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Vaccine development

DEVELOPMENT OF A NOVEL SIX-VALANT RECOMBINANT PROTEIN VACCINE AGAINST TOXOPLASMOSIS USING ANTIGENS DISCOVERED BY HIGH-THROUGHPUT PROTEIN MICROARRAY ANALYSIS OF PATIENT AND INFECTED MURINE MODEL SERA

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Objectives: The existing traditional vaccine development strategies are not likely to generate new and safe vaccines against major global diseases. Although novel vaccination strategies (DNA and recombinant protein vaccines) and adjuvants are developed, the selection method(s) of antigens to be used in new vaccines has not evolved yet. To date almost all of the vaccine studies aimed to use one of the most antigenic protein which is frequently expressed on the surface of the microorganism. In the era of modern vaccinology, the initial step of the vaccine development pipeline is the discovery of the antigen to be used in a vaccination study. In the present study, a novel high-throughput vaccine antigen discovery method using proteome microarrays to profile the humoral immune response to toxoplasmosis has been utilized to discover immunogenic antigens. Thereafter, a group of discovered antigens were used to generate a protective multivalant recombinant protein vaccine against toxoplasmosis.

Methods: Initially, protein microarray slides containing 2705 expressed exons derived from *T. gondii* genome were probed with sera of patients to discover the IgM/IgG responsive antigens. Group 1 comprised recently acute toxoplasmosis cases from an outbreak (n:27). Group 2 and 3 patients had chronic toxoplasmosis with/without IgM (n:27). In second stage, two groups of mice were perorally infected with *T. gondii* tissue cysts and oocysts to mimic the natural route of infection. Sera were collected from mice prior to infection and 1, 2, 3, 6, 10, 15, 40, and 120 days post-infection. Then, proteome chips containing 240 *T. gondii* reactive proteins were probed with murine sera to discover IgM/IgG responsive antigens. Then, six immunogenic antigens were selected based on microarray data as well as expression and purification quality. Thereafter, mice were vaccinated with six-valant recombinant protein adjuvanted with Montanide ISA50V. Humoral and cellular immune responses were analyzed from sera and splenocytes of vaccinated mice by Western blot and flow cytometer.

Results: Initial screening of human sera discovered 240 potential targets. After the analysis of microarray data obtained from murine screening, potential 53 overlapping antigenic targets were selected for vaccine formulation. Expression and purification studies in *E. coli* prioritized six antigens. Analysis of sera obtained from mice vaccinated with adjuvanted six-valant recombinant protein vaccine showed strong IgG response. The ratio of CD8 T lymphocytes secreting IFN-gamma significantly increased compared to controls ($P<0.01$), indicative of protection against toxoplasmosis.

Conclusions: Overall, the present study identified more than 240 antigenic proteins that can be used in vaccine formulations against toxoplasmosis. In addition, a novel six-valant recombinant protein vaccine has been developed against toxoplasmosis based on the microarray data first time, complying the requirements of modern vaccinology.
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