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Research Focus

# Molecular diagnosis of experimental Chagas disease

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**Diagnostic methods for detecting *Trypanosoma cruzi* infection are important to allow administration of chemotherapy and as an experimental tool when trying to understand the pathogenesis of Chagas disease. A nested polymerase chain reaction (PCR)-based approach was recently used to demonstrate preferential heart and skeletal muscle tropism in mice of a Mexican *T. cruzi* isolate. The authors of this study also demonstrated higher sensitivity for this PCR setup compared with a commercially available enzyme-linked immunosorbent assay kit.**

*Trypanosoma cruzi* is the causative agent of Chagas disease, a chronic inflammatory condition mainly affecting the heart and gastrointestinal tract [1]. The pathogenesis of this disease remains poorly understood and has been attributed to parasite persistence [2], autoimmunity [3], microvascular changes [4] or neurogenic disturbances [5]. The pathogenesis remains controversial by the simple fact that there exists no definite proof that any of these factors would be both necessary and sufficient to cause chronic Chagas disease. The necessity for persistence of *T. cruzi* in Chagas disease has recently become increasingly accepted. The evidence supporting this line of thought can be summarized as follows: (i) there is an almost perfect association between parasite persistence and disease; (ii) there is a correlation between inflammation and parasite DNA and/or antigens; and (iii) regimens that lessen parasite burden alleviate or even abrogate chronic Chagas disease, whereas immunosuppression aggravates the inflammatory disease. How these persisting parasites affect the immune response remains unknown, but several hypotheses have been put forward including providing a continuous stimuli for a host–parasite crossreactive immune response, affecting local peripheral tolerance or even being sufficient to cause disease. The principles of how pathogen persistence facilitates chronic inflammation are of fundamental importance, as persisting pathogens also appear to be involved in several other chronic inflammatory diseases. An obvious prerequisite for being able to study how persistence affects the immune response is the ability to demonstrate persistence easily and effectively.

### PCR-based diagnosis of *T. cruzi* infection

Several different polymerase chain reaction (PCR)-based approaches, which aim to amplify either kinetoplast DNA or nuclear satellite DNA, have been successfully used to diagnose *T. cruzi* infection [1]. Recently, Vera-Cruz *et al.* added to previously existing PCR-based diagnostic approaches by designing a nested PCR method that amplifies a gene for a flagellar protein of *T. cruzi*, Tc-24, and thus directly demonstrates the presence of *T. cruzi* [6]. Utilizing this method, the authors then continued to assess the growth kinetics and parasite persistence in mice infected with a Mexican isolate of *T. cruzi*, JALGO. The methodology in itself is not truly quantitative (as no housekeeping genes are amplified) but does indicate that parasites preferentially persist in heart, skeletal muscles and, to a slightly lesser extent, colon. Inflammatory disease was apparent in several organs at four months post-infection, including heart, skeletal muscles and colon, but also in kidneys, ileum and liver. The presence of inflammation in some of these organs might reflect the systemic nature of the acute phase of the disease [7]. The authors suggest that the preferential tropism of the JALGO isolates for skeletal muscle in mice might in part be responsible for the lack of clinical symptoms demonstrated by some patients that had come into contact with the parasite. This conclusion is most likely flawed as the host genotype affects tropism and disease manifestations [8,9]. Thus, if the authors had chosen to infect another mouse strain, the outcome might have been completely different. Consequently, it is impossible to predict the clinical symptoms of humans from murine infections. It is also interesting to note that skeletal muscle is almost always the primary target organ in mice, in contrast to humans in which it is most often the heart.

In principle, nested PCR is a good way to assess *T. cruzi* persistence. It is fairly simple compared with other methods such as *in situ* PCR and *in situ* hybridization, the second PCR step being seen as a confirmation that it

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indeed was the appropriate parasite DNA that was amplified in the initial reaction. The usefulness of this method will to a large extent depend on its sensitivity when compared with others. Previous 'epidemiological' studies suggest that the sensitivity is indeed excellent [10,11]. However, it would also be interesting to see a comparison in sensitivity in animal models. Parasite diversity is less of a problem in a well-defined animal model, so the efficiency of a diagnostic method is rather dependent on how few parasites it can detect.

### Comparing PCR and ELISA as diagnostic methods

Infection with *T. cruzi* is, in the main, diagnosed indirectly by using serological tests such as indirect hemagglutination, indirect immunofluorescence and enzyme-linked immunosorbent assay kit (ELISA) [1]. These tests have traditionally been chosen because of their simplicity, low cost and good performance. One problem associated with these methods is the antigenic crossreactivity between *T. cruzi, Leishmania*spp. and *Trypanosoma rangeli*, which results in false positive tests and reduced sensitivity. By contrast, xenodiagnosis, another diagnostic method of *T. cruzi* infection, frequently presents false negative results. The problem with false positive and negative results has resulted in a continued interest to improve current methods and to develop alternative diagnostic methods such as PCR-based approaches, to improve the ability to diagnose *T. cruzi* infection correctly. Recent studies suggest that the use of recombinant *T. cruzi* proteins instead of crude epimastigote extracts as antigens solve this problem to a large extent [12]. Nonetheless, it appears that some patients exposed to *T. cruzi* are not discovered using conventional serological tests [13]. Considering that North American isolates are poorly characterized, it would be interesting to compare the difference in sensitivity between serological tests and other diagnostic methods.

In the study by Vera-Cruz *et al.*, the Tc-24-based PCR reaction was also compared with a commercially available ELISA [6]. In total, 39 blood samples collected in an endemic area in Mexico were evaluated for the presence of *T.cruzi*-specific antibodies and *T. cruzi* DNA using ELISA and PCR, respectively. Of these, 21 out of 39 were positive by ELISA and 33 out of 39 were positive by PCR. These results raise the possibility that ELISA-based methods underestimate the prevalence of *T. cruzi* infections. Whether this finding is limited to Mexican isolates of *T. cruzi* remains to be seen, but it would definitely be interesting to repeat these experiments with larger numbers of blood samples, possibly including some from other parts of the world. It would also be appropriate to compare the PCR method not only with ELISA, but also with indirect hemagglutination or indirect immunofluorescence, as it is common procedure to use at least two of these tests in parallel when trying to diagnose *T. cruzi* infection. It is also important to include blood samples from a well-defined group of patients suffering from chronic Chagas disease to ensure that increased sensitivity of PCR is medically relevant, rather than just resulting in the detection of a group of patients that will always remain in the indeterminate phase of the disease and will never suffer any deleterious consequences from the infection.

One surprising aspect of the Vera-Cruz *et al.* study is that the second PCR step in the nested PCR seems to be used sparingly. Thus, instead of consistently using a nested PCR, it appears that many of the experiments are based on the conventional Tc24 PCR as first described by Ouaissi *et al.* [14]. A failure to include the second PCR step results in decreased specificity of the method and, after scrutiny of some of the figures in the paper, it appears that one or two of the PCR products do not have the appropriate size (550 bp).

In conclusion, the nested PCR assay designed by Vera-Cruz *et al.* has potential to be a useful experimental tool when investigators attempt to address the cellular and molecular mechanisms involved in the selective tropism of *T. cruzi*, as well as questions concerning the pathogenesis of Chagas disease. It might also be an excellent diagnostic method in epidemiological studies in which it could complement serological assays. Future studies concerning the sensitivity of the method both in animal models and in clinical settings will determine its usefulness.

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