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Serodiagnosis of Chagas Disease Using Recombinant Antigens

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The advances in molecular biology, genomics, proteomics, and bioinformatics have supported the design of new strategies for the development of more sensitive and specific diagnostic tests. This has originated the identification of genes coding for actives biologically proteins, later analysed in fine detail, transferred from one organism to another and expressed under controlled conditions so as to synthesize efficiently the polypeptides [1]. The recombinant DNA technology provides methods for producing proteins that constitute antigens well characterized, this system enable the overexpression of the gene of interest, its purification with higher production efficiencies, what guarantees, consequently, lower costs of the final product. In addition, they do not require manipulation of the infective agent as it happens when the antigens are obtained by purification procedures from microorganism culture, moreover, the production and evaluation can be highly standardized and great amounts of extremely purified proteins are obtained through the use of efficient tag-based purification system, which are easily retrieved through affinity columns [2].

The use of an overexpression system causes excessive production of recombinant proteins in relation to cellular proteins, and for this reason, the proteins of interest can be easily purified with minimal contamination of proteins of the host cell. The objective of the production of recombinant proteins is to obtain as many quantities as possible of a high quality product, with reproducible results, that is, the repetition of the same protocol yields identical products, thus reducing the variability observed when the proteins are purified [3].

Recombinant antigens can be produced in heterologous expression systems, as *Escherichia coli* cells, one of the organisms of choice for the production of recombinant proteins, what represent a cell factory and constitute a benchmark for comparison among the other expression platforms [4]. This is due to the multiple advantages that shows *E. coli* as the host organism 1) has fast growth kinetics, in the optimal conditions its doubling time is about 20 min [5] 2) is a organism of simple genetic manipulation 3) favor availability of optimized expression plasmids 4) provide high level expression 5) simple and inexpensive media requirement, 6) high cell density cultures are easily obtained, 7) the transformation with exogenous DNA is quick and simple [6,7]. The use of recombinants antigens expressed in this host cell for human serodiagnosis has presented the problem of presenting antibodies that have cross reaction against fusion partners or proteins of *E. coli* [8]. This inconvenient has been solved by the use of a His-Tag, which gives the recombinant protein a label of six repeated histidines in tandem, which allows to be easily purified using a one-step chromatography procedure [9].

The advent of recombinant DNA technology and its application in the biotechnology industry has revolutionized healthcare. Its use in the diagnosis of diseases has improved the speed, specificity and sensitivity of various serological tests. By expressing the genes cloned in cell cultures, specific antigens are produced that maintain their native conformation without altering the tertiary or quaternary structures, therefore, the epitopes are conserved [10]. This methodology comprises altering genetic material outside an organism (e.g. *Trypanosoma cruzi*) to obtain improved and desired characteristics in living organisms, which are termed host cells (e.g. *E. coli*). This technology involves the insertion of DNA fragments from an organism of interest for a plasmid, which is introduced into a host organism,

and cultured to produce multiple copies of the incorporated DNA fragment. This plasmid has special characteristics, a selectable marker (generally in the form of antibiotic resistance genes) to distinguish host cells that transport vectors from host cells that do not contain a vector, a gene that encodes an affinity tag (e.g. His-Tag) that allows its easy and rapid purification using an affinity column, one of the most used is the immobilized metal affinity column (IMAC). Polyhistidine is the affinity tag most offered by most companies that provide expression vectors or protein expression systems. Histidine readily forms coordination bonds with immobilized transition metal ions. The Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Ca^{2+} and immobilized Fe^{3+} can be used to purify the fusion proteins of polyhistidine, but Ni^{2+} is the most used [11,12]. The new molecular tools have revolutionized the diagnosis of parasitic diseases such as Chagas disease.

