The time has come for a vaccine against Chagas disease

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Summary

Despite many studies, there is still no vaccines for Chagas Disease (CD), which affects approximately 7 million people mainly in Latin America. To make matters worse, the only two drugs available have proved efficacy only when administered during the acute phase of the disease. Here, we discuss recent advances towards the development of a CD vaccine including (i) better understanding of the role of elements involved in immune responses and host defense against *Trypanosoma cruzi*; (ii) molecular characterization of parasite genetic diversity and the biochemical nature of *T. cruzi* antigens involved in protective immune responses; and (iii) the use of novel vaccine platforms that show high efficacy in experimental models. Other aspects such as the role of parasite-induced inflammation in the pathogenesis of CD is also under intense scrutiny. An essential issue discussed in this Viewpoint refers to the main use of a vaccine for CD, i.e., whether this vaccine should be used for prophylactic purposes, combined therapy to improve drug efficacy and parasitological cure, or to re-orient the immune and inflammatory response. Finally, we emphasize the necessity to attract the interest of both private and public pharmaceutical companies to translate all the pre-clinical studies into a much-needed CD vaccine.

Keywords: Chagas disease; Prophylactic vaccine; Therapeutic vaccine; Immune response

Challenges facing a Chagas disease vaccine: parasite genetics and biology

Chagas disease (CD), caused by *Trypanosoma cruzi* (*T. cruzi*), is considered a neglected disease that affects an estimated 6–7 million people mainly in Latin America countries. Due to increased human migration, CD has spread to other continents, and it is estimated that approximately 75 million people worldwide are at risk of infection.[1] In endemic countries, CD is primarily transmitted by the triatomine insects also known as "kissing bugs". Although less common, other forms of contamination also occur, including transmission through blood transfusion or organ transplantation, oral transmission via ingestion of food or beverages contaminated with *T. cruzi*, vertical transmission, and accidental exposure in laboratories where the parasite is cultured.[1]

Despite the success of vector control in many areas, non-vectorial transmission including organ transplantations, blood transfusions, congenital transmission and oral infection by contaminated food remain a significant threat.[1] During its life cycle, four main forms of the parasite alternate between its various hosts: epimastigotes and metacyclic trypomastigotes are present in the insect vector, whereas bloodstream trypomastigotes and intracellular amastigotes, are found in different mammals.[1]

During human infection, an acute symptomatic phase characterized by high parasitaemia, that lasts about 2 months, is followed by a life-long chronic asymptomatic infection. In the chronic phase, although parasitaemia is barely detectable, amastigotes that persist in the tissues lead to the digestive and/or cardiac forms of the disease in approximately 30% of infected individuals. Curable if treatment is initiated early, the two drugs used, benznidazole and nifurtimox, are less efficient in the chronic phase of the disease. In addition, the drugs have unwanted side effects that often result in early termination of treatment.[1] Current gaps in the treatment for CD have been discussed by Pinazo *et al.* (2024).[2]

A remarkable variability is observed among different *T. cruzi* strains and isolates, including differences in morphology, virulence, response to drugs and tissue tropism in animal models. This enormous genetic diversity has not only an impact on transmission cycles and the severity of the disease but also poses great challenges for the development of a CD vaccine. Molecular markers revealed the existence of six discrete groups, called 'discrete typing unit'' or DTU I to VI. Importantly, DTUs TcV and TcVI include hybrid strains, and some DTUs can be associated with geographical distribution and clinical manifestations.[3] In addition to the genetic variability of its population, each parasite genome contains multigene families encoding highly variable surface parasite antigens.[4] With estimated gene copy numbers from hundreds to

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few thousands, the three largest *T. cruzi* multigene families encoding trans-sialidases (TSs), mucin-associated surface proteins (MASPs), and mucins (TcMUCs) surface antigens, are all targets of the immune response. It can be assumed that sequence variability among surface parasite antigens represents an immune evasion mechanism that poses an extra layer of complexity for the design of vaccine candidates.[4] Nevertheless, using bioinformatic tools and sequencing data from an increasing large number of *T. cruzi* genomes, it is possible to find conserved regions among these genes that are also conserved among various *T. cruzi*.[4]

