

New Advances in the Management of a Long-Neglected Disease

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(See the articles by Bern et al, on pages 1667–74, and Laucella et al, on pages 1675–84.)

Two major articles in the current issue of *Clinical Infectious Diseases* report important advances on diagnosis [1] and evaluation of treatment efficacy [2] of American trypanosomiasis or Chagas disease. This condition is a chronic parasitosis caused by the kinetoplastid protozoan *Trypanosoma cruzi*, which has afflicted humanity since its earliest presence in the Americas [3] and remains today the largest parasitic disease burden of the continent [4, 5]. The name derives from Carlos Chagas, a Brazilian physician who, 100 years ago, simultaneously discovered the parasite, the insect vector that transmits it, and the that disease it causes in humans. Chagas disease is technically a zoonosis, because the natural reservoirs of *T. cruzi* are placental and marsupial mammals autochthonous to the American continent, the parasite being transmitted among them by hematophagous insect vectors. Human disease results from the invasion of natural ecotopes and from the establishment of the vectors in human dwellings due to the poor socioeconomic conditions of most rural human populations

from Mexico to Argentina, where it is an endemic condition [4]. The parasite can also be transmitted by transfusion of contaminated blood and congenitally from infected mothers to newborns [4, 6], and these routes of transmission, together with intense international migrations in the past 15 years, have led to the spread of the disease to areas where it is not endemic, including the United States and western Europe [7–10].

The initial acute phase has a low mortality rate (<10%, mostly among children, because of acute myocarditis) and generally mild and unspecific symptoms [11, 12]. This phase is followed by a life-long chronic condition in which the cellular immune response limits the parasite's proliferation inside the host's tissues but is unable to eradicate the infection, leading to a sustained inflammatory response that underlies the development of ≥ 1 of the symptomatic chronic forms of the disease in 30%–40% of patients; the forms include chronic Chagas cardiomyopathy, digestive problems, and neuropathies [11, 13, 14]. The most severe of these manifestations is chronic Chagas cardiomyopathy, which can lead to congestive heart failure and death and which is the leading cause of cardiac disease and cardiac death in poor rural and rural-originated urban populations in Latin America [12].

Although great advances has been made since Chagas' discovery in our under-

standing of the epidemiology, immunology, and pathogenesis of the disease and in the biochemistry, physiology, and genetics of its etiological agent, many challenges remain for the control of this serious condition [6, 15]. During the past 20 years, we have witnessed important progress in the control of the vectorial and transfusional transmission of *T. cruzi*, mainly among the countries of the southern cone of South America [5, 16], but in other areas of endemicity, the progress has been uneven, because of the lack of maintenance of previously successful control programs (eg, in Venezuela), limited economic resources (in Central America), or the fact that control programs have never been officially implemented (eg, in Mexico and Peru) [5].

On the other hand, the treatment of persons who are already infected with the parasite (currently estimated to be 10–15 million persons) has traditionally received much less attention, with abysmally low specific treatment coverage levels—currently estimated to be <1% [17]. The reasons for such low treatment coverage for Chagas disease, even among tropical neglected diseases, include controversies on the relevance of specific treatment in the chronic stage, limitations of current diagnostic methods and available anti-*T. cruzi* drugs, lack of biomarkers to evaluate treatment efficacy, and limited political

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and economic support for these efforts [6, 18].

The article by Bern et al [1] describes a comparative study of molecular (polymerase chain reaction [PCR]-based) methods with conventional parasitological tests for the diagnosis of congenital Chagas disease in Santa Cruz, Bolivia. The diagnosis of Chagas disease in newborns and other early acute *T. cruzi* infections present special difficulties, because the immunoglobulin G response has not yet developed, making serological methods useless, whereas the critical need for fast results precludes the use of traditional parasitological methods, such as hemoculture or xenodiagnosis. The standard procedure in Bolivia and most other countries where Chagas disease is endemic is direct microscopic examination of blood cells concentrated in microhematocrit tubes (the “microhematocrit method”); newborns from seropositive mothers who test negative using this method are retested with use of conventional serological methods at 6 months of life to decide on potential specific treatment. In the present study, 530 women were analyzed before delivery by 3 independent serological methods, and 151 had confirmed *T. cruzi* infection (prevalence, 28.5%). Of these, 10 newborns (6.5%) received a diagnosis of congenital infection by qualitative *T. cruzi* PCR during the first month of life (mean age at diagnosis, 4 days), whereas only 4 of these children received a positive diagnosis by the microhematocrit method during the first month of life (2 others received such diagnoses at 103 and 189 days of life), for a mean age at diagnosis of 131 days; only 5 children were treated before the age of 6 months (including 1 who experienced treatment failure), whereas 2 of them refused or were unavailable for treatment. Other important result was the strong correlation of high blood parasite levels in mothers, evaluated by quantitative PCR, with congenital transmission. The bottom line of the study, which confirmed previous results from other regions of endemicity [19, 20],

