by the host defence such as, the protein A which interferes with opsonophagocytosis and superantigens which bind to MHC complex protein and stimulate T cells to proliferate non-specifically leading to toxic shock and other acute reactions, capsular polysaccaride, the fibronectin-binding protein, collagen binding protein, fibrinogen binding protein and non- toxic alpha toxin.[31]

ASL acts like a specific subunit vaccine where a few surface proteins of *S. aureus* are used which apparently as per our study act as potent antigens to evade staphylococcal infections. The efficacy of these surface proteins acting as potent vaccines were investigated using immunological mechanisms as a basis and computational programmes as tools. In subunit vaccine design it is important to search antigenic regions in an antigen that can stimulate T cells called as T cell epitopes.[32] A large number of databases are available which provide comprehensive information related to T-cell epitopes.[33-36] CTL epitopes can be used in subunit vaccine design for various diseases.[37]

The multiple sequence alignment of MHC 1 and staphylococcal antigens when analysed showed that a few antigens had similarity with MHC 1 of which extracellular fibrinogen binding complex had highest similarity, with the alignment score being 206, while the least alignment was found to be collagen binding protein A and B with the scores being – 249 and -367 respectively.

Upon docking the antigenic sequences with high similarity, with MHC 1 it was seen that extra cellular fibrinogen binding complex formed the most stable complex with MHC 1, the energy minimization value being - 697.3 KJ/mol. Followed by collagen binding

protein with energy value being - 682.7KJ/mol, hence these proteins could effectively bind with MHC 1 and initiate cell mediated immune response.

The CASTp results indicate that extracellular adhesion protein has the highest area binding pockets value being 4043.2 and highest volume of binding in the complex is that of collagen binding surface protein with a volume of 39688. These results indicate good binding pocket and that these antigens could be a good elicitors of immunological response.

Further the evaluation of the most stable docked structures namely fibrinogen binding protein, protein G, staphylocoagulase, collagen binding protein by docking with CD8+ and MHC alpha domain the scores obtained showed effective energy minimization values thus further confirming that not only do these proteins bind to MHC 1 but they are also potent in binding to CD8+ molecules on the CTL cell via the T-cell receptor.The next immunological step would be the process of binding the TCR which when analyzed by docking showed a maximum energy minimized value with protein G followed by collagen binding protein and staphylocoagulase.

As the ASL is a combination of all these antigens it can effectively bind to MHC 1 present on all nucleated cells in humans and once bound by the protein the cell presenting the MHC-1 acts as an APC and draws the CD8+ T cells and the entire complex binds to the TCR[38] and initiates the gene transcription which enables the T-cells to synthesize and secrete interleukins which bring about a cascade of events which bring about activation of subsets of T-cells and evade the pathogenic staphylococci.

In conclusion in ASL multiple antigenic components are involved which direct the immune system to protect the host against staphylococcal infections. By understanding the MHC binding regions which are specific to the antigens present in ASL we can eluciate the immunogenic ability of ASL. One of the problems encountered in subunit vaccine design is to search antigenic regions in an antigen that can stimulate T cell epitopes. This has been overcome by using insilico methods that integrates prediction of peptide MHC I class binding, CD8+ and MHC I binding and identification of peptides that can stimulate CTLs. The ASL which is a suspension of entire killed organism serves as an effective sub-unit vaccine, and as the causes for *S.aureus* infection is multifactorial it can act effectively leading to reducing the morbidity and mortality of staphylococcal infections.

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Table 1 : SEQUENCE ALIGNMENT SCORES OF MHC 1 WITH THE SURFACE ANTIGENS OF *Staphylococcus aureus*

PROTEIN	2noj	1nu7	2gi9	1edk	1yn3	2b71	1amx	1 n67	1d20
ALIGNMENT SCORE	206	182	101	63	10	-57	-141	-154	-249

Table II: DOCKING RESULTS OF MHC 1 WITH THE SURFACE PROTEINS

PROTEIN	2noj	1d20	1n67	2b71	1nu7	1yn3	1amx	2gi9	1edk
ENERGY VALUES (E values)	-697.3	-682.7	-665.1	-634.9	-627.6	-537.4	-513.9	-442.3	-420.3

Table III: BINDING POCKETS ANALYSED BY CASTp

PROTEIN	1amx	1yn3	2gi9	1d20	1edk	1n67
AREA	2004.3	4043.2	1218.3	3083.3	3479.3	2299
VOLUME	8999.3	25648	2252.6	39688	12493	9291.7

Table IV: DOCKING RESULTS OF MHC 1 WITH CD8+ CO-RECEPTOR					
PROTEIN	2noj	2gi9	1nu7	1d20	1n67
ENERGY VALUES (E values)	-541.9	-669.9	-523.8	-365.6	-643.6

Table V: DOCKING RESULTS OF MHC 1 CD8+ COMPLEX WITH TCR					
PROTEIN	2noj	2gi9	1nu7	1d20	1n67
ENERGY VALUES (E values)	-154.9	-669.7	-523.8	-409.1	-606.2

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