The immune responses in host resistance and disease caused by *T. cruzi* infection

In asymptomatic CD patients, the immune system seems to maintain an effective and balanced response, while a group of chronically infected patients develop an intense inflammatory process especially in the heart or the digestive tract. Previously attributed to the development of autoimmune antibodies and T cells generated by molecular mimicry or bystander activation,[5] the autoimmunity hypothesis was weakened by studies showing parasite persistence in the tissues. However, autoantibodies against cardiac myosin, tubulin, β-adrenergic receptors, and muscarinic receptors have been detected in patients with Chagas disease, potentially influencing heart pathology.[6,7] Thanks to highly sensitive methods such as polymerase chain reaction (PCR) and immunocytochemistry, a positive correlation between the presence of parasite in the tissues and inflammation was established.[6] These in-depth studies have elucidated a central role of parasite in eliciting myocarditis rather than an autoimmune process [for a review see reference[6]]. In addition, these studies helped defining key elements of host immune response mediating resistance and disease in CD including elements involved in the innate immune response [for a review see reference[8]].

Among the several Pathogen-Associated Molecular Patterns (PAMPs), described as critical for controlling *T. cruzi* infection, are glycosylphosphatidyl inositol (GPI) anchors from mucins, glycoinositolphospholipids (GIPLs) as well as parasite DNA and RNA. GPI anchors and GIPLs activate Toll-like receptors (TLRs) 2/6 and 4, respectively.[9] Parasite DNA, especially CpG-rich oligonucleotides, activate TLR 9, whereas parasite RNA is recognized by TLR 7.[10] Other innate immune receptors and pro-inflammatory mediators were shown to mediate both resistance and disease triggered by *T. cruzi*. These receptors trigger the secretion of inflammatory signals, e.g. IL-12 and TNF by dendritic cells and macrophages, leading to the production of IFN-γ by NK and T cells. These events culminate in the release of reactive nitrogen intermediates (RNI)[11,12] and activation of other effector mechanisms that control and/or destroy the parasite (for a review see reference[8]).

The inflammatory environment induces T cell differentiation into the Th1 and Th17 phenotypes, with the production of IFN-γ by CD4+ T lymphocytes that stimulates the cytolytic activity (CTL) of CD8+ T lymphocytes. These cells constitute key factors to host resistance, through secretion of IFN-γ and cytotoxic granules that kill intracellular parasites.[13,14] The Th1 response is also important for IgG1 and IgG3 specific for *T. cruzi* that facilitate phagocytosis by opsonization or direct parasite killing by complement-mediated lysis.[15] Although Th1 polarization is crucial for controlling parasitaemia and tissue parasitism, an unbalanced inflammatory response can cause a deleterious effect in the tissue, contributing to the immunopathology of CD.[16,17] Anti-inflammatory cytokines (e.g., IL-10 and TGF-β) or the inhibitory receptors may be essential to prevent the pathologic immune response; however, they may also contribute to parasite escape of the immune response.[18]

Vaccines based on live attenuated parasites

Only four years after the discovery of the disease, the first attempt to test a vaccine against *T. cruzi* using an attenuated strain was described.[19] Due to its capacity of inducing a broad immune response with the involvement of different PAMPs, CD4+ T and CD8+ T lymphocytes (T-cells) as well as antibodies, and because they persist in the host, vaccination with live attenuated parasites results in high protection efficacy and potentially life-long protection. Evidently, a prophylactic vaccine for CD based on live attenuated parasites raises significant concerns due to the potential for the parasites to revert to a virulent state, a risk that is particularly significant for immunocompromised individuals. Regardless, a field trial vaccination in dogs, carried out in 1993 in Argentina, with the attenuated TCC strain, showed significant reduction in natural *T. cruzi* infection.[20] In addition to strains that have lost virulence by culture in vitro, naturally occurring highly attenuated strains such as the CL-14, have also been tested as vaccine candidates.[21] Interestingly, ectopic expression of the cancer testis antigen (NY-ESO-1) in genetically modified CL-14 strain was shown to induce a robust and sustainable antigen-specific T-cell response and protection against melanoma, evidencing its potential use as a vaccine vector.[22]

