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Multiple peptide antigen (MAP) approach for V antigen of *Y. pestis* as a vaccine candidate

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Yersin pestis is the causative agent of the most deadly disease plague. F1 & V antigens are the vaccine candidates. We were the first to map the B and T cell epitopes on F1 and V antigen and when we linked B-T and studied humoral, cellular and mucosal immune responses and *in vivo* protective study during challenge experiments, few B-T constructs showed complete protection as compared to native antigens. In this study, we followed multiple antigen peptide (MAP) approach towards development of sub-unit vaccine in which protective epitopes were assembled on a lysine backbone. MAP was synthesized containing seven peptides of length varying from 15-25 aa on the lysine backbone. The authenticity of MAP was verified by amino acid analysis, SDS-PAGE and immunoblot. Palmitate was coupled at the end of amino terminus. MAP was encapsulated in microspheres (polylactide/glycolide beads) and immunized via intranasal with two immunoadjuvants murabutide (MB) and CpG ODN 1826 (CpG), in two strains of mice while humoral and mucosal immune responses were studied till day 120 and memory response was checked by immunizing with native V antigen.

Epitope specific serum, mucosal washes IgG, IgA, IgG subclasses and specific activity were measured by indirect ELISA and sandwich ELISA respectively. MAP in saline and MAP in microspheres showed similar titres with maximum on day 60 with a titre of 1,02,400 which maintained till day 90 and declined to 51,200 on day 120. On day 135 the titres increased to 1,02,400 showing good memory response after challenge with native V antigen. Out of, MAP +MB and MAP +CpG, and MAP +MB+CpG formulations MAP in CpG showed maximum peak titres 4,05,600 on day 60. The MAP +MB and MAP +CpG showed similar peak titres with maximum 2,04,800 on day 60. The specific activity levels, which indicate local antibody production, correlated well with the antibody levels. When IgG subclasses were estimated, the MAP in saline and MAP +MB showed higher levels of IgG1 indicating Th1 directing immune response whereas MAP in microspheres, and MAP+CpG+MB showed a mixed IgG1 and IgG2a/2b indicating a mixed Th1/Th2 directing immune response.

In the mucosal washes, MAP+CpG showed maximum IgG peak titres of 6,400 and 3,200 while MAP +MB and MAP +MB+CpG showed 3,200 and 1,600 on day 60 in intestinal washes and lung washes respectively and the IgA peak titres for MAP+CpG were maximum with 3,200 and 1,600 while MAP +MB and MAP +MB+CpG showed 1,600 and 800 in intestinal washes and lung washes respectively. Secretory component in mucosal washes was also detected showing the presence of SIgA. This study revealed that MAP is highly immunogenic with high and long lasting antibody titres in serum as well as in mucosal washes with good recall response and this approach can be used for sub unit vaccine development for plague. Currently, we are performing *in vivo* protective study.

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