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Peptide-based self-assembled nanovaccine system targeting Group A Streptococcus infection

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The use of traditional vaccines based on whole microorganisms is often associated with the serious drawbacks such as allergic responses or difficulties in pathogen cultivation. These obstacles can be overcome by peptide-based vaccines. However, as peptide itself has almost no immunogenicity, adjuvant that could evoke and enhance the immune response against a supplied antigen is necessary for peptide subunit vaccine. Currently, the high toxicity and low immunogenicity of current existed adjuvants are the biggest obstacle blocked the application of peptide subunit vaccine. In this project, a novel self-adjuvanting system for peptide subunit vaccine was proposed. This system applied hydrophobic amino acids sequence to which was expected to induce self-assembly of vaccine candidates into nanoparticles. As nanoparticles are known to elicit immune response, this system should find application in vaccine development. GAS B-cell epitope J14 was used in this project to evaluate the efficiency of this system, and T-helper epitope PADRE was also applied. J14, PADRE and poly phenylalanine sequence were linked covalently through the lysine moiety to product the vaccine candidates.

Two approaches were attempted to obtain the desired compound. The segment condensation based synthesis approach was unsuccessful due to poor solubility of poly phenylalanine unit in examined solvents. Stepwise synthesis approach was conducted afterwards. The whole desired compounds were synthesized successfully. Formation nanoparticles (10-70nm) were confirmed by dynamic light scattering (DLS) and transmission electron microscopy (TEM). The efficacy of particles to induce immune responses will be analysed in mice model in near future.

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Anti-idiotype mimicking outer membrane proteins of Pasteurella multocida B:2 in the control of haemorrhagic septicaemia

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Haemorrhagic septicaemia (HS) is a fatal systemic disease of livestock most commonly affected are buffalo, cattle, pigs and camels, meanwhile all ungulates are equally susceptible. It is caused by a capsular strain of *Pasteurella multocida* type B:2 in southern Asia. Outer membrane proteins (OMPs) are virulent factor which remained less immunogenic due to low molecular weight and high lipoprotein contents, therefore anti-idiotypes were developed against *P. multocida* B:2. OMPs were extracted with 1% Sarkosyl method, the purified protein content obtained 21.3 mg/100 ml and idiotypes were raised in rabbits followed by anti-idiotypes in sheep. The fragments of antibody attachment (Fab) were separated through pepsin digestion and the idiotypes were adjuvanted in Montanide. P. multocida anti-idiotype 400 µg/100 kg body weight through S/C route was evaluated and compared with OMPs subunit alone and alum-adsorbed bacterin in buffaloes and cattle. It was recorded that OMPs-anti-idiotype vaccine induced high levels of geomean antibody titres (GMT) detected using indirect haemagglutination (IHA) test at 100th day post vaccination, buffaloes and cattle groups revealed the GMT antibody titre 147 and 137.2 respectively. The OMPs alone exhibited antibody titre of 137.2 in buffaloes and 119.4 in cattle. The bacterin showed least antibody titre both in buffaloes (27.9) and in cattle (32.0). Moreover, the OMPs anti-idiotype vaccine provoked better protection (100%) against homologous strain of *P. multocida* and humoral immunogenic response throughout till 370 days and the OMPs alone could achieved 67% protection at 160 th day post vaccination in cattle and buffaloes.

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