Immune epitopes of pneumococcal endolysins Cpl-1 and Pal and their role in shaping immunoreactivity of the enzymes

In presented work I investigated the potential for improving endolysin performance *in vivo*. I determined pre-clinical safety assessment of Cpl-1 and Pal endolysins. They induce a normal profile of specific IgG production, and no health threatening effects were noted. I developed a new method based on the use of DNA dye to precisely measure bacteriolytic activity of endolysins and their variants, including *ex vivo* conditions in biological samples. This method focuses on killing efficiency of an bacteriolytic agent. I also provided mathematical description of results that allows for direct comparison of activity for both endolysins and their variants in various environments or concentrations. I further identified amino acids that interact with the endolysin-specific IgG's in immunogenic regions of CPl-1 and Pal. I used this knowledge to propose potentially deimmunized variants of Cpl-1 and Pal. I designed and produced eight variants that retained antibacterial activity. All these variants were relatively immunogenic, but at the same time recognized weaker by wild type endolysins-specific antibodies, five of them demonstrated markedly decreased reactivity. All variants showed bacteriolytic activity: in five of them this activity was weaker than that of wild type endolysins, two retained the same activity, and one (Pal v3) showed increased bacteriolytic activity in comparison to wild type endolysin. Two variants of Pal endolysin (Pal v3 and pal v9) demonstrated no decrease in bacteriolytic activity when treated wild-type specific murine serum, while wild type Pal showed decrease in its enzymatic activity in the same conditions. Thus, I propose Pal v3 as the new highly active variant of endolysin Pal with improved performance *in vivo*, particularly less prone to neutralization by specific antibodies without losing bacteriolytic activity.