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Highly conserved exposed immunogenic peptides of Omp34 against *Acinetobacter baumannii*: An innovative approach

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ABSTRACT

Omp34, also known as Omp34kDa or Omp33–36 is a virulence factor associated with *A. baumannii* metabolic fitness or its adherence and invasion to human epithelial cells. This protein is also introduced as a specific antigen which could induce strong antibody responses. In the present *in silico* study, recent vaccine design strategies such as 'antigen minimization' and 'high epitope density' were invoked to design a soluble immunogen with higher antigenicity. As an advantage, the tools employed in the current study are easily available. Exposed peptides in linear B-cell epitopes were predicted and their conservancy and immunogenicity were evaluated. In this regard, constructs were designed by removal of inappropriate regions. Based on the obtained results the external loops (L1–L7) were exclusively considered of which L3, L6 and L7 were the most appropriate of which the most appropriate were in L3 > L6 > L7 order while L2 was assigned as an inappropriate peptide. The final construct, named Omp34-4, encompasses three copies of L3, two copies of L6 and L7 and one copy of L1, L4 and L5. The designed construct is predicted to be a soluble antigen with enhanced epitope density and antigenicity. Omp34 is present in > 1600 strains of *A. baumannii* with ≥ 98% identity. So, it could be applicable in diagnostic kits and an immunotherapy choice against *A. baumannii*. It could be presumed that co-administration of Omp34-4 and a recently designed OmpA-derived antigen could confer sufficient protection against *A. baumannii*-associated infections. *In vitro* and *in vivo* experiments are needed to confirm all these data. The innovative approach could be generalized to vaccine designs focused on OMPs.

1. Introduction

The nosocomial pathogen *Acinetobacter baumannii* is becoming an increasingly serious health threat such that it has been assigned as one of the six most dangerous microbes by the Infectious Diseases Society of America (IDSA) (Huang et al., 2016). Pneumonia, meningitis, bloodstream infections, skin and soft tissue infections and urinary tract infections are considered among multiple infection types caused by this highly successful pathogen (McConnell et al., 2013). Rapid emergence of multidrug resistant (MDR) strains of the pathogen with no available commercial effective antibiotic leads to difficulty of *A. baumannii* clinical management (Pachón and McConnell, 2014). These implications suggest active and passive immunization as a cost-effective approach against the notorious pathogen. In this regard, various immunogens have been investigated among which, protein antigens (*e.g.* OmpA (Fajardo Bonin et al., 2014, Lin et al., 2013, Luo et al., 2012), OmpW (Huang et al., 2015), Bap (Fattahian et al., 2011) and Omp34 (Fajardo Bonin et al., 2014)) are highly appreciated as promising immunogens (reviewed in (Ahmad et al., 2016, Chen, 2015)).

The outer membrane protein (OMP), Omp34, is also known as Omp34kDa and Omp33–36. Omp34 is a specific antigen which could serve for detection of *A. baumannii* (Islam et al., 2011). Islam et al. (2011) demonstrated Omp34 could be uniquely recognized by IgM, IgA, and IgG from patients infected by *A. baumannii* with no cross-reaction with sera from patients infected by other bacteria. Moreover, this OMP was enriched in outer membrane vesicles (OMVs) employed by McConnell et al. (2011) as a vaccine candidate against *A. baumannii*.

Recently, Omp34 was highlighted as a potential vaccine candidate against *A. baumannii via* an immunoproteomic study (Fajardo Bonin et al., 2014). Furthermore, this highly immunogenic protein plays a role

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in adherence and invasion of the pathogen to human epithelial cells (Smani et al., 2012). In addition to fibronectin binding (Smani et al., 2012), this virulence factor is associated with *A. baumannii* cytotoxicity (Smani et al., 2013) as well as its metabolic fitness (Smani et al., 2012). This protein induces apoptosis and inhibits autophagy in human cells. Inhibition of autophagy modulated by Omp34 enables the pathogen to persist inside autophagosomes (Rumbo et al., 2014). Although OMPs have been proposed as strong immunogens by several studies (Fajardo Bonin et al., 2014; Huang et al., 2015; Huang et al., 2016; Lin et al., 2013; Luo et al., 2012; Toobak et al., 2013a, 2013b), recombinant OMPs are not sufficiently soluble, limiting their attractiveness as powerful immunotherapeutic preparations (Ahmad et al., 2016).

Nowadays, bioinformatics and immunoinformatics are exhorting researchers to employ rational vaccine design approaches instead of conventional empirical vaccine development strategies (Khalili et al., 2014). In this regard, “antigen minimization” could be of interest as an effective approach in which domains of a given protein containing only protective epitopes are focused upon elicit responses against the epitopes of interest (Kulp and Schief, 2013). The protective epitopes could be revealed through experimental or *in silico* studies. These epitopes could be increased in a single protein molecule by a novel strategy known as “high epitope density” (Liu and Chen, 2005; Pei et al., 2009). In addition to epitopes predictions (Jahangiri et al., 2012; Rahbar et al., 2012; Sefid et al., 2013) and vaccine designs (Farhadi et al., 2015; Hajighahramani et al., 2017; Jahangiri et al., 2011; Jahangiri et al., 2017; Khalili et al., 2015; Nazarian et al., 2012; Nezafat et al., 2016; Shahbazi et al., 2016), discerning of three-dimensional protein structures (Khalili et al., 2016, 2017a, 2017b; Sefid et al., 2013) and protein functions (Khalili et al., 2017a, 2017b; Mohammadpour et al., 2015; Mohammadpour et al., 2016) could also benefit from valuable bioinformatic tools. Moreover, *in silico* studies could obviously reduce effort, time, expense and inevitable inherent ethical conflicts of experimental studies. In the present study, we integrate various easily available bioinformatic and immunoinformatic tools to predict exposed linear B-cell epitopes within the Omp34 sequence. Then, novel Omp34-derived antigens were designed to arrive at a final putatively soluble antigen with enhanced predicted immunogenicity as well as the B-cell epitopes density.