The development of genetic manipulation protocols allowed the generation of attenuated parasites resulting from the disruption of different genes. These parasite mutants have impaired ability to infect mice but are able to protect against a virulent strain. Using CRISPR/Cas9, we were able to disrupt multiple copies of the TSs multigene family and generated a highly attenuated parasite that lacks TS activity. Known to be an essential

virulence factor, only a sub-group of the TS gene family encodes active enzymes, which are responsible for transferring sialic acid from host glycoconjugates to parasite mucins, shielding the parasite against host anti-*T. cruzi* antibodies. An unexpected finding of this study, parasites with no TS activity, remain infective, but are unable to egress the host cells. Importantly, the TS mutants were harmless even in highly immunocompromised IFN-γ KO mice. Different from other mutants derived from single gene knockout, immunization with TS knockout parasites resulted in undetectable parasitaemia or tissue parasitism and induced sterile immunity to a challenge with the highly virulent Y strain of *T. cruzi*.[23]

Recombinant protein and glycoproteins as vaccine candidates for Chagas disease

Members of the trans-sialidase (TS) family are one of the most frequently tested vaccine candidates for CD. In addition to being expressed at the surface of mammalian forms of the parasite, they contain immunodominant epitopes that are recognized by CD8+ T cells. Mice immunization with recombinant TS resulted in significant protection, including the induction of both mucosal and systemic immunity.[24] Notably, immunization protocols using a chimeric protein consisting of the Amastigote Surface Protein-2 (ASP-2), also a member of the TS family that is highly expressed in amastigotes, fused to a TS that is expressed on trypomastigotes, induced a robust protective immunity both in mice and dogs. Associated with Poly ICLC (a synthetic double stranded RNA), this chimeric recombinant protein, named TRASP, elicited protection mediated mainly by CD8+ T cells and IFN-γ.[25] Also, a chimeric antigen, Traspain, which contains the N-terminal domain of Cruzipain, the major cysteine protease, ASP-2 and TS, was effective in eliciting CD8+ T cell responses, leading to significant reduction in parasite load, chronic inflammation and heart damage in infected mice.[26]

Several other parasite antigens, including the cysteine protease Cruzipain, Tc52 protein, a protein with glutathione transferase activity, the Tc80 prolyl oligopeptidase and the Tc24 calcium-binding protein have been tested using different adjuvants and combinations of immunization/challenge protocols, see reference[27] for a comprehensive review. Importantly, treatment of mice chronically infected with *T. cruzi* using recombinant Tc24 induced a Th1 immune response that eliminates parasites in the blood, as demonstrated by qPCR, and reduces cardiac fibrosis.[28] As a significant advance towards human clinical trials, the safety and immunogenicity of the recombinant Tc24 and TSA-1 proteins formulated as an emulsion with a TLR4 agonist, was also demonstrated in rhesus macaques.[29]

Different from most mammals, humans, along with Old World primates, do not make the terminal carbohydrate linkage of the α-Gal trisaccharide (Qβ-α-Gal) and, therefore, express high levels of anti-α-Gal antibodies, prior to bacteria and protozoan infections. *T. cruzi* mucins expressed in trypomastigotes contain the immunodominant α-Gal glycotope that induces high levels of anti-α-Gal antibodies in CD patients. However, mice, customarily employed in *T. cruzi* infection studies, naturally express the α-Gal epitope and therefore do not produce anti-α-Gal antibodies. Using a C57BL/6 α-1,3-galactosyltransferase knockout mice, which does not synthesize the α-Gal epitope, it was shown that immunization with a virus-like particle vaccine displaying the immunogenic α-Gal induced high levels of protective anti-α-Gal antibodies. Among the beneficial effects, the authors showed decreased parasite load after a lethal *T. cruzi* challenge, lower tissue inflammation and myocyte necrosis.[30,31] In addition to providing a proof-of-concept demonstrating the efficacy of a prophylactic α-Gal-based glycovaccine, these studies also suggested that the association of recombinant proteins with the α-Gal epitope may be a valuable strategy to obtain a highly efficacious subunit vaccine.