The studies carried out by several researchers have allowed the cloning of many *T*. cruzi antigens to be used in serological studies. The DNA sequences encoding the *T. cruzi* proteins are inserted into a bacterial plasmid, which is transformed into competent bacteria. The proteins encoded by the plasmid are expressed in the bacterial culture and then purified to obtain a product of the highest quality. The main objectives were to solve problems related to the existence of false positive diagnoses, detect early infections and establish potential diagnoses to distinguish the different stages of the disease [13]. The cloned of *T. cruzi* antigens correspond to different parasite stages, trypomastigote, amastigote and epimastigote. Several of these antigens were obtained by immunoscreened of cDNA expression libraries using chagasic patient sera, as well as from immunized animals [14-16]. The antigen codifying genes have been identified from cDNA present in the libraries accomplished from epimastigote or trypomastigote forms [17,18]. Da Rocha., *et al.* have proposed the use of proteins of amastigotes since this is the intracellular parasite form, being these antigens more significant for serodiagnosis [19].

Various recombinant antigens have been used to improve serodiagnosis of Chagas disease: shed acute-phase antigen (SAPA) [20], flagellar repetitive antigen (FRA) [21] and cytoplasmic repetitive antigen (CRA) [21]. SAPA detect antibodies present mainly in acute and congenital sera [20,22] and is mainly expressed in the infective (trypomastigote) stage of the parasite [20]. This antigen has been used for the diagnosis of neonates with congenital transmission and for follow-up serological treatment with benznidazole infection Chagas [23,24]. In a study conducted by Gil., *et al*. (2011), the sensitivity calculated for ELISA-SAPA was elevated (97.1%) and some works published by different authors evidence heterogeneous results regarding antigenic reactivity of SAPA against sera of people in chronic phase (32% and 38%) or indeterminate (81% and 88%) [13,25,26]. Among the recombinant antigens, repetitive proteins (RP) represent very promising targets [27]. Two well characterized RP's are CRA and FRA, what are they highly antigenic and induce the production of several classes of antibody in humans [28]. CRA is a 225 kDa protein composed of a 14-amino acid repeat that is distributed in the cytoplasm of the replicating epimastigote and amastigote forms. FRA is a 300 kDa protein composed of a 68-aminoacid repeat that is located at the flagellum in all life cycle stages of the *T. cruzi* [29].

The results obtained by some laboratories indicate that the recombinant (CRA+FRA) ELISA was better than the conventional ELISA in the diagnosis of Chagas disease, providing 100% specificity and sensitivity in all sera tested [21,30]. Numerous investigations have shown that mixtures of recombinant antigens provide better results than the use of a single protein. This is because the use of several antigens provides better sensitivity values, which results in a more reliable and safe test [31,32].

In a study performed with samples of sera from 142 nonchagasic persons (62 with others diseases) were obtained the specificities of JM, MT, and MJT recombinants proteins with 99.3, 96.5 and 98.6%, respectively. Instead, the specificity of EAE (in-house ELISA sensitized with the epimastigote form of *T. cruzi*) was of 95.8%, this is due to the fact that 6 of 10 sera from patients with leishmaniasis showed cross-reactive, that is, they showed false positive results [33]. The use of recombinants antigens from quality prevents the appearance of false-positive results, that is, avoid cross-reactivity between *T. cruzi* and parasites of related diseases caused by protozoa, particularly leishmaniasis and infection with *T. rangeli* [34,35]. One of the advantages of using this type of antigens is the absence of host cellular proteins in the recombinant antigen preparations that dramatically decrease the rate of false-positive reactions [10].

These antigens contain specific *T. cruzi* epitopes that elicit an immune response in the majority of chagasic patients [36,37]. Recombinant antigens can also be used in serological techniques to evaluate the usefulness of chemotherapeutic drugs. They have been used in the follow-up of patients on treatment with amiodarone and itraconazole, in a study conducted by Paniz *et al*. (2009), using PGR31-His, PGR30-His or rTc24-His (Parasitic Enzyme Laboratory, University of Los Andes, Mérida, Venezuela) to perform several serological tests. The results show a decrease of the anti-rTc24 titers by ELISA and of the signal by Immunoblot, 6 months after starting treatment with amiodarone and itraconazole the optical density is below the limit for ELISA and the signal becomes undetectable in the format Immunoblot, indicating the patient has been cured [38].

Diseases can be controlled through the implementation of programs of prophylaxis and studys epidemiologic, whose effectiveness improve with the development of high quality diagnoses. In addition, the fight against emerging diseases in the care of both human and animal health is a challenge and depends on the execution of safe and efficient diagnoses, whose usefulness increases with the use of specific, sensitive recombinant antigens, to low costs and produced in large quantities, through the use of biotechnological and molecular advances.