2. Methods

2.1. Sequence availability and topology prediction

A reference sequence of Omp34kDa protein with accession no. WP_000733005.1 was extracted from NCBI at http://www.ncbi.nlm.nih.gov/protein in FASTA format. FASTA format is a standard text-based format representing protein sequences as single-letter codes. Further analyses were carried out on this sequence.

Topology of the protein was predicted by PRED-TMBB2 (Tsirigos et al., 2016) at http://www.compgen.org/tools/PRED-TMBB2. PRED-TMBB2 is an updated version of the PRED-TMBB method with improved performance and new features. This is a newly developed method with high accuracy and reliability for predicting topology of beta-barrel outer membrane proteins and discriminating these proteins from water-soluble proteins (Tsirigos et al., 2016). This server could also ascertain signal peptide within a given sequence.

2.2. Linear B cell epitope predictions

In order to generate the most reliable results, linear B cell epitopes of Omp34 were predicted by various algorithms and tools: BCPREDS (EL-Manzalawy et al., 2008), EPMLR (Lian et al., 2014), BepiPred (Larsen et al., 2006), SVMTriP (Yao et al., 2012) and LBtope (Singh et al., 2013). BepiPred at http://www.cbs.dtu.dk/services/BepiPred/ combines a propensity scale method with a hidden Markov model to predict linear B-cell epitopes (Larsen et al., 2006). The threshold was kept as default (0.35) in which the sensitivity and specificity of the predictions are 0.49 and 0.75 respectively. BCPREDS at http://ailab.ist.psu.edu/bcpred/ benefit from the subsequence kernel (EL-Manzalawy et al., 2008). Fixed length epitope mode of BCPred was set. All epitope lengths were assessed. The specificity was set as default (75%). SVMTriP at http://sysbio.unl.edu/SVMTriP/ combines Support Vector Machine (SVM) with the Tri-peptide similarity and Propensity scores (SVMTriP). The AUC value of SVMTriP is 0.702 (Yao et al., 2012). All available epitope lengths provided as options were set for the Omp34 sequence. EPMLR at http://www.bioinfo.tsinghua.edu.cn/epitope/EPMLR/ employs multiple linear regression (MLR). The AUC value of EPMLR is 0.728 (Lian et al., 2014). Default threshold of -0.15 was kept. All available various lengths of the epitopes provided as options were set for the Omp34 sequence. LBtope at http://www.imtech.res.in/raghava/lbtope/index.php has three different models that can be selected (LBtope_Fixed, LBtope_Variable and LBtope_Confirm models). Since validity of a created dataset is a challenge in algorithm development, LBtope_Confirm model was harnessed to predict potential linear B-cell epitopes of Omp34. This model is developed based on only epitopes (1042 unique B-cell epitopes) or non-epitopes experimentally validated at least by two studies. This model can also predict variable length epitopes (Singh et al., 2013). The default size of window length (15 aa) was set for the prediction.

2.3. Epitope screening and selection

The external loops contained in epitopes assigned by at least 3 harnessed tools of previous section (*i.e.* Section 2.2 Linear B cell epitope predictions) were considered as the most reliable peptides to be selected. All external loops of Omp34 served as separate queries to VaxiJen (Doytchinova and Flower, 2007) with default threshold of the server (0.4) at http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html in order to determine their individual antigen probability. VaxiJen is the first alignment-independent antigen predictor with accuracy of 70–89% (Doytchinova and Flower, 2007).

2.4. Peptide conservancy

A protein BLAST search restricted to *A. baumannii* was run against non-redundant protein sequences on the Omp34 sequence at https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome. Hits with E-value < 0.001 were considered as reliable results. Among those hits, hits with query coverage $\geq 99\%$ and identity $\geq 99\%$ were considered. To analyze epitope conservancy, all external loops of Omp34 were used as individual inputs at http://www.iedb.org/. The search was restricted to linear peptidic epitopes with $\geq 70\%$, $\geq 80\%$, or $\geq 90\%$ identity to the input sequences.

2.5. Construct designs, analyses and refinement

Two strategies (‘antigen minimization’ and ‘high epitope density’) were integrated to achieve constructs of interest. In the following, in order to arrive at the best regions as well as decreasing false negative and positive results, two approaches were tracked and their resulting constructs were evaluated. In the first selection and design, only topology and linear B-cell epitope predictions were invoked by which, the most reliable exposed epitopes were incorporated. In the second selection and design approach, in addition to topology and linear B-cell epitope predictions, antigen probability of loops was also considered. The first construct was designed as follows: External loops contained in reliable linear B-cell epitopes (L3, L4, L5, L6 and L7) were retained. The remaining loops along with their flanking transmembrane β-strands and internal turns (residues 1–115) were removed. The remaining transmembrane β-strands served as linkers instead of using artificial ones. Then, remaining internal turns (in3, in4, in5 and in6) were replaced by