Recombinant vaccine for prevention of *Streptococcus pneumoniae* infection

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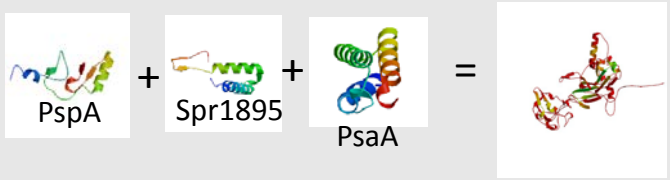
Streptococcus pneumonia (*S.pneumoniae*) is a leading cause of serious illness, including bacteremia, meningitis, and pneumonia among children and adults. Vaccine prevention of *S.pneumoniae* infections is recommended in many countries but all the commercial vaccines on the market (PPV or PCV) are targeted against capsular antigens of bacteria (Nuorti JP et al 2010). In spite of the proved effectiveness of the known commercial vaccines, vaccination against the list of serotypes causes the appearance of the new clinically important strains with different capsular antigens ("Red Queen Dynamics" Jefferies G.M. et al. 2011). At present over 90 serotypes of pneumococci exist. Present work suggests a new approach of making chimerical vaccine based on the conserved surface proteins.

Materials and Methods

Making the construct

Chimeric protein was generated from the completely artificial DNA template generated via elongation of the overlapping oligonucleotides (Majumder, 1992).



59 primers were used for making 1623 bp long DNA molecule which was cloned into E.coli expression vector PET24a.

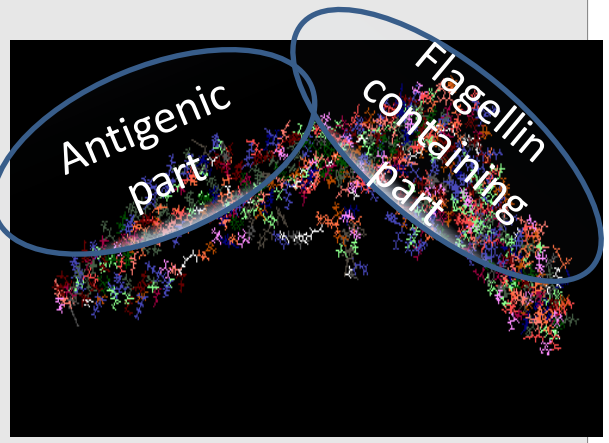
For generating the chimeric protein model 5 procedures were used: estimation of the domain boarders, construction of a model of the full protein for estimation of the domain orientation, construction of models for each domain (using examples of 3D structures and *ab initio*-based modeling), docking of the models using the model of the full protein. I-Tasser algorithm was used for the structure prediction.

Immunization and protection

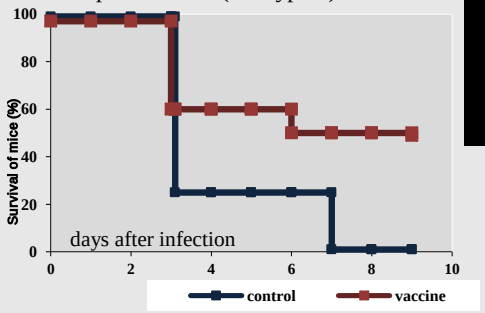
Balb/c mice were immunized with different doses of vaccine. The level of the IgG in blood was monitored by ELISA. For the protection assay *S.pneumoniae* was introduced either intra peritoneum or intra nasally. Prevnar-7 vaccine was used as control. Protection was monitored by the number of survived animals or by the bacterial titer in lungs and spleen. All the animal work was done in the certified facility with all the necessary ethical requirements.

Results

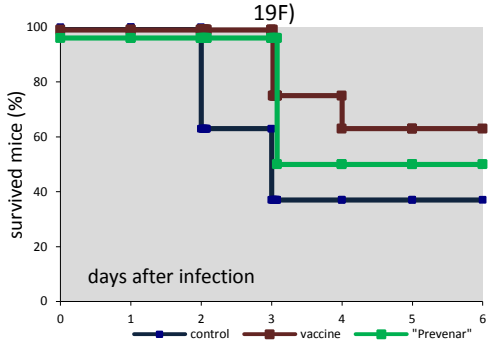
Highly purified chimeric protein containing immunogenic fragments of proteins PspA , Sprl895 and PsaA together with two portions of *E.coli* flagellin molecule (FliC1 and FliC2) was injected in Balb/c mice and tested for immunogenicity and protection against *S.pneumoniae* infection.



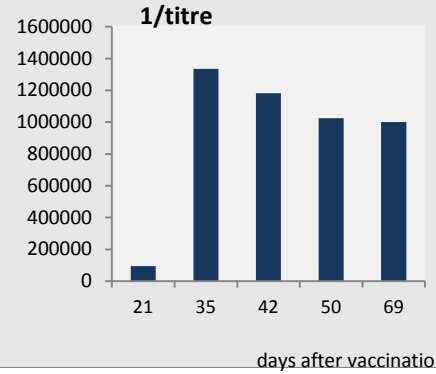
Intra peritonium infection
S. pneumoniae (serotype 3)



Lung infection of *S.pneumoniae* (serotype 19F)



Immune response after vaccination



References

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2. Majumder K.Ligation-free gene synthesis by PCR: synthesis and mutagenesis at multiple loci of a chimeric gene encoding ompAsignal peptide and hirudin. Gene. 1992 Jul 1;116(1):115-6.

3. Nuorti JP, Whitney CG. Prevention of pneumococcal disease among infants and children -use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine -recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR RecommRep. 2010 Dec 10;59(RR-11):1-18

Conclusions

Chimeric protein vaccine composed of three immunogenic epitopes corresponding to the surface proteins was immunogenic and provided protective immunity against *S.pneumoniae* infection.