is the perturbing conclusion that ~50% of children born with active *T. cruzi* infection may have their infections go undiagnosed if the standard procedure is used during their first months of life and must wait for the serological tests at 6 months to receive specific treatment, if that possibility is ever available to them. PCR-based methods can dramatically reduce the age at diagnosis and guarantee early treatment, which has consistently proven to be more effective and to have less-adverse side effects [19]. Unfortunately, as the authors point out, no PCR-based method adapted to field conditions is currently available; this should be a first priority for improving both diagnosis and the evaluation of treatment efficacy (see below).

The article by Laucella et al [2] describes the results of a study of potential great significance in the field that was aimed at the identification of surrogate biomarkers of treatment efficacy and parasite elimination. The lack of such biomarkers has been a major stumbling block for the development of new anti-*T. cruzi* drugs, as well as for the evaluation of the real activity of currently available drugs in patients with chronic disease, because the clinical evolution of the disease can only be evaluated by follow-up of many years to decades in duration, and conventional serological test respond slowly to parasite elimination, with the lag time increasing with the duration of the original infection [6, 21–23]. The authors had shown in a previous study that individuals with chronic *T. cruzi* infection display a functional profile of interferon- γ -only secreting T cells, characteristic of effector/effector memory T cells (T_E/T_{EM}). In the present study, they sought to investigate the effects of benznidazole treatment on the frequency, function, and phenotype of these *T. cruzi*-specific T cells, as well as on the patients' B cell response, evaluated using both conventional serological testing and a novel multiplex assay that uses a panel of recombinant antigens derived from the vertebrate stages of the parasite [24]. Forty-three patients treated with

benznidazole, a 2-nitroimidazole that is the most frequently used anti-*T. cruzi* drug, at 5 mg/kg per day for 30 days and 32 age-matched, untreated individuals were observed for 3–5 years. It was found that, within 12 months of treatment, levels of *T. cruzi*-specific interferon- γ - and interleukin-2-secreting T cells decreased to less than the level of detection (47%) or decreased substantially (25%) in benznidazole-treated individuals, whereas no response was seen in untreated patients. The modification of the specific T cell response in treated subjects was strongly correlated with a significant decrease in the antibody titers assayed using the novel multiplex assay—a change that, again, was not observed in untreated individuals. As expected, no significant correlation was observed between treatment and the levels of anti-*T. cruzi* antibodies detected by conventional methods. In total, 33 of the 43 treated subjects showed significant changes in either or both T cell and B responses, a fact that the authors interpret as indicative of a significant benznidazole-induced reduction of the parasite loads in the responder individuals; they further hypothesize that the drug could have induced parasitological cure in a substantial portion of these patients. Although no independent parasitological data were provided to support this last assertion, it agrees with the results of a previous study by the same group in a murine model of the disease [25], which found that radical parasitological cure (verified by immunosuppressing the treated animals) was consistently associated with the disappearance of specific T_E/T_{EM} cells and the development of a stable central memory (T_{CM}) response. In contrast, such a conclusion is in conflict with those of several other studies that have unequivocally shown, using PCR-based *T. cruzi* assays, that benznidazole is unable to induce parasite elimination in the majority of patients with established chronic *T. cruzi* infection [26–30]. The discrepancy could be explained by distinct drug susceptibility [31] and/or loads of the infecting parasite

populations; unfortunately, changes of the specific anti-*T. cruzi* T cell responses of the treated patients were investigated in none of the previous studies. However, independent of the capacity of benznidazole to induce true parasitological cure in patients with chronic infection, which may indeed vary among the zones of endemicity [32], the significance of the present study lies in the fact that it is the first to document marked and fast *T. cruzi*-specific T and B cell responses resulting from specific antiparasitic treatment in chronically infected adult subjects. If confirmed by other independent studies, such responses may become the long-sought surrogate biomarkers for treatment efficacy.

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