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Immunity and vaccine development efforts against *Trypanosoma cruzi*

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Abstract

Trypanosoma cruzi (*T. cruzi*) is the causative agent for Chagas disease (CD). There is a critical lack of methods for prevention of infection or treatment of acute infection and chronic disease. Studies in experimental models have suggested that the protective immunity against *T. cruzi* infection requires the elicitation of Th1 cytokines, lytic antibodies and the concerted activities of macrophages, T helper cells, and cytotoxic T lymphocytes (CTLs). In this review, we summarize the research efforts in vaccine development to date and the challenges faced in achieving an efficient prophylactic or therapeutic vaccine against human CD.

Keywords

Chagas; Prophylactic; Therapeutic; Vaccine; Immunity; Adjuvants

1. Introduction

Chagas disease is a chronic degenerative illness inflicted by the protozoan *Trypanosoma cruzi*. This infectious agent is prevalent in South Central America and Mexico, and the

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disease is continually spreading due to worldwide human migration efforts. An estimated 6–7 million people, globally, are infected with *T. cruzi* and many more are exposed to risk of infection. Routes of primary infection consist of direct transmission by triatomine vector, consumption of food contaminated with feces of infected triatomines, transfusion of blood from infected donors, and vertical transmission from infected mothers to their newborns (WHO: World Health Organization, 2019; Coura, 2015). Other routes of transmission, e.g., transplantation of organs from infected individuals, sexual transmission, laboratory accidents, are also described (Coura, 2015). The oral route of transmission is noted to result in a higher mortality rate of 8–35% than is generally observed by vector transmission which is <5–10% (Antunes et al., 2019). *T. cruzi* infected mothers can vertically transmit the parasite to the fetus in 5–10% of pregnancies (Messenger and Bern, 2018). Benznidazole and nifurtimox are the currently available chemotherapeutic treatment options (Meymandi et al., 2018). These treatments were developed over 40 years ago and benznidazole is the only drug that is approved by US Food and Drug Administration to treat *T. cruzi* infection (CDC, 2019). These anti-parasitic drugs are most effective in treating the disease in children and acutely infected adults. However, it is common for patients to suffer from unspecific symptoms, or be asymptomatic, leading to lack of treatment. Patients will encounter a high parasitic load within blood circulation during acute stage and a low to intermittent parasite load during the chronic phase of the disease (D'Ávila et al., 2018). Studies in mice infected with 10^5 trypomastigotes of *T. cruzi* (Y strain) showed peak parasitemia developed by 5 days post infection (dpi) with a median range of 9.3×10^4 parasite equivalent/mL (par. eq/mL); and parasite levels fell below 10^{-1} par eq/mL at 100- and 260-dpi in infected mice (Mateus et al., 2019). Acutely infected patients from Colombia had median parasite loads of 6.68 par eq/mL (Hernández et al., 2018). Chronic patients from Southern Brazil, tested for parasite load via blood culture and analyzed using quantitative real-time PCR (qPCR), had a median parasite load of 1.18 par eq/mL of blood (D'Ávila et al., 2018). Another study of chronic patients from various locations of Brazil determined a median load of 2.54 par eq/mL (Rodrigues-dos-Santos et al., 2018). Chronic disease progression occurs due to *T. cruzi* amastigote infiltration into organs, specifically cardiac and digestive tissues. Approximately, 30–40% of chronically affected Chagas patients will experience cardiomyopathies, and to a lesser extent, gastroenterological and neurologic disorders. Chagas disease can ultimately cause death due to heart failure (Meymandi et al., 2018).

Although the immune system is required to control acute infection (discussed below), low-grade systemic infection that persists during intermediate-to-chronic phase supports consistent activation of pathologic immune reactions and contribute to the pathogenesis of Chagas heart disease (reviewed in (Marin-Neto et al., 2007; Gutierrez et al., 2009; Rassi and Marin, 2017)). Indeed, with the advent of modern techniques, e.g., PCR and qPCR, parasite DNA is detected in blood and heart tissue biopsies of chronically infected animals and human patients (D'Ávila et al., 2018; Gutierrez et al., 2009). Others have reported transmission of *T. cruzi* via blood transfusion and transplantation of infected organs obtained from asymptomatic individuals (Leiby et al., 2000; Schmunis and Cruz, 2005; CDC, 2007; Angheben et al., 2015). The reactivation of acute parasitemia in chronic individuals, following immunosuppression after heart transplantation (Campos et al., 2008; Gray et al., 2018), AIDS (Cordova et al., 2008; Lattes and Lasala, 2014) or drug therapy (D'Almeida et

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al., 1996; Ribas et al., 2016), illustrates that parasites persist for years after initial infection. It is, thus, accepted by the research community that parasite persistence provides consistent antigens that continue to trigger immune responses, cause pathologic tissue injury leading to cardiac inflammation and fibrosis, and, subsequently, cardiac insufficiency (Fonseca et al., 2018).

