Neglected Tropical Diseases, Bioinformatics, and Vaccines

Mauricio Martins Rodrigues[1,2] and Jonatan Ersching[1,2]

[1]Centro de Terapia Celular e Molecular, and [2]Departmento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo-Escola Paulista de Medicina, Brazil

(See the major article by Teh-Poot et al on pages 258–66.)

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More than 2.5 billion individuals living in the tropics are estimated at risk of contracting at least 1 neglected tropical disease. Half of these individuals may be exposed to or have concomitantly ≥2 neglected tropical diseases, including those caused by helminths, schistosomes, parasitic protozoans, and viruses. Although the death toll caused by all neglected tropical diseases is not as high as for AIDS or tuberculosis, neglected tropical diseases still affect more than a million individuals, most of whom live in the poorest regions of Africa, Asia, and the Americas. In addition to the high mortality, most neglected tropical diseases are due to chronic infections and, therefore, have an enormous impact on childhood growth, disability-adjusted life-years, and productivity-associated economic losses targeting mainly the rural and poorest urban areas of developing countries [1].

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is a prototypical example of an neglected tropical disease of Latin America, with an estimated 10 million people chronically infected, causing a large burden of disability-adjusted life-years and billions of annual costs [2]. Chagas disease is endemic in 21 countries, and, in some instances, the number of individuals coming in contact with the parasite (estimated on the basis of seroprevalence) can be as high as 6.75% of the population, as in Bolivia [2, 3]. In addition, because of the migration of chagasic individuals, the disease has also been considered a health problem in developed countries where the disease is not endemic, such as the United States and Spain [4].

Upon contact with the parasite, human hosts may develop a patent parasitemia (acute phase) that, in most cases, will resolve after a few weeks.

Two-thirds of individuals will become serologically positive for *T. cruzi* antigens but will never develop symptoms (the indeterminate form of the disease); a decade or more later, one-third of patients will develop chronic forms of the disease, which can be either cardiac (most common), digestive (megaesophagus and megacolon), or cardiodigestive.

The pathogenesis of chronic Chagas disease is still a matter of intense debate. Some researchers initially proposed that there is an autoimmune response supporting the chronic inflammatory process [5, 6]. More recently, the identification of parasite DNA in the lesions has led to the hypothesis that parasite persistence is the main driving force leading to tissue inflammation and destruction [6, 7]. However, very recent observations have described heart tissue pathology in the absence of living parasites [8].

Treatment for Chagas disease is effective for acute cases and for children up to 14 years old, with cure rates as high as 100%, and is therefore recommended by the World Health Organization [9]. In adults, treatment success depends on the type of evaluation, using clinical and/or serologic indicators. Although definitive results of large randomized clinical trials (TRAENA and BENEFIT) are still forthcoming, based on previous nonrandomized studies, the Latin American Network for Chagas Disease proposes that treatment should be mandatory [10].

Similar to other neglected tropical diseases, prevention needs to be inexpensive, considering the poor economic situation of the regions where much of the disease is transmitted. The most-effective traditional methods for the prevention of Chagas disease are based on control of the triatomine vector, using insecticides and blood screening prior to transfusions. Southern Cone countries (Argentina, Brazil, Chile, and Uruguay) have developed

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Correspondence: Mauricio Martins Rodrigues, PhD, Centro de Terapia Celular e Molecular, UNIFESP–Escola Paulista de Medicina, Rua Mirassol, 207, São Paulo-SP 04044-010, Brazil (mrodrigues@unifesp.br).

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successful programs of vector control/elimination and blood screening before transfusion, leading to a significant reduction in transmission of the infection in these countries in the past 30–40 years [11].

Vaccine development as part of the strategy for disease control and eradication was once commonly not considered for Chagas disease [2], and the reasons for this are many. First, there are the successes of vector control and blood bank screening programs. Second, despite numerous efforts, no human vaccine against any parasitic disease has been successfully developed. These factors, combined with the shortage of resources for Chagas disease research, have kept vaccine development off priority lists.

Against this historical trend, recent studies have added a different perspective of the problem. These studies have indicated that vaccination can be cost-effective and economically feasible for a wide range of scenarios, even when the risk of infection is as low as 1% and vaccine efficacy is as low as 25% [12]. These studies support the concept that, despite all of the difficulties, the development of a vaccine against Chagas disease should be pursued. These studies have raised the interest of a nonprofit foundation devoted to the development of vaccines against neglected tropical diseases [13].