New generation vaccines based on nucleic acids vectors

Since the first attempt, in 1998, of genetic immunization with a plasmid DNA containing sequences of a TS gene,[32] various studies have described heterologous and homologous prime-boost protocols using plasmid DNA and adenoviruses as vectors encoding members of the TS family. Immunization with homologous prime-boost protocol with replication-deficient human type 5 recombinant adenoviruses (rAd5), or with heterologous DNA-rAd5 expressing TS and ASP-2 elicits high levels of antigen-specific IgG antibodies and secretion of IFN-γ by T lymphocytes in mice. A robust reduction in parasitaemia and 100% survival in the highly susceptible BALB/c mice was also shown.[33,34] The protective immunity induced by these vaccinations is mediated essentially by effector memory CD8+ T cells, with an important role of cytotoxic granules.[14,34] Importantly, the immunization with rAd5 TS and rAd5 ASP-2 was capable to reduce heart injury and electrical abnormalities in mice chronically infected with the myotropic Colombian strain.[35]

Likewise, immunization with DNA formulations containing other antigens, such as TcG2 and TcG4, also induced high titters of antigen-specific IgG antibodies, Th1 response, and reduction in parasite burden in the heart and skeletal muscle.[36] Attenuated Salmonella carrying plasmids encoding cruzipain (Cz), Tc52, and Tc24 antigens was also evaluated as an alternative approach for oral administration, with significant protection demonstrated by reduced parasitaemia and

cardiomyopathy in immunized mice.[37] In addition to demonstrating the protection capacity of a plasmid DNA encoding the trypomastigote surface antigen TSA-1 and the flagellar calcium-binding Tc24 antigen in mice, the safety and efficacy of a therapeutic protocol that prevents cardiac alterations in *T. cruzi*-infected macaques was also described using the DNA vaccine formulation.[38]

Thus far, only two published studies have explored the mRNA platform for the development of a CD vaccine. Lessons learned from the COVID-19 mRNA vaccines showing that they induce a strong T cell immunity and long-term immunological memory, brought new hope for the development of a CD vaccine. Poveda *et al.*, 2023, tested homologous and heterologous immunization of mice with formulations containing recombinant Tc24 flagellar protein and the corresponding mRNA formulated within lipid nanoparticles.[39] These authors showed that heterologous immunization using mRNA/protein induced increased levels of cytokine-producing CD4+ and CD8+ T-cells, and a balanced Th1/Th2/Th17 secreted-cytokine profile. In a more recent study, mRNA encoding Tc24 and ASP-2 were tested as monovalent or bivalent therapeutic vaccines in a *T. cruzi* infected chronic mouse model of CD. Mice immunized with the bivalent vaccine containing both antigens exhibited a robust immune response, characterized by sustained production of inflammatory cytokines as well as a significant reduction of parasite burden.[40]

Questions to be answered if we plan to test a vaccine for Chagas disease

In 1986, Professor Zigman Brenner asked why we not have a CD vaccine[41] and some of the reasons he indicated included the limited characterization of relevant antigens, limited knowledge of mechanisms of protection and parasite immune evasion and the ethical dilemma involving clinical trials in areas of active transmission. 36 years later, returning to the same question, Camargo *et al.*, 2022[42] suggested that, although there was no lack of interest of the scientific community, the absolute requirement for sterile immunity for a prophylactic vaccine together with the autoimmune theory for CD may have added few more obstacles to the road in spite of the enormous advances in the understanding of different aspects of the infection. These issues have also been discussed in two recent reviews.[2,43]

The development of vaccines depends on preclinical studies in animal models to evaluate toxicity, immunogenicity and efficacy. Although they are essential for these initial evaluations, animal models for CD have limitations regarding the translation to clinical studies in terms of pathophysiology, immune response and genetics. Clearly, inbred mice do not represent the human genetic polymorphisms that influence in the immune response and susceptibility to CD. Dogs have been used as an appropriate experimental model for acute and chronic phases of Chagas' disease, since it can mimic the long-term effects of the disease seen in humans. Moreover, in the different preclinical studies of CD vaccines, which were mainly done in mice, the use of different parasite strains makes it even harder to compare their efficacy and to predict the results in humans.[44]

