The development of DNA vaccines against different serotypes of *Actinobacillus pleuropneumoniae* using exotoxins and surface antigen ApfA

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ABSTRACT

*Actinobacillus pleuropneumoniae*, a Gram-negative bacterium that all of the 15 serotypes are known can cause infection and lead to lung lesion in pigs with high mortality. Several virulence factors are known including capsule, lipopolysaccharide, exotoxins (Apx toxins), fimbriae, and various outer membrane proteins. The Apx toxins are considered the most important virulence factors. ApxI has strongly hemolytic and cytolytic activity, and ApxII has weakly hemolytic and cytotoxic activity. However, ApxIII is not hemolytic but strongly cytotoxic to porcine neutrophils and pulmonary alveolar macrophages, and is secreted by serotypes 2, 3, 4, 6, and 8. There are 32 amino acids differences in the C terminus of ApxIII of serotype 2 comparing with other serotypes and they should influence the immunogenicity of ApxIII. Fimbriae are usually with highly antigenicity and mediated the attachment ability of bacteria to the host cell. The structural protein gene of *A. pleuropneumoniae* fimbria, *apfA*, was found with highly conserved in all serotypes. In the previous study of our laboratory showed that a 4-valent DNA vaccine containing ApxIA, ApxIIA and two outer membrane proteins can provide 70% protection against serotype 1 challenge. To extend the DNA vaccine against different serotypes infection, ApfA and ApxIIIA of serotypes 2 and 8 were chosen as the target antigens for DNA vaccine candidates. These genes were cloned into a mammalian cell expression vector and confirmed by DNA sequencing. The expression of these proteins was first verified in prokaryote by Western blot analysis and further confirmed their expression in NIH/3T3 cells by immunofluorescence assay. The ApfA and ApxIIIA DNA vaccine were injected intramuscularly into mice using monovalent or bivalent formulation. A commercial inactivated *A. pleuropneumoniae* vaccine was used as positive control as
well as the empty vector and PBS were used as negative control. The survival rate of each group after challenged with \textit{A. pleuropneumoniae} serotype 2 was as following: 30\% for ApfA vaccine; 50\% for commercial vaccine and ApxIIIA of serotype 2 vaccine; 60\% for ApfA plus ApxIIIA of serotype 2 vaccine; 70\% for ApfA plus ApxIIIA of serotype 8 vaccine; 0\% for empty vector and PBS groups. These results showed that the bivalent vaccine provided better protective efficacy than monovalent vaccine or commercial inactivated vaccine. A tetravalent DNA vaccine containing ApxIIIA of serotype 8, ApfA, ApxIA and ApxIIA were used to evaluate the protective efficacy for different serotypes. The survival rate was 70\% for serotype 2 and 30\% for serotype 1 challenged, respectively. These results indicate that the protective efficacy of tetravalent vaccine against \textit{A. pleuropneumoniae} serotype 2 is more significant than serotype 1. Taken together, the present study suggests that these DNA vaccines are promising candidates for prevention different serotypes infection and it is necessary to search the best antigens combination or through the adjuvant administration to improve the protective efficacy.

\textbf{Keywords:} \textit{Actinobacillus pleuropneumoniae}, ApfA, ApxIIIA, DNA vaccine