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TITLE

EXPRESSION AND PURIFICATION OF A RECOMBINANT CHIMERIC ANTIGEN FOR DIAGNOSIS OF SCHISTOSOMIASIS MANSONI

AUTHORS

Costa, N.A.R.S.*[1]; Aussourd, L.A.A.[1]; Correia, D.M.C.[1]; Ruas, A.C.L.[2]; Pereira, M.E.S.[1]; Fujiwara, R.T.[2]; Junior, J.C.S.F.[3]; Neto, O.P.M.[1]; Gomes, E.C.S.[1]

AFFILIATIONS

[1] Fundação Oswaldo Cruz – Instituto Aggeu Magalhães
[2] Universidade Federal de Minas Gerais
[3] Universidade Federal de Pernambuco

ABSTRACT

Introduction: Schistosomiasis is a neglected disease caused by the helminth Schistosoma mansoni. The Kato-Katz parasitological diagnostic method, which is recommended by the WHO and the Brazilian Ministry of Health, has low sensitivity for detecting infected individuals with a low parasite load. Thus, the ELISA detection method may be indicated for alternative or complementary diagnosis in patients with suspected S. mansoni infection. However, the known antigens used in ELISA have low sensitivity and cross-react with other diseases. An alternative is the use of recombinant chimeric antigens, which consist of a combination of immunodominant antigenic epitopes from S. mansoni proteins, which allows for an increase in the sensitivity and specificity of the test. Objective: This work aimed to optimize the expression and purification of a recombinant chimeric antigen in order to evaluate it and use it in an ELISA immunological test for diagnosing patients with schistosomiasis mansoni. Methods: To optimize the expression of the chimeric antigen, induction was evaluated at temperatures of 30°C for 3h, 37°C for 2h and 18°C for 16h in the BL21 strain and 18°C for 24h in the Arctic strain. Next, a solubility test was carried out with the antigen, in which the cells were resuspended with solution without and with different concentrations of urea (2M, 4M, 6M and 8M). After assessing solubility, a new induction was carried out and the antigen was purified by affinity column chromatography using AKTA start equipment. Once quantified on a gel, the chimeric antigen was used in an ELISA immunoassay at a concentration of 100 ng and a dilution of 1:50 of the sera of five schistosomiasis-positive patients with different parasite loads and five negative patients. Along with the chimeric antigen, the soluble egg (SEA) and adult worm (SWAP) antigens were used as positive controls for S. mansoni infection. The serum samples used were obtained from the Biorepository of the Schistosomiasis Reference Laboratory of the Aggeu Magalhães Institute, approved by the Human Research Ethics Committee under opinion numbers 5.905.584 and 7.020.057. Results: Induction showed good results at all temperatures in both strains, but the best was at 30°C for 3 hours in the BL21 strain, which presented a higher concentration of the protein after the induction period. Regarding the solubility test, the concentrations that yielded the most protein levels in the supernatant were 4, 6 and 8 M. The ELISA test showed a cutoff of 0.120 for the chimeric antigen, with no false positives or false negatives, but there was no differentiation between the parasite loads. Conclusion: The chimeric antigen has the potential to be used in the ELISA diagnostic test, as it can be detected in serum samples from patients with the disease. However, it is still necessary to test with a larger number of samples to evaluate its sensitivity and specificity.

KEYWORDS

ELISA; Chimeric Antigen; Diagnosis; Schistosomiasis

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