Important questions regarding the clinical design to test a vaccine for CD remain. Natural transmission has been eliminated in various countries of Latin America, and a low frequency of new cases is found in endemic areas. These areas are of difficult access for patient monitoring and would be unethical not keeping vector control measures in the homes of the population at risk, which would be recruited for testing a prophylactic vaccine. Considering the limitation posed by testing a prophylactic vaccine in humans, and the difficulties of designing a phase 3 clinical trial, a therapeutic vaccine, for which some of these logistic and ethical issues do not apply, may be less difficult to plan. Moreover, considering the low efficacy of the currently available drug treatment for chronically infected individuals, a therapeutic vaccine is recognized as being more of a priority than a prophylactic vaccine. Fig. 1 presents the different protocol possibilities. Considering a CD therapeutic vaccine, two scenarios can be anticipated: first, a vaccine that reverses cardiac disease, which, in the more advanced cases, may not be efficient, since the pathology and the functional alterations could be irreversible, due to the processes of fibrosis, denervation and myocardium cells damage. Secondly, a more plausible approach would be a vaccine immunization associated with chemotherapy, particularly for asymptomatic CD patients and those with early stages of a cardiac disease, to enhance parasitological cure and prevent development of cardiac disease. Several studies have shown that immunization of acutely infected or mice chronically infected with *T. cruzi* with different recombinant parasite antigens associated with a low dose of benznidazole reduces cardiac pathology[45] [for a review see[2]] and it is clear that any decrease in the administered dose of BZ would be interesting to mitigate the adverse effects caused by the drug. Our studies have also shown that the use of IL-12, as an immunostimulant, enhances the efficacy of BZ to cure mice infected with the natural BZ-resistant Colombian strain[46] and that this treatment was less efficacious in infected mice deficient in iNOs, CD4+ T, CD8+ T and IFN-γ.[47,48]

Regarding the design of a clinical trial, which should be the parasitological and clinical profile of CD patients recruited to the study? For safety reasons, we suggest that only asymptomatic patients and patients with initial stages of cardiac alteration should be enrolled. Should we avoid recruiting individuals with significant levels of circulating markers of inflammation and myocardium damage? This issue must be carefully considered as

