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Reverse-vaccinology strategy for designing T-cell epitope candidates for *Staphylococcus aureus* endocarditis vaccine

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ABSTRACT

Staphylococcus aureus is an opportunistic pathogen causing various inflammatory diseases from skin and tissue local infections, to serious life threatening infections including endocarditis. Experimental models for endocarditis demonstrated that virulence factors of *S. aureus,* that are very important in infection of heart vegetations, are surface proteins which promote bacterial adherence. Until now, efforts to develop effective vaccines against *S. aureus* were unsuccessful, partly due to the fact that different vaccine formulations have targeted mainly B-cell immunity. Reverse vaccinology is applied here, in order to identify potential vaccine epitope candidates. The basic epitopes prediction strategy relied on detection of a common antigenic 9-mer epitope meant to be able to stimulate both the B-cell and T-cell mediated immunity. Ten surface exposed proteins were chosen for antigenicity testing. Using a web-based system, five T-cell epitopes corresponding to fibronectin binding protein A (FDFTLSNNV and YVDGYIETI), collagen adhesin (FSINYKTKI), serine-rich adhesin for platelets (LTFDSTNNT) and elastin binding protein (FAMDKSHPE) were selected as potential vaccine candidates. Epitopes sequences were found to be conserved among the different *S. aureus* genomes screened from NCBI GenBank. *In vitro* and *in vivo* immunological tests will be performed in order to validate the suitability of the epitopes for vaccine development.

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1. Introduction

Infective endocarditis (IE) is defined as an infection of the inner surface of the heart or the heart valves occurring when bacteria circulating in the blood stream adhere to the damaged tissue developing characteristic lesions known as vegetations. This is an amorphous mass containing proteins (especially fibrin) and platelets. Bacteria adhere to this lesion, and protected by the fibrocellular matrix multiply and proliferate generating a progressive infection [1].

Risk patients are those with congenital hearts disease, chronic rheumatic disease, mitral valve prolapse, but also those with prosthetic devices, patients undergoing hemodialysis and drug consumers. The bacteremia is often caused by medical procedures (gastro-intestinal, ophthalmic, dental, etc.) [1–3].

In an international collaboration study on endocarditis conducted between the years 2000–2003, *Staphylococcus aureus* (*S. aureus*) was identified as the most prevalent etiological agent, being responsible for 31.4% of the 1779 studied cases. Most infections were of nosocomial or healthcare-associated origin (39.1%), followed by community-acquired (37.5%) and intravenously drug user's cases (21%) [4]. Another study, mentioned that *S. aureus* was isolated as the causative agent in 46% of 87,300 patients with EI investigated during 1999–2008 in US [5].

S. aureus IE, involving native valves, is often characterized by a sudden onset and absence of peripheral events. Complication rate is higher than for other etiological agents and mortality may even reach 43.9% [6].

IE severity may have several explanations based on the circumstances of infection onset, but also tightly related to the characteristics of this pathogen.

On one hand, as revealed in the two studies mentioned above, most *S. aureus* IE cases are infections related to hospital care units. Patients are usually immunocompromised and have different comorbidities; therefore they are more vulnerable to *S. aureus* strains, hosted by the hospital environment that could enter the blood stream through various injuries or following therapeutic procedures.

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On the other hand, the severity of clinical symptoms may be explained by the microbiological characteristics of this opportunistic pathogen. *S. aureus* shows a great pathogenic potential, mediated by numerous structural and secreted virulence factors, involved in different stages of infection, i.e.: colonization, invasion, dissemination and avoiding immune system effectors.

Due to the severity of *S. aureus* IE, adequate prophylaxis is desirable for patients enrolled in risk groups which will be subjected to conditions that could favor the occurrence of *S. aureus* bacteremia. Antibiotic prophylaxis is often given to patients, but many staphylococcal strains, especially of nosocomial origin, are multi-resistant [7]. Also, some patients may be allergic to certain classes of antibiotics. Several studies aiming at developing a prophylactic vaccine were conducted these days, but so far, they have failed in finding a safe and effective vaccine for *S. aureus* IE [8,9].

In the present work, reverse vaccinology has been applied to identify new potential vaccine candidates. Reverse vaccinology is a web-based vaccine system that predicts candidates starting from the genome sequence, having access to the entire proteomic repertoire. Single or multiple proteins can be used as vaccine candidates. Usually the whole protein is not essential to elicit an immune response and peptides derived epitopes can be also used. The protein (and derived epitopes), which could act as a vaccine candidate, must be surface exposed, highly antigenic and responsible for the generated pathogenicity.

Since the attachment of *S. aureus* on the vegetation is the first step in IE onset, a number of virulence factors involved in bacterial attachment were analyzed here using reverse vaccinology technique, in order to detect appropriate epitopes for a new vaccine development. For some of them, i.e. clumping factors A and B (ClfA, ClfB), fibronectin binding proteins A and B (FnbPA, FnbPB) or collagen adhesin (Coa), evidences about their involvement in IE were previously published [10–14].