Our work suggests that Chagas myocardium sustains oxidative stress associated with mitochondrial dysfunction. A functional decline in the respiratory chain and increased generation of reactive oxygen species (ROS), coupled with an inability to efficiently scavenge mitochondrial free radicals, predisposed the heart to oxidative insult during infection and disease development (Garg, 2005; Zacks et al., 2005; Wen and Garg, 2008; Wen and Garg, 2010; Lopez et al., 2018). The sustained occurrence of oxidative adducts was shown in the myocardium of experimental models of Chagas disease (Wen et al., 2008; Wen et al., 2004; Dhiman et al., 2012; Paiva et al., 2018) and in peripheral blood of rodents and human Chagas subjects (Wen et al., 2006; de Oliveira et al., 2007; Dhiman et al., 2008; Dhiman et al., 2009) that was exacerbated by inefficient antioxidant capacity (Wen et al., 2004; Wen et al., 2006; Wen et al., 2006; Perez-Fuentes et al., 2003; Wen et al., 2017; Wan et al., 2016; Dhiman et al., 2013). Recent studies demonstrating ROS signaling of cytokine and chemokine production in infected cardiomyocytes and murine hearts (Gupta et al., 2009; Ba et al., 2010; Wen et al., 2010; Barroso et al., 2016) have provided a potential mechanistic link between ROS generation and chronic inflammation in Chagas cardiomyopathy.

Readers interested in further understanding the complexity of Chagas pathogenesis for all stages of infection and disease are referred to excellent recent reviews (Pinazo et al., 2015; Nunes et al., 2018).

2. Immunity to *Trypanosoma cruzi* infection

The innate immune system is the first to actively elicit a defensive role followed by a cascade of components from the adaptive immune response. Briefly, innate immunity is comprised of an anatomic barrier, physiologic boundary, phagocytic cells and inflammatory components. Skin and mucosal surfaces are anatomic barriers that prevent foreign materials initial entry, while the complement system provides a physiologic defense. Macrophages and neutrophils are the primary phagocytic cells which consume pathogenic organisms. Dendritic cells are also capable of phagocytosis and operate as antigen presenting cells during an adaptive immune response. Basophils and mast cells are responsible for initiating an inflammatory response due to damage or infection. Eosinophils have both inflammatory and phagocytic properties specific to the removal of parasites. Natural killer cells are important for the destruction of infected cells and provide the cytokine interferon-gamma (IFN-γ). The adaptive immune response utilizes two cell types: antigen specific T cells and B cells. Antigen presenting cells signal the B cells differentiation into plasma cells that generate antibodies. Dendritic cells, macrophages, and B cells are able to present antigens to $CD4^+$ and $CD8^+$ T cells. $CD8^+$ T cells directly destroy infected cells, while $CD4^+$ help amplify the immune response by releasing cytokines. Cytokines and chemokines secreted by innate and adaptive immune cells are responsible for cell recruitment, cell-to-cell

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communication, and facilitating pathogen clearance by various mechanisms (Marshall et al., 2018).

T. cruzi interacts with different arms of the host immune defense in a complex manner and is capable of establishing a persistent infection. Recent reviews have discussed how parasite evades classical, lectin and alternative complement pathways to establish infection (Acevedo et al., 2018; Ferreira et al., 2014; Lidani et al., 2017). Studies in a variety of experimental models showed that *T. cruzi* surface glycoproteins (mucins) and/or glycophospholipids (GIPLs) are recognized by innate immune cells and stimulate the production of multiple cytokines, e.g., IFN-γ, TNF-α, IL-1β, and IL-6; and chemokines, e.g., MCP-1, RANTES/CCL5, and IP-10 (interferon gamma-induced protein 10) in macrophages (Almeida et al., 2000; Aliberti et al., 2001; Almeida and Gazzinelli, 2001; Buscaglia et al., 2004; Fonseca et al., 2019). These cytokines/chemokines induce macrophage production of superoxide ($O_2^{\bullet -}$) and nitric oxide (NO) that are important free radicals for direct killing of *T. cruzi* (Martins et al., 1998). However, these free radicals are produced at low levels, and not sufficient to clear the infection (Koo and Garg, 2019). Others showed that IFN-γ promoted infection of astrocytes by *T. cruzi* Colombian strain (Silva et al., 2015).