Because parasites have multiple developmental forms, which transiently express distinct antigens, immunity to them is very complex. Not only do parasites shift their antigens during their life cycles, they also strongly modulate the immune response in their favor. The complex balance between immune response and parasite escape mechanisms allows the survival of both parasite and host and the establishment of chronic infection [14]. The development of a successful vaccine will depend on how this equilibrium can be shifted in favor of the host, leading to elimination of the parasite.

More than 20 years ago, $CD8^+$ T cells were described as being critical for naturally acquired resistance against experimental infection with *T. cruzi* [15]. Accordingly, the epitopes recognized by $CD8^+$ T cells from mice and humans have been identified, and immunodominant epitopes have been shown to be present on proteins of the *trans*-sialidase family [16–19]. The immunodominant CD8 epitopes are certainly important targets for protective immunity. Nevertheless, the fact that *trans*-sialidases are a family of highly diverse polypeptides may complicate their use as part of broad subunit vaccines.

On the other hand, several proteins also have subdominant CD8 epitopes [20, 21]. These subdominant epitopes elicit weaker immune responses that can, nonetheless, promote host resistance [22, 23]. Most relevant for the purpose of vaccine development is the fact that subdominant epitopes may be more conserved across distinct parasite strains.

Corroborating the studies on the importance of $CD8^+$ T cells during naturally acquired resistance against experimental infection with *T. cruzi*, studies of vaccination using recombinant proteins, plasmid DNA, recombinant viruses, bacteria, and synthetic peptides have provided strong evidence that host protection can only be achieved in the presence of $CD8^+$ T cells. Protective immunity is simply not observed in gene-deficient animals without $CD8^+$ T cells or upon depletion of these cells by use of treatment with specific antibodies [24–31]. These findings reinforce the concept that the development of a successful vaccine against Chagas disease should, indeed, stimulate $CD8^+$ T cells.

$CD8^+$ T cells recognize short peptides bound to major histocompatibility complex (MHC) class I molecules. Because humans are highly polymorphic for MHC I alleles, a number of peptides will have to be discovered and assembled to generate a subunit vaccine to elicit broad immunity in a large, heterogeneous population. These multiple peptides must bind to a variety of MHC I alleles and be present in a vast number of parasite strains. To search and identify these short epitopes, a number of computer programs are being developed, and these bioinformatics tools are used for the identification of $CD8^+$ T cell epitopes within viruses and other pathogens [32]. Parasites, however, have large genomes, and some, as in the case of *T. cruzi,* have recently been sequenced [33]. In the current issue of *The Journal of Infectious Diseases*, Teh Poot et al use such bioinformatics tools to search for $CD8^+$ T-cell epitopes in the large genome of *T. cruzi* [34].

Using this approach, the authors identified a number of candidate $CD8^+$ T-cell epitopes within hypothetical proteins and proteins with putative functions. These epitopes as synthetic peptides were then tested for their ability to stimulate interferon γ production by specific T cells obtained from mice previously infected with *T. cruzi*. Because of their ability to restimulate T cells that were primed during infection, they were selected to be part of a subunit therapeutic vaccine against *T. cruzi* infection.

The authors' prediction was that these peptides would constitute important targets for the development of a $CD8^+$ T cell-based therapeutic vaccine against this parasitic disease. The prediction was confirmed, as therapeutic vaccination with a mixture of these peptides in the presence of the Toll-like receptor 4 agonist monophosphoryl lipid A led to a significant control of the infection in vaccinated mice. This disease control was estimated by significant reductions in parasitemia, parasite burden in the tissue of the infected mice, and cardiac tissue damage, as well as an increase in mice survival.

Similar strategies of reverse vaccinology have been proposed in the past for parasites with large genomes [32]. However, to date, the use of bioinformatics has never been as successful as in the study by Teh Poot et al. This success opens new avenues to a general strategy that may greatly facilitate the development of vaccines against parasites and provides a new tool for the prevention and treatment of neglected tropical diseases.

Notes

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Potential conflict of interest. M. M. R. is a named inventor on patent applications covering *T. cruzi*–vectored vaccines and immunization regimens. J. E. certifies no potential conflicts of interest.

Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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