Bibliography

1. World Health Organization. "Guidelines for assuring the quality of pharmaceutical and biological products prepared by recombinant DNA technology". Annex 3 in: WHO Technical Report Series No 814 (1991).
2. LaVallie E and McCoy J. "Gene fusion expression systems in *Escherichia coli*". *Current Opinion in Biotechnology* 6.5 (1995): 501-506.
3. Lawrence J and Klass M. "Antigens produced by recombinant DNA Technology". *Clinical Chemistry* 35.9 (1989): 1838-1842.
4. Rosano G and Ceccarelli E. "Recombinant protein expression in *Escherichia coli*: advances and challenges". *Frontiers in Microbiology* 5 (2014): 172.
5. Senozov G., *et al*. "*Escherichia coli* physiology in Luria-Bertani broth". *Journal of Bacteriology* 189.23 (2007): 8746-8749.
6. Wingfield P. "Overview of the purification of recombinant proteins". *Current Protocols in Protein Science* 80 (2015): 6.1.1-35.
7. Balamurugan V., *et al*. "Biotechnology in the production of recombinant vaccine or antigen for animal health". *Journal of Animal and Veterinary Advances* 5.6 (2006): 487-495.
8. Tenter A and Johnson A. "Recognition of recombinant *Toxoplasma gondii* antigens by human sera in an ELISA". *Parasitology Research* 77.3 (1991): 197-203.
9. Hiszczynska-Sawicka E., *et al*. "Efficient production of the *Toxoplasma gondii* GRA6, p35 and SAG2 recombinant antigens and their applications in the serodiagnosis of toxoplasmosis". *Acta Parasitologica* 50.3 (2005): 249-254.
10. Balamurugan V., *et al*. "Recombinant protein-based viral disease diagnostics in veterinary medicine". *Expert Review of Molecular Diagnostics* 10.6 (2010): 731-753.
11. Kimple M., *et al*. "Overview of affinity tags for protein purification". *Current Protocols in Protein Science* 73 (2013): Unit-9.
12. Costa S., *et al*. "Fusion tags for protein solubility, purification and immunogenicity in *Escherichia coli*: the novel Fh8 system". *Frontiers in Microbiology* 5 (2014): 63.
13. Breniere S., *et al*. "Immune response to *Trypanosoma cruzi* shed acute phase antigen in children from an endemic area for Chagas disease in Bolivia". *Memórias do Instituto Oswaldo Cruz* 92.4 (1997): 503-507.
14. Lafaille J., *et al*. "Structure and expression of two *Trypanosoma cruzi* genes encoding antigenic proteins bearing repetitive epitopes". *Molecular and Biochemical Parasitology* 35.2 (1989): 127-136.

15. Cotrim P., *et al*. "Expression in *Escherichia coli* of a dominant immunogen of *Trypanosoma cruzi* recognized by human chagasic sera". *Journal of Clinical Microbiology* 28.3 (1990): 519-524.

16. Gruber A and Zingales B. "*Trypanosoma cruzi*: characterization of two recombinant antigens with potential application in the diagnosis of Chagas´disease". *Experimental Parasitology* 76.1 (1993): 1-12.

17. Zingales B., *et al*. "Molecular approaches to diagnosis of Chagas´ disease: use of defined antigens and a target ribosomal RNA sequence". *Biological Research* 26.1-2 (1993): 89-100.

18. Godsel L., *et al*. "Utility of recombinant flagelar calcium-binding protein for serodiagnosis of *Trypanosoma cruzi* infection". *Journal of Clinical Microbiology* 33.8 (1995): 2082-2085.

19. DaRocha W., *et al*. "Characterization of cDNA clones encoding ribonucleoprotein antigens expressed in *Trypanosoma cruzi* amastigotes". *Parasitology Research* 88.4 (2002): 292-300.

20. Affranchino J., *et al*. "Identification of a *Trypanosoma cruzi* antigen that is shed during the acute phase of Chagas´ disease". *Molecular and Biochemical Parasitology* 34.3 (1989): 221-228.

