A New Development in *Trypanosoma cruzi* Detection

Herbert B. Tanowitz, Louis M. Weiss
Department of Pathology, Division of Tropical Medicine and Parasitology, and Department of Medicine, Division of Infectious Disease, Albert Einstein College of Medicine, Bronx, New York, USA

**ABSTRACT** Chagas disease is caused by the parasite *Trypanosoma cruzi* and is an important cause of morbidity and mortality in areas of Latin America where Chagas disease is endemic and among infected individuals who have migrated to nonendemic areas of North America and Europe. There are many diagnostic tests that are employed in the serological diagnosis of this infection. In this issue of the *Journal of Clinical Microbiology*, Bautista-López et al. provide characterization of excretory vesicles (EVs) from Vero cells infected with *T. cruzi* and provide data on the EVs produced by trypomastigotes and amastigotes (N. L. Bautista-López et al., J Clin Microbiol 55:744–758, 2017, https://doi.org/10.1128/JCM.01649-16). Their proteomic study defines potential targets to evaluate for improved diagnostic tests, effects on host cell biology that contribute to the pathogenesis of infection, and vaccine candidates. If any of the EV-associated proteins identified were to be correlated to cure of infection, this would be a major advance.

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is an important cause of heart disease. While it is endemic in non-Caribbean Latin America, there has been a large migration of infected individuals to nonendemic areas of the world, including North America and Europe (1, 2). This has led to the diagnosis of entities such as Chagasic heart disease, congenital Chagas disease, and transfusion-associated Chagas disease in these areas of the world where the disease is nonendemic. The diagnosis of *T. cruzi* infection can be straightforward or elusive, depending on the stage of infection, recognition that infection can occur in nonendemic regions, and availability of diagnostic testing. During acute Chagas disease, whether vector-borne, acquired via the oral route, or transmitted via blood or organ donation, blood-form trypomastigotes are usually visible in the bloodstream. However, once the acute phase of infection has subsided, trypomastigotes are no longer observed in the blood, and the presence of antibody to the parasite may be the only laboratory evidence that an individual is infected. If blood from an asymptomatic but seropositive blood donor is administered to another individual, the recipient may develop transfusion Chagas disease. Infection is lifelong, with parasites persisting in reservoirs within the body in many tissues and organs, including adipose tissue (3, 4). When such seropositive persons with chronic infection become immune suppressed, due to medication or HIV infection, there is an exacerbation of *T. cruzi* infection and trypomastigotes are usually visible in blood films.

The current methods employed for the diagnosis of *T. cruzi* infection include microscopy, xenodiagnosis, quantitative PCR (qPCR), and serological methods, such as enzyme-linked immunosorbent assays (ELISAs) and immunoblotting techniques, that detect circulating *T. cruzi*-specific antibodies. Microscopy and PCR are the preferred methods for the diagnosis of acute infection, congenital Chagas disease, and immunosuppression-induced *T. cruzi* reactivation (5, 6). Xenodiagnosis, while useful for the diagnosis of chronic infection, requires the use of live triatomid vectors and is not useful in most settings. PCR, while available and highly specific, has sensitivity problems, and therefore a negative PCR test does not exclude...
infection with a high probability. For the diagnosis of chronic Chagas disease, serological methods are usually used, and these employ parasite-derived antigens, recombinant proteins, or synthetic peptides (7). Some of these serodiagnostic tests lack specificity, because they cross-react with Leishmania spp. and with Trypanosoma rangeli. Therefore, agencies such as the Pan American Health Organization recommend that two different assays be employed for the diagnosis of this infection. For example, serological testing using trypomastigote excretory-secretory antigens (TESA) can be performed as part of an ELISA platform or an immunoblotting assay for the detection of antibodies reactive with proteins or glycoconjugates released by the parasite.

The manuscript by Bautista-Lopez and colleagues provides a characterization of excretory vesicles (EVs) from Vero cells infected with T. cruzi and provides data on the EVs produced by trypomastigotes and amastigotes (8). EVs have been increasingly recognized among infectious diseases as important modulators of the host-pathogen relationship, including T. cruzi infection (9, 10). In their study, the T. cruzi infection EVs were purified utilizing standard centrifugation methods, such as those used to produce TESA. The tryptic peptides obtained from these EVs were analyzed using a standard proteomics approach and employing a Velos Pro LTQ-Orbitrap mass spectrometer. About 90% of the 766 proteins identified were from Vero cells, with the remaining 10% from T. cruzi, with both trypomastigote and amastigote proteins being identified. Overall, this is a reasonable yield for this type of analysis. It is possible that EVs from infected cells contain both Vero cell and T. cruzi proteins or that there are two types of EVs. The authors did not provide any data that would allow one to distinguish between these possibilities. To identify proteins recognized by the host, an immuno-proteomics approach was utilized. In this proteomics experiment, purified EV proteins were affinity purified using antisera from humans with Chagas disease, and the purified proteins were identified by mass spectrometry. The results provided a list of EV proteins which are recognized by the host and could be useful for the development of new serological assays. Overall, this proteomic study has defined a list of potential targets to evaluate for improved diagnostic tests, their effects on host cell biology that contribute to the pathogenesis of infection, and possible vaccine candidates. Further research on EV components as host-pathogen modulators is important and is likely to yield important insights into disease pathogenesis.

The retrotransposon hot spot (RHS) proteins that Bautista-López et al. identified and characterized as diagnostic proteins in this proteomic survey may be quite useful in limiting cross-reactions in serological studies for T. cruzi infection in patients with leishmaniasis; however, RHS proteins were not quite as sensitive as TESA, as demonstrated in Fig. 6 of their report. It remains to be seen, even for endemic regions, how significant the problem of cross-reaction is in clinical use, as opposed to epidemiological studies. The Ortho ELISA test system and the Abbott Prism Chagas assay are the only two assays approved for donor testing in the United States, and in extensive premarket evaluation trials, both appeared to have a sensitivity of 100% and a false-positive rate of about 1 in 8,000 samples tested (11, 12). A major limitation of all current serologic assays is that there is no test that correlates with cure of chronic infection. If any of the EV-associated proteins that the Bautista-López group identified were to be correlated to cure of infection, this would be a major advance and would clearly merit commercial development.

ACKNOWLEDGMENT

The work of H.B.T. is supported in part by NIH grant AI124000.

REFERENCES