In mice acutely infected with *T. cruzi* Y strain, IFN-γ of NK origin and IL-12 of macrophage origin supported the protective adaptive immunity constituted of parasite-specific type 1 T cells (Abrahamsohn and Coffman, 1996). Parasite-specific $CD4^+$ T cells assist in the control of *T. cruzi* through secretion of Th1 cytokines (IFN-γ, IL-2), amplification of phagocytic activity of macrophages, stimulation of B cell proliferation and antibody production, and differentiation and activation of $CD8^+$ T cells (Brener and Gazzinelli, 1997). A strong lytic antibody response by activated B cells enhances the opsonization, phagocytosis, and complement-dependent killing of the parasites (Lidani et al., 2017; Krautz et al., 2000). Yet, B cells also manage the inflammatory/antiinflammatory balance. Mice deficient in B cells responded to *T. cruzi* (Y strain) with an increased frequency of TNF-α producing $CD4^+$ T cells and a decline in anti-inflammatory factors, including IL-10^+, Fox3^+, and IL-17^+ $CD4^+$ T cells, which ultimately led to early death instigated by toxic levels of inflammation (Gorosito Serrán et al., 2017). Humans infected with *T. cruzi* display protection through antibodies that target the parasites α-Gal epitope containing the Galα1,3-Galβ1,4-GlcNAc. This epitope is present in a majority of the mucin glycoproteins expressed on the surface of *T. cruzi* trypomastigotes. Antibodies that target the α-Gal epitope are expressed in high concentrations during acute infection, detectable during chronic phase, and are capable of killing the parasite independently of the presence of complement or immune cells (dos Santos Virgilio et al., 2014). *T. cruzi* antigen-specific $CD8^+$ T cells are frequently present in infected mice and humans (Wizel et al., 1997; Wizel et al., 1998), and suggested to contribute to *T. cruzi* control through cytolysis of the infected cells and secretion of Th1 cytokines (IFN-γ) that induce trypanocidal activity (DosReis, 1997).

Some studies in chronically infected mice and patients suggest that immune exhaustion of $CD4^+$ and $CD8^+$ T cells contribute to reduced cytokine production and parasite persistence (Mateus et al., 2019; Pérez-Antón et al., 2018). Conversely, others used mice chronically infected with Brazil, TCC or Colombian strains of *T. cruzi* to show that $CD8^+$ T cells in

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infected organs were immunologically non-exhaustive; and concluded that $CD8^+$ T cell exhaustion was the unlikely culprit for long-term parasite persistence (Pack et al., 2018). Other studies indicate that too much of IFN-γ, IL-17, TNF-α cytokines correlates with tissue damage and clinical disease in chronically infected patients (Chevillard et al., 2018). Recent studies have also identified a role for interleukin 27 (IL-27) in Chagas disease. IL-27, composed of epstein-Barr virus-induced gene 3 (EBI3) and IL-27p28, is a member of the IL-12 cytokine family, expressed by antigen presenting cells, and it induces differentiation of diverse T cell populations and also upregulates IL-10. In human Chagas patients, IL-27 expression was significantly associated with cardiac protection. In mice chronically infected with *T. cruzi* Y strain, IL-27 regulated the proinflammatory IFN-γ and inhibited cardiac muscle inflammation (Medina et al., 2017).

To sum up, these studies indicate that an efficient protective response to *T. cruzi* infection requires elicitation of Th1 cytokines, lytic antibodies, and the concerted activities of phagocytes, T helper cells, and cytotoxic T lymphocytes; and an absence of either of these components can result in parasite persistence associated pathologic events contributing to cardiomyopathy and heart failure in the host.

