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TITLE

SCREENING AND EVALUATION OF SCHISTOSOMA MANSONI PEPTIDES AS TARGETS OF SEROLOGICAL TESTS FOR THE SCHISTOSOMIASIS DIAGNOSIS

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ABSTRACT

Despite advances in schistosomiasis control, it remains a serious global public health issue. New diagnostic strategies are crucial for its control and elimination since the current Kato-Katz test has low sensitivity. Serological tests are promising alternative in the diagnosis of active Schistosoma mansoni infection, necessitating the identification of antigens for these tests. Our group identified potential target proteins, including a protein named PPE, through immunoproteome and bioinformatics studies. Therefore, this project aims to screen and evaluate antigenic peptides from these S. mansoni proteins for new serological diagnostic tests for schistosomiasis. The experimental strategy is defined in two axes. The first involves in silico prospecting of PPE peptides, and the second uses microarrays of peptides derived from PPE and other proteins selected through bioinformatics in immunoassays. In silico analysis selected six potentially antigenic PPE peptides(P1-6). These peptides were synthesized, and P6 was used in ELISA assays to evaluate antibody reactivity in serum samples from S. mansoni infected and non-infected individuals, previously characterized by Helmintex and Kato-Katz parasitological tests. After standardization, the assay was conducted as follows: 96-well Maxisorp plates were coated with 100µL of P6 (6µg/mL) in carbonate-bicarbonate buffer pH 9.6, overnight at 4°C, then blocked with 10% FBS in PBS-T for 2h at 4°C and washed. Serum samples diluted 1:80 in PBS-T were added in duplicate and incubated at 37°C for 2h. Following washing, plates were incubated at 37°C for 1h with anti-human IgG-HRP secondary antibody at 1:60.000 dilution in PBS-T and then washed again. TMB susbtrate (100µL) was added for 30 min in the dark at room temperature, and 50µL of 4M sulfuric acid were used to stop the reactions. The colorimetric signal was read in a microplate spectrophotometer at 450nm. Under these conditions, the assay showed a coefficient of variation lower than 5%. However, peptide P6 failed to distinguish infected from non-infected individuals, regardless of their residence in an endemic area. In parallel, the custom-made high-density peptide microarrays were synthesized containing 726 peptides of 15 aminoacids derived from five S. mansoni proteins: 340 peptides from PPE, 179 from Smp33, 130 from Smp13, 56 from Smp18 and 21 from Smp19. ELISA-like immunoassays were performed using pooled serum samples from S. mansoni infected and non-infected individuals, and anti-human IgG and IgG4 fluorescent antibodies. Two larger peptides from regions highly recognized by antibodies were selected for synthesis and further ELISA assays. Additionally, 120 of the 726 peptides showing the greatest reactivity differences between serum pools from infected and non-infected individuals were selected for a new microarray, which will be used to determine their performance in a schistosomiasis diagnostic test.

KEYWORDS

Serological Diagnosis; Peptide Microarray; Schistosomiasis

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