The basic epitopes prediction strategy presented here relies on detection of a 9-mer common antigenic epitope, selected for being able to stimulate both the B-cell and T-cell mediated immunity.

2. Material and methods

2.1. Target proteins1D and 3D sequence retrieval

In this study 10 surface exposed proteins with a high degree of conservation and representative among the *S. aureus* strains were selected.

The 1D sequences for ClfA (GenBank: NP_645581.1) – 946 aa, ClfB (GenBank: NP_647368.1) – 907 aa, FnbPA (GenBank: NP_647238.1) – 1015 aa, FnbPA (GenBank: NP_647237.1) – 943 aa, Cna (GenBank: NP_647429.1) – 1183 aa, EbpS – elastin binding protein (GenBank: NP_646186.1) – 486 aa, SraP – serine-rich adhesin for platelets (Genbank: NP_647392.1) – 2275 aa, Eap/Map – extracellular adherence protein/MHC analog protein (GenBank: NP_646697.1) - 581 aa, Efb – extracellular fibrinogen binding protein (GenBank: NP_645857.1) – 165 aa and Eno – enolase, laminin binding protein (GenBank: NP_645555.1) – 434 aa were retrieved from NCBI database – GenBank (www.ncbi.nlm.nih.gov). The sequences correspond to *S. aureus* MW2 strain (GenBank: NC_003923.1). Other protein sequences from NCBI database were used to check if the selected epitopes were conserved among the different *S. aureus* strains.

Phyre 2 (Protein Homology/analogy Recognition Engine v2.0server (www.sbg.bio.ic.ac.uk/phyre2) was used for 3D modeling structures of proteins. PHYRE 2 use the alignment of hidden Markov models *via* HH search to significantly improve accuracy of alignment to known 3D structure models. It also incorporates an *ab initio* folding simulation called Poing to model regions of proteins with no detectable homology [15]. Best models were selected based on superfamilies, confidence key, coverage and amino acid identities.

2.2. Antigenicity and topology testing of the whole protein

VaxiJen v2.0 (www.ddg-pharmfac.net/vaxijen/) was used as an antigen prediction server, which is based on transformation of protein sequences into uniform vectors of principal amino acid properties. The default parameters (threshold = 0.4, ACC output) were used against each full length protein [16]. Proteins having antigenic score > 0.4 were selected and subjected to TMHMM v2.0server (www.cbs.dtu.dk) in order to identify exo-membrane topology of amino acid sequences of each protein and to confirm surface exposure [17].

2.3. Identifications of B-cell epitopes

B-cell epitope identification is the first step in epitope designing. Antigenic linear non-overlapping 20-mer B-cell epitopes were predicted from whole antigen using BCPreds software (http://ailab.cs.iastate.edu/bcpreds). As it is often valuable to compare predictions of multiple methods, and consensus predictions are more reliable than individual predictions, two methods: AAP method, based on amino acid pair antigenicity [18] and BCPreds, using the subsequence kernel [19], provided by BCPreds were followed. Only B-cell epitopes having a score >0.8 were accepted. Selected B-cell epitopes were then subsequently checked for antigenicity and exo-membrane topology using VaxiJen and TMHMM v2.0servers.

2.4. Identification of T-cell epitopes

Several1D sequence-based screening servers were used to identify T-cell epitopes. ProPred-1 software (www.imtech.res.in/raghava/propred1/) was used to identify the MHC Class-I (47 alleles) binding regions [20] and ProPred software (www.imtech.res.in/raghava/propred/) was used to predict MHC Class-II (51 alleles) binding regions in antigen sequences [21]. These computational tools use matrix-based prediction algorithm. The obtained matrices are multiplication matrices, where the scores are calculated by multiplying and summing the score of each amino acid position. Promiscuous T-cell epitopes binding to at least 7 MHC alleles, both class I and II, were selected. The selected epitopes were analyzed by VaxiJen server for antigenicity. Then, MHCPred was used to predict the binding affinity of MHC class I and II molecules and the value was given in terms of inhibitory concentration (IC50) [22]. MHCPred use partial least squares based approach for the prediction of binding affinity to MHC molecules. IC50 <50 nm indicate a good binder, IC50 = 50–500 nm an intermediary binder, IC50 = 500–5000 nm a week binder and IC50 > 5000 a non-binder. As the DRB1*0101 is the commonest bound allele, epitopes with IC50 value less than 100 nm were selected.

Second, binding affinity to A*0101, A*0201, A*0204, A*2705, DRB1*0401 and DRB1*1101 were checked using MHCPred server and T-epitope designer. The last one is based on a model that defines peptide binding pockets using information gleaned from X-ray crystal structures of HLA-peptide complexes, followed by the estimation of peptide binding to binding pockets. Thus, the prediction server enables the calculation of peptide binding to HLA alleles [23].

Beside DRB1*0101, the mentioned alleles are the most frequent MHC alleles in human population. Protein Digest site was used to identify the enzymatic and chemical restriction sites in T-cell epitopes sequences (www.db.systemsbiology.net:8080/proteomicsToolkit). In order to be an ideal candidate for vaccine developing, it is important

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