3. Vaccine development against *T. cruzi* and Chagas disease

Given the detailed knowledge about the status of anti-*T. cruzi* protective immunity discussed above, a successful vaccine against the parasite is envisioned to elicit lytic antibody response, type 1 cytokines, and cytotoxic T lymphocytes (dos Santos Virgilio et al., 2014; Gupta et al., 2019). It is generally accepted by the research community that a vaccine should include antigens targeting all stages of the parasite and should be useful as a prophylactic and therapeutic vaccine (Dumonteil et al., 2019; Bustos et al., 2019; Quijano-Hernandez and Dumonteil, 2011). Computational modeling studies show that a vaccine against *T. cruzi* would be economically viable and provide net cost savings, even with stringent simulation parameters of 1% infection risk, treatment efficacy of 25%, and a cost of $20/dose (Lee et al., 2010; Lee et al., 2012). With an efficacy of >50% and infection risk of 20%, vaccine costing even >$200 would also be economically advantageous (Lee et al., 2010; Lee et al., 2013). A safe, effective, and reliable vaccine will also alleviate the costs for vector control and drugs used to dispel this infection in endemic countries (Ma et al., 2015).

3.1. Live and attenuated whole organism vaccines

The proponents of the live and attenuated vaccine believe that such a vaccine will provide a full spectrum of antigenic epitopes for stimulation of protective immunological responses. Early efforts utilized whole parasites killed by chemical, physical or irradiation methods or sub-cellular fractions (e.g., flagellar, soluble, or parasite membrane fractions) for their prophylactic vaccine potential in various animal models, including mice, guinea pigs, dogs, and monkeys. While chemically treated *T. cruzi* provided no protection from lethal challenge infection, other approaches generated a degree of resistance to *T. cruzi* and a large percentage of immunized animals survived from acute infection (reviewed in Bhatia et al., 2009; Vazquez-Chagoyan et al., 2011; Rodriguez-Morales et al., 2015). Live vaccines, constituted of *T. cruzi* strains attenuated by treatment with pharmacological agents or by

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serial passage in cultures have also been tested for their potency in experimental animals (reviewed in Sanchez-Valdez et al., 2015). With the advancement of CRISPR and other technologies to knockdown genes in trypanosomes, recent efforts have also focused on utilizing the genetically modified *T. cruzi* knocked down in one or more virulence genes as live vaccines. These live vaccines were largely effective in controlling challenge infection; vaccinated animals exhibited decreased parasitemia and increased survival rates compared to the unimmunized animals (Quijano-Hernandez and Dumonteil, 2011). Further efforts will determine if a live vaccine protective against one lineage of *T. cruzi* would be efficacious in providing protection against infection from the other five lineages of *T. cruzi*. Some researchers also remain concerned that an attenuated live vaccine may trigger a full-blown infection and disease in immunocompromised individuals (Sanchez-Valdez et al., 2015).

Phytomonas serpens, a tomato parasite that shares antigens with *T. cruzi* but is non-pathogenic to humans, has also been used as a potential prophylactic vaccine. Authors noted that mice immunized with *P. serpens* followed by challenge infection with lethal dose of *T. cruzi* Y strain (5×10^3 or 10^5 trypomastigotes) exhibited a significant decrease in parasitemia and mortality (Bregano et al., 2003). Basso and colleagues have promoted the use of *Trypanosoma rangeli,* which shares significant sequence homology with *T. cruzi* and infects triatomines and mammals but does not cause disease in humans, as a prophylactic vaccine (Basso and Marini, 2015). Indeed, mice immunized with glutaraldehyde-fixed *T. rangeli* epimastigotes were protected from challenge infection with a virulent isolate (Tulahuen) of *T. cruzi* (10^2 trypomastigote inoculum) (Introini et al., 1998). In our studies, we found that glutaraldehyde fixed *T. rangeli* itself did not offer protection from challenge infection in mice and dogs. Including fixed *T. rangeli* with a subunit DNA vaccine consisting TcG2 and TcG4 did not enhance the vaccine's efficacy in mice (Gupta et al., 2019). However, DNA vaccine (consisting TcG1, TcG2 and TcG4 subunits) adjuvanted with fixed *T. rangeli* provided significant protection against Sylvio X10/4 strain of *T. cruzi* that is highly pathogenic in dogs (Aparicio-Burgos et al., 2015).

Overall, we believe that the concerns in the use of *T. rangeli* or *T. cruzi* based attenuated vaccines appear to outweigh the benefits offered by whole organism vaccine in the context of Chagas disease.

