Development of new tools to the serodiagnosis of Chagas' disease: a chimeric antigen design and new method based on flow cytometry

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The diagnosis of Chagas disease is largely based on serological techniques. Several recombinant proteins have proven to be promising targets, however none of them used individually has satisfactory sensitivity and specificity values. In this work, we propose the production and validation of a chimeric protein of *T. cruzi* that was assembled from peptides previously validated for use in the specific serodiagnosis of Chagas disease. A total of six peptides were selected to compose the chimeric molecule, which was expressed in Arctic Express (DE3) *E. coli* and used in ELISA assays using sera from animals experimentally infected with Colombian (TCI), Y (TCII), and CL Brener (TcVI) strains. Sera from mice infected with *Leishmania* spp were also used to verify the occurrence of cross-reaction. The recombinant *T. cruzi* chimeric protein had a sensitivity of 94.44% and specificity of 100%. Besides assessing the performance of this chimeric molecule, in this work we also proposed the validation of a new serodiagnosis methodology based on flow cytometry. This methodology has potential specificity for T. cruzi diagnosis allowing multiplex analysis of co-infections. To standardize this methodology, three recombinant proteins derived from the MASP protein family, which is expressed in trypomastigotes of *T. cruzi,* were coupled to fluorescent microspheres. To confirm the coupling, anti-MASP sera produced in BALB/c mice were used. We have also performed two tests with these MASP recombinant proteins coupled to different fluorescent microspheres and with sera from mice infected with *T. cruzi* CL Brener strain. In the first test, each individually coupled MASP was incubated with the sera. In the other one, all MASPs coupled to beads with different fluorescence, forming a mix, were incubated simultaneously with the sera. The profile of reactivity was very similar in both tests, indicating that both methods are effective and could potentially to be used in the serodiagnosis of Chagas disease.