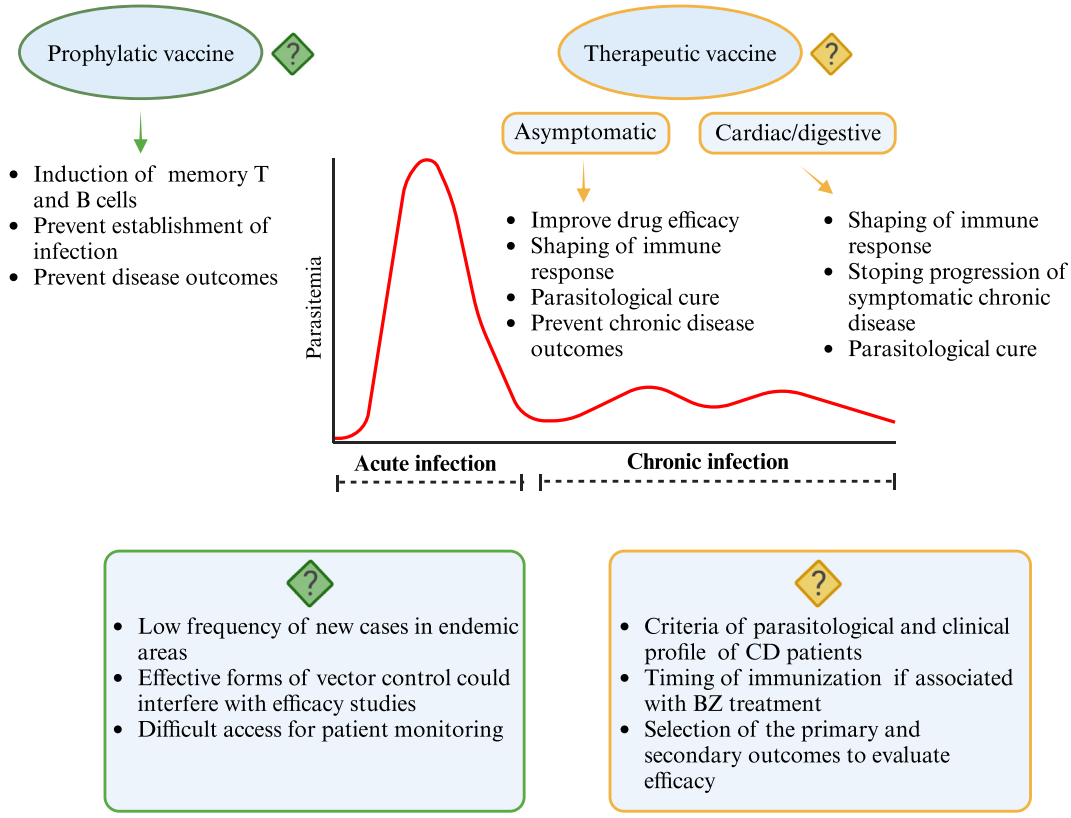

***Fig. 1:* Expected outcomes and challenges in different protocols for a CD vaccine.** The curve represents the course of the infection, with an acute phase and a chronic phase. The main vaccine protocols for CD, one for a prophylactic vaccine and two therapeutic vaccine protocols are represented. A prophylactic vaccine, represented on the left part of the figure (green section), is intended for administration prior to infection. The two proposed therapeutic vaccines protocols indicated on the right (yellow sections), are designed for patients with chronic infection. One protocol is aimed at asymptomatic patients, while the other targets individuals exhibiting symptoms of chronic disease. Arrows indicate potential outcomes of immunization, while the question marks underscore the challenges associated with each vaccination protocol. Created in BioRender. Castro, J. (2025) https://BioRender.com/y26u221.

immunization with *T. cruzi* could potentially amplify an inflammatory response. What about the timing of immunization doses regarding the BZ treatment? Should the vaccine be given prior, during or after drug treatment? Which are the main goals, lower parasitism before treatment, potentiate drug efficacy, or avoid parasite relapses after treatment with BZ, if parasite cure is not achieved? If immunization is initiated after one or two weeks of treatment, this would lower parasitism before boosting the immune response, which may avoid eliciting a strong inflammatory reaction at the sites where parasite nests remain. Last, but not least, what would be the primary and secondary outcomes to evaluate the efficacy of the therapeutic protocol? Parasite load by PCR and haemoculture and cardiac function evaluation seem to be the key primary outcomes. Should we also consider using immunological surrogates? It is known that anti-*T. cruzi* antibodies persist for many years post-treatment in a large percentage of CD patients. Therefore, which are the adequate immunological surrogates that might indicate parasitological cure, or re-oriented protective immune responses? Are antibody levels to specific parasite antigens, cytokine profile of parasite-specific T cell responses, and activation markers expressed by T cells adequate biomarkers? Also, for how long these immunological markers will be detectable in patients and what would be an acceptable duration of immune response to be considered long-lasting protection? Which kind of evidence should be considered when testing different vaccine formulations to ensure a long-term immunity? The only certainty is that much will be learned with these translational studies, which are irreplaceable if we want to move forward with the development of a CD vaccine.

Finally, it cannot be forgotten that Chagas disease remains a highly neglected disease that primarily affects poor population in less developed countries. Therefore, access and affordability are two important issues when planning to develop a CD vaccine. Among the different

vaccine platforms, the use of recombinant antigens may be the one that will result in the least expensive vaccine to produce and that can be delivery to remote areas away for major medical centres in large cities. Maybe for this reason, a vaccine for CD is not an attractive product for the pharmaceutical industry. Neither the industry, nor funding agencies or even the international agencies that define the priorities for translational research for neglected diseases, such as World Health Organization (WHO), Drugs for Neglected Diseases Initiative (DNDI), and public health governmental agencies, have listed a CD vaccine as a priority. This is essential, as the laboratories wishing to advance from pre-clinical tests to clinical trials of a therapeutic CD vaccine need to be supported by national and international funding agencies and make this initiative economically viable.

Contributors

SMT and RTG conceived the idea, searched for published work, were the mentors for the Figure and wrote most of the report. JTC prepared the Figure and GAB and JTC searched for published work, contributed to writing, review and editing of the report. All authors have seen and approved the submitted version.

Declaration of interests

We declare no competing interests.

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