Expression and Purification of Recombinant Thiol-specific Antioxidant (TSA) Protein from Leishmania Majoras a Vaccine Candidate

Narges Khabazzade Tehrani*[1]; Fateme Tabatabaee[2]; Abbas Ali Imani Fooladi[3]; Hamid Sedighian Rad[3]; Mehdi Mahdavi[4]; Zahra Abrehdari[4]

1-Department of Microbiology, Science and Research Branch, Islamic Azad University, Tehran, Iran

2- Department of Parasitology and Mycology, School of Medicine, Iran

3-Applied Microbiology, Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

4- Department of Immunology and Virology , Pasteur Institute of Iran, Tehran, Iran

nargesskhabazzz@gmail.com

Background & Objectives: Leishmaniasis is caused by parasitic protozoa of the genus Leishmania which, in the infected host are obligate intracellular parasite. Many efforts have been done to develop an effective Leishmaniavaccine but failed.In the past 15 years, many groups have analyzed DNA-derived proteins from Leishmania. Large amounts of data obtained by these groups can be collated to direct future research into Leishmania and to find novel immunological mechanisms and information about its pathogenic molecules. TSA is the immuno-dominant antigen of Leishmania major which is considered as the most promising molecule for a recombinant or DNA vaccine against leishmaniasis.

Methods: In the present study the TSA sequence was optimized using CLC,Dnasis and online software and then the sequence was synthesized. Plasmid contain tsa gene were sub-cloned into the pet28A expression vector. In order to express the recombinant vector, TSA/pet28A expression vector was transformed into the E. coli BL21 using electroporation technique. After selection the bacteria containing plasmid the culture of E. coli BL21 with TSA/pet28A recombinant plasmid was done in the present of IPTG 1 mM for 4 h. The expression of recombinant protein was confirmed with SDS page and also via western blot technique. Mass production of recombinant protein carried out through nickel affinity chromatography.

Results: We results showed that recombinant TSA protein is produced in the *E. coli* BL21 containing TSA/pet28A plasmid after induction with 1mM IPTG and in the western blot analysis a ~22 k.D band was observed. In the next we intended that use this protein as a vaccine candidate againstLeishmania infection in Balb/C mouse model.

Keywords: Thiol-specific Antioxidant; Leishmania; Vaccine