20% of adult population in Bangkok may be at risk for acquiring JE when traveling to high risk/endemic area e.g. rural or upcountry.

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Immunogenecity of a chimeric protein of Bacillus anthracis protective antigen and lethal factor in murine model

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Background: Anthrax, a disease of bioterrorism and public health importance is caused by the Gram positive, spore-forming bacterium, *Bacillus anthracis*. Anthrax toxin, a tripartite toxin is composed of protective antigen (PA), lethal factor (LF) or edema factor (EF). PA is the major protein which facilitates the entry of toxin component either of lethal factor or edema factor. Recombinant PA has been a suitable target for anthrax vaccine worldwide. However, instead of full PA, its domains are reported to provide protection. LF also contributes to immuno-protection against anthrax. Therefore, in this study, a chimeric protein consisting of both, PA and LF was developed as candidate vaccine for anthrax.

Methods & Materials: A chimera was made by fusion of immunodominant portion of PA (Domains 2-4) and LF (Domain 1) genes. . The construct was cloned in *pET32a+* vector and expressed in *E. coli* host. The recombinant chimeric protein was purified by immobilized metal affinity chromatography. The 4-6 week old Balb/c mice were injected intraperitonealy with three doses of chimeric protein (20 μg each mouse) at two week interval. The first dose was given with Freund's complete adjuvant and the subesequent doses were given with incomplete Freund's adjuvant. The serum IgG and its subtypes were determined by plate ELISA.

Results: The chimeric protein (PA-LF) was purified up to homogeneity and the production yield was 15 mg/l of the shake flask culture. The chimera elicited good immune response against both the toxins i.e. PA as well as LF. The end point titre of chimeric protein was 1:1024000 by plate ELISA. An antibody titre of 1:512000 was observed in mice serum for PA protein. The same serum exhibited the titre of 1:256000 against LF protein. The end point titres of IgG1, IgG2a, IgG2b and IgG3 were 1: 512000, 1:128000, 1:256000 and 1:32000, respectively. Thus, IgG1 was predominant among all subtypes indicating that PA-LF chimera induced Th2-type immune response

Conclusion: The chimera consisting of partial sequences of PA and LF can be better vaccine candidate than individual PA or LF proteins. In the present study, the recombinant protein elicited very good immune response in mice and showed Th2 type of immune response.

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Antibody response to various domain of protective antigen in cutaneous anthrax cases in India

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Background: Anthrax, caused by *Bacillus anthracis* is a well known biothreat disease. Besides, cutaneous anthrax is a public health disease also several countries where agriculture is the major source of income. Being a zoonotic disease, it primarily infects herbivorous livestock and wildlife species and then spreads to human through contact with infected animals or contaminated animal products. The virulence of *B. anthracis* is attributed to two major factors, i.e. a tripartite toxin and the poly-g-D-glutamic acid capsule. The anthrax toxins are secreted as three distinct proteins, namely protective antigen (PA), lethal factor (LF) and edema factor (EF) and their activities have been well described. PA is the pivotal protein of the anthrax toxin complex and therefore, has been a major target for vaccine development.

Methods & Materials: PA is a 83 kD protein which has 4 different domains. In this study, the 3 different domains of PA were cloned and expressed. The recombinant proteins were used to develop ELISA to determine the anti-PA IgG for individual domain in human cutaneous anthrax serum samples. End-point titers were defined as the highest serum dilutions that yielded an OD_{450nm} value 2-fold the value for the corresponding dilution of the control serum.

Results: Full PA protein (83 kD) and different domain proteins (PAD1, 46 kD; PAD2, 43 kD and PAD4, 33 kD) were purified to the homogeneity. A total of 41 cutaneous anthrax serum samples were examined for immuno-reactivity with PA protein and its domains. The whole PA protein was found to give maximum immunoreactivity followed by domain 4, 2 and 1.

Conclusion: The immunoreactivity of human cutaneous serum samples with individual PA domains showed that besides full PA protein, individual domain 4 and 2 can also be good targets for vaccine development as well as for serodiagnostic assays.

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