3.2. Selection of T. cruzi antigens for subunit vaccines

Early studies utilized *T. cruzi* cDNA expression libraries and performed immune screening with sera from infected animals or human patients to detect the antigenic candidates (referenced below). This approach identified the candidates that were recognized by anti-parasite antibodies but may or may not consist of epitopes for T cell activation. Subsequently, Garg et al. (1997) utilized recombinant *T. cruzi* expressing a model antigen (chicken ovalbumin) in different cellular compartments to demonstrate that parasite GPI-anchored proteins (glycosylphosphatidylinositol), that are abundantly expressed in infective and intracellular stages of *T. cruzi* and released in host cell cytoplasm during parasite differentiation, are the most likely source of peptides for immune activation of B and T cells. Accordingly, several surface proteins of *T. cruzi* that belong to the abundantly expressed surface antigens of the large families, e.g., trans-sialidase (TS) super family (737 genes),

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mucins (662 genes), mucin-associated surface proteins (MASPs, 944 genes), and glycoprotein 63s (GP63s, 174 genes) (El-Sayed et al., 2005) have been selected as vaccine candidates. For example, among the members of the TS superfamily, trypomastigote surface antigen (TSA1, 85 kDa), TS, and amastigote surface proteins (ASP1, ASP2, ASP9 (ASP-2 like clone 9)) were shown to be recognized by antibody response and $CD8^+$ T lymphocytes in infected mice and humans (Wizel et al., 1997; Wizel et al., 1998; Low and Tarleton, 1997; Pereira-Chioccola et al., 1994; Ribeirao et al., 2000; Santos et al., 1997; Boscardin et al., 2003; Low et al., 1998). Mucins contain a large proportion (85%) of O-glycosidic-linked carbohydrates (Schenkman et al., 1993), and GPI component of GP35/50, SSP3 and other mucins was noted to be a powerful inducer of polyclonal B cells, inflammatory cytokines and macrophages (Bhatia et al., 2009; Acosta-Serrano et al., 2001; Cardoso et al., 2013; de Paulo Martins et al., 2010). Other antigens that do not belong to large families, but nevertheless were tested for their antigenic potential include complement regulatory protein (CRP or gp160) (Norris et al., 1994), a lysosomal cysteine proteinase (cruzipain, 60 kDa) (Fonseca et al., 2005), flagellar calcium binding protein (FCaBP or Tc24, 24 kDa) (Godsel and Engman, 1999, Krautz et al., 1998), GP90 (90 kDa) and GP82 (82 kDa) (Yoshida et al., 1993), kinetoplastid membrane protein (KMP-11, 11 kDa) (Trujillo et al., 1999; Maranon et al., 2001), LYT1 (61 kDa) (Fralish and Tarleton, 2003), paraflagellar rod proteins (70–86 kDa) (Wrightsman et al., 2002; Michailowsky et al., 2003), and TC52 (52 kDa) (Fernandez-Gomez et al., 1998). These antigens were shown to be recognized by antibodies and IFN-γ-producing $CD8^+$ T cells in experimental models of *T. cruzi* infection and/or Chagas subjects. These *T. cruzi* surface membrane proteins are discussed in recent reviews (Pech-Canul et al., 2017; De Pablos and Osuna, 2012).

We have employed a computational algorithm to screen the *T. cruzi* sequence database for desirable B and T epitopes (Bhatia et al., 2004; Bhatia and Garg, 2008), and then performed a biological screen to identify those that were recognized by IgGs and elicited type 1 $CD8^+$T cell response in infected mice, dogs, and humans (Bhatia et al., 2004; Bhatia and Garg, 2008; Gupta et al., 2013; Aparicio-Burgos et al., 2011; Bhatia et al., 2004; Garg and Bhatia, 2005). Of the 11 antigens thus selected, TcG1, TcG2, and TcG4 passed additional criteria for vaccine design. The three candidate antigens were (a) expressed (mRNA/protein) in mammalian stages of *T. cruzi* (Bhatia et al., 2004; Bhatia and Garg, 2008), (b) released in host cell cytoplasm during parasite differentiation (Bhatia et al., 2004; Bhatia and Garg, 2008), and (c) consisted of epitopes presented by MHC alleles of mice, dogs (Aparicio-Burgos et al., 2015; Aparicio-Burgos et al., 2011), and humans (Gupta et al., 2013). Notably, TcG2 and TcG4 were conserved in five of the six *T. cruzi* lineages (80–96% homology), providing confidence that TcG2/TcG4-based vaccine would provide protection against diverse *Tc* isolates in the US and Latin America (Gupta et al., 2019; Bhatia et al., 2004). Immune properties of some of the antigenic targets selected for vaccine efficacy studies are listed in Table 1.

3.3. Subunit vaccines

Several investigators have utilized a protein immunization approach to test the vaccine potential of *T. cruzi* antigens (Wizel et al., 1997; Low et al., 1998; Serna et al., 2014). Others favored the DNA immunization approach due to the ease of construction and production of

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the vectors, the stability of DNA and the ability to enhance the immune response by the co-delivery of genes encoding cytokines (Gupta et al., 2019; Bhatia and Garg, 2008; Aparicio-Burgos et al., 2011; Vasconcelos et al., 2003). Most importantly, antigen delivery by DNA vaccination has proved to be efficient in eliciting antibodies, Th1 cytokines and $CD8^+$ T cell immune responses to encoded antigens (Aparicio-Burgos et al., 2011; Gupta and Garg, 2015). Efforts were also made to enhance the protective efficacy of recombinant protein and DNA vaccines by use of alternative routes of antigen delivery, by increasing the amount or the number of doses, and heterologous prime-boost strategies (Gupta and Garg, 2015). In parallel with the efforts towards testing of vaccine candidates, adjuvants were tested to enhance or skew the immune responses toward desirable Th1 type. Accordingly, studies have examined the utility of IL-12, GM-CSF, CD40, HSP70, and CpG oligodeoxynucleotides (ODN-CpG), among others, in enhancing the Th1 responses to defined antigen vaccines (Corral and Petray, 2000). Several reviews have discussed the vaccine potential of antigenic candidates in experimental models (Vazquez-Chagoyan et al., 2011; Rodriguez-Morales et al., 2015; Arce-Fonseca et al., 2015). Herein, we discuss in brief the past literature and add the updates as relevant.

A majority of studies have tested the efficacy of genes/proteins as prophylactic vaccine in murine models (summarized in Table 2). A few studies that have tested the therapeutic immunization are discussed where relevant, and also mentioned in Table 2. Some investigators tested immunization with a single gene/protein, and others have utilized multi-component candidate vaccines. The ability of the individual or mixed genes to elicit protection from challenge infection depended upon the amount of the given recombinant protein or antigen-encoding plasmid sufficient to elicit protective B and T cell responses. For example, we have tested various dilutions of *ASP1, ASP2,* and *TSA1* (0.001–33 μg/mouse), and showed that the level of resistance to *T. cruzi* infection correlated with the amount of DNA delivered, the maximal protection being obtained with 1 μg (or higher) amount of each candidate with IL-12 and GM-CSF cytokine adjuvants (Garg and Tarleton, 1998). Yet, the level of protection from *T. cruzi* infection induced in mice immunized with these antigens (individually or in combination) (Fralish and Tarleton, 2003, Garg and Tarleton, 2002) was not sufficient to offer sterile immunity and no protection was observed in mice immunized with a pool of genes encoding mucin family members (Fralish and Tarleton, 2003). Researchers postulated that genes of large families express shared epitopes that when delivered together may not offer additive protective benefits in in-bred mice. Yet, it was predicted that the potential synergistic immunologic benefit of a combination of epitopes from many genes would stimulate a larger frequency of immune effectors in heterogeneous host populations and provide efficient immunity against diverse parasite strains (Vazquez-Chagoyan et al., 2011), to be verified in future studies.

Other investigators have tested the vaccine efficacy of non-family proteins in a variety of in-bred mouse models. For example, Cazorla et al. used a *Salmonella* carrying Cz-DNA (SCz) and adjuvants (CpG-ODN, GM-CSF, MALP-2) in mice. SCz oral doses induced a mucosal immune response (IgA) but weak systemic immune response, while including a boost with recombinant CZ protein and CpG-ODN produced a strong systemic immune response (i.e., antigen-specific IgG, splenic lymphocyte proliferation and IFN-γ production) that provided significant protection from challenge infection and tissue damage (Cazorla et al., 2008).

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Immunization of mice with PFR2 and PFR3 genes (encode for paraflagellar rod proteins) alone or fused to the 70 kilodalton heat shock protein (HSP70) induced high levels of anti-PFRs IgG2a; however, only the PFR2-HSP70 immunization induced a significant increase in the IL-12 and IFN-γ expression and a degree of protection against *T. cruzi* experimental infection (Egui et al., 2012; Morell et al., 2006).

Recently, prophylactic vaccine efficacy of α-Gal epitope, Galα13Galβ14GlcNAc bound to human serum albumin (HSA) was investigated. C57BL/6 mice immunized with this vaccine elicited anti-α-Gal antibody-mediated humoral reaction and were protected from lethal challenge infection with *T. cruzi* Y strain (1×10^5 parasite inoculum). Authors also observed a decline in parasite burden, T cell permeation in tissues, necrotic myocytes and cardiac inflammation in the heart of vaccinated (vs. non-vaccinated) mice (Portillo et al., 2019).

Screening of a cDNA expression library with anti-amastigote monoclonal antibodies identified FCaBP (Tc24), Tcb3 and LYT1 genes as potential antigens for vaccine design. The three gene peptides induced a response of cytotoxic T cells in chronically infected mice; however, the immunization with only *LYT1* gene protected 80% of mice from *T. cruzi* lethal challenge (Fralish and Tarleton, 2003), thus, suggesting that proteins that may be recognized by immune responses in an infected host may not always be the best candidates for vaccine development.

Other groups used recombinant adenovirus serotype 5 as vector and evaluated the protection raised by immunization of mice (C57BL/6 and C3H/HeJ) with recombinant Ad5 encoding amastigote surface protein 2 (ASP2). This immunization protocol elicited a robust expansion of $CD8^+$ effector T cells producing TNF-α, IFN-γ, and a higher mobilization of surface marker CD107a that is indicative of cytolytic activity and provided significant protection against challenge infection with *T. cruzi* Y strain (Barbosa et al., 2013). Afterwards, the therapeutic effects were evaluated in mice simultaneously infected with *T. cruzi* (Y strain) and immunized by AdASP-2. A significant control of tissue amastigote nests associated with a rapid immune response characterized by the expression of inflammatory genes, such as iNOS, TNF-α, TLR-4, and IL-10 was noted in mice therapeutically vaccinated with Ad-ASP2 (Ribeiro et al., 2019). Pereira et al. (2015) also used recombinant Ad5 encoding ASP2 and TS sequences and tested their prophylactic and therapeutic efficacy in C57BL/6 mice that were acutely or chronically infected with *T. cruzi* Colombian strain. These authors found there was an increase in $CD8^+$ T cells and production of IFN-γ, accompanied by a decrease in cardiac electrical alterations and cardiac tissue parasite burden in mice given prophylactic vaccine. Therapeutic treatment of chronic mice with Ad5-ASP2 / Ad5-TS enhanced survival, reduced electrocardiogram abnormalities, maintained IFN-γ concentrations, and decreased $CD107a^+$ $CD8^+$ T cells and plasma nitric oxide production (Pereira et al., 2015). Taken together, these results suggest promising application of ASP2-expressing recombinant virus as a prophylactic and therapeutic vaccine against acute and chronic infection.

In a recent study, Bivona et al. examined the 80 kDa prolyl oligopeptidase (Tc80) as a novel immunogen for Chagas vaccine. Authors immunized mice with recombinant Tc80 protein and recombinant *Salmonella* encoding Tc80 (STc80) in a prime-boost approach, and showed

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that immunized mice elicited Tc80-specific, complement-dependent trypanolytic antibodies, splenic production of Th1 cytokines, such as IFN-γ, IL-2 and TNF-α, as well as polyfunctional $CD4^+$ T cells and cytotoxic T lymphocytes associated with significant protection from challenge infection and chronic pathology (Bivona et al., 2018).

We have followed the protective efficacy of three antigens TcG1, TcG2, TcG4 in two experimental models (mice and dogs). Studies in mice were conducted with delivery of the three antigens (individually or in combination) by several prime / boost approaches (e.g., DNA/DNA, DNA/protein, or DNA/ Modified Vaccinia Ankara (MVA)). In all cases, we showed that vaccine provided 80–90% protection from acute parasite burden and chronic myocarditis in mice (Bhatia et al., 2004; Bhatia and Garg, 2008; Gupta and Garg, 2010). The overall extent of protection was associated with elicitation of trypanolytic antibodies, Th1 cytokine response, and $CD8^+$ T cells cytolytic activity. In further studies, we also found that TcG1 is dispensable, and TcG2/TcG4-based two component candidate vaccine was highly effective (Gupta and Garg, 2015). Importantly, these studies showed an increase in inflammatory responses in the vaccinated mice immediately after challenge infection; however, chronic inflammatory infiltrate was decreased