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Cloning and expression of immunogenic molecules of L. major promastigote by Patient's immune sera

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Leishmaniasis is caused by parasitic protozoa transmitted by the bite of female sand fly and is currently endemic in 88 countries, affecting 12 million people worldwide and threatening 350 million more. The susceptibility of human to parasite has been attributed in part to the expansion of Th2 cells and production of their cytokines, IL-4 and IL-10 and down-regulation of Th1 cytokine, INF-γ. Leishmaniasis treatment involves pentavalent antimony or amphotericin B, but with diverse side-effects and generation of drug-resistant organisms. Therefore, it is necessary to discover new drugs and an effective vaccine. In this report we tried to identify the immunogenic leishmania major proteins using human immune sera that were infected and cured from the leishmania major infection. mRNA of L. major promastigotes of stationary phase was isolated and cDNA was made using Invitrogen CloneMiner kit. cDNA was inserted into kanamycin resistant pDONR222 plasmid and then transferred into DH5a competent E. coli. The positive plasmids were inserted into pDEST17 vector and transferred into BL-21 cells for expression. The colonies were monitored for expressed proteins by overlapping nitrocellulose filters impregnated with 10 mM IPTG. Filter was incubated with patient's immune sera and after washing, HRP-conjugated goat anti-human Ig was added and finally expressed proteins were detected with DAB substrate. The immunopositive clones were picked, and stored for further expression and purification of proteins to be testes as candidate vaccine.

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Development of transgenic brassica juncea expressing normal and mutated edema factor gene for the development of vaccine against athrax

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Anthrax is a zoonotic disease caused by the spore-forming bacterium Bacillus anthracis. It produces three toxin components, protective antigen (PA), lethal factor (LF) and edema factor (EF). Lethal toxin (LF+PA) and edema toxin (EF+PA) are known to cause lethality in anthrax infected animal models. The recent bioterrorism attack and the revival of the disease enforce an urgent need for effective prophylactic measures and therapeutic formulations to combat the disease. The currently used PA based anthrax vaccine is associated with several side effects due to its potential reactogenecity and requires protracted and complicated dosages schedule. Since the expression of hepatitis B surface antigen in model plant tobacco, a number of antigenic proteins have been expressed in different plant systems. These plant based vaccines are especially attractive as plants are free of human or animal diseases, reducing screening and purification costs. Also, they offer a palatable oral delivery system that stimulates both systemic and mucosal immune response. Attempts are being made to develop improved anthrax vaccines using antigens other then PA. Several immunization studies have shown that LF and EF alone, produce substantial amount of antibodies and in combination with PA, strengthen PA mediated immunoprotection. Studies have also highlighted the need of using mutants of anthrax toxin components in vaccine preparations. We have successfully cloned normal and mutated EF gene in a plant expression vector and Indian mustard plant Brassica juncea was transformed via Agrobacterium mediated transformation. Molecular and immunological studies have shown the stable integration and expression of EF gene in the plants.

Biography

This is Prerna Chaudhary, a Ph.D student in school of life sciences, Jawaharlal Nehru University, New Delhi. I am working on edible vaccine against anthrax and recently communicated a paper on PA based edible vaccine.

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