21. Krieger M., *et al*. "Use of recombinant antigens for the accurate immunodiagnosis Chagas' disease". *American Journal of Tropical Medicine and Hygiene* 46.4 (1992): 427-434.

22. Reyes M., *et al*. "Fetal IgG specificities against *Trypanosoma cruzi* antigens in infected newborns". *Proceedings of the National Academy of Sciences of the United States of America* 87.7 (1990): 2846-2850.

23. Flores-Chavez M., *et al*. "Polymerase chain reaction detection and serologic follow-up after treatment with benznidazole in Bolivian children infected with a natural mixture of *Trypanosoma cruzi* I and II". *American Journal of Tropical Medicine and Hygiene* 75.3 (2006): 497-501.

24. Mallimaci M., *et al*. "Early diagnosis of congenital *Trypanosoma cruzi* infection, using shed acute phase antigen, in Ushuaia, Tierra del Fuego, Argentina". American Journal of Tropical Medicine and Hygiene 82.1 (2010): 55-59.

25. Gil J., *et al*. "Reactividad del antígeno GST-SAPA de *Trypanosoma cruzi* frente a sueros de pacientes con enfermedad de Chagas y Leishmaniasis". *Medicina (Buenos Aires)* 71.2 (2011): 113-119.

26. Lorca M., *et al*. "El diagnóstico de la enfermedad de Chagas crónica mediante antígenos recombinantes de *Trypanosoma cruzi*". *Revista Medica de Chile* 121 (1993): 363-368.

27. Valiente-Gabioud A., *et al*. "Effect of repetitiveness on the immunogenicity and antigenicity of *Trypanosoma cruzi* FRA protein". *Experimental Parasitology* 127.3 (2011): 672-679.

28. Pereira V., *et al*. "Evaluation of the immune response to CRA and FRA recombinant antigens of *Trypanosoma cruzi* in C57BL/6 mice". *Revista da Sociedade Brasileira de Medicina Tropical* 36.4 (2003): 435-440.

29. Goto Y., *et al*. "Immunological dominance of *Trypanosoma cruzi* tandem repeat proteins". *Infection and Immunity* 76.9 (2008): 3967-3974.

30. Almeida E., *et al*. "Use of recombinant antigens for the diagnosis of Chagas' disease and blood bank screening". Memórias do Instituto Oswaldo Cruz 85.4 (1990): 513-517.

31. Carvalho M., *et al*. "Chagas´ disease diagnosis: evaluation of several tests in blood bank screening". *Transfusion* 33.10 (1993): 830-834.

32. Levin M., *et al*. "Recombinant *Trypanosoma cruzi* antigens and Chagas´disease diagnosis: analysis of a workshop". *FEMS Microbiology Immunology* 4.1 (1991): 11-19.

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33. Umezawa E., *et al*. "Serodiagnosis of chronic and acute Chagas´disease with *Trypanosoma cruzi* recombinant proteins: results of a collaborative study in six Latin American countries". *Journal of Clinical Microbiology* 42.1 (2004): 449-452.

34. Caballero Z., *et al*. "Evaluation of serological tests to identify *Trypanosoma cruzi* infection in humans and determine cross-reactivity with *Trypanosoma rangeli* and *Leishmania spp*". *Clinical and Vaccine Immunology* 14.8 (2007): 1045-1049.

35. Ramírez J., *et al*. "Evaluation of adult chronic Chagas´heart disease diagnosis by molecular and serological methods". *Journal of Clinical Microbiology* 47.12 (2009): 3945-3951.

36. da Silveira J., *et al*. "Chagas disease: recombinant *Trypanosoma cruzi* antigens for serological diagnosis". *Trends in Parasitology* 17.6 (2001): 286-291.

37. Umezawa E., *et al*. "Evaluation of recombinant antigens for serodiagnosis of Chagas' disease in South and Central America". *Journal of Clinical Microbiology* 37.5 (1999): 1554-1560.

38. Paniz-Mondolfi A., *et al*. "Amiodarone and itrazonazole: A rational therapeutic approach for the treatment of chronic Chagas´ disease". *Chemotherapy* 55.4 (2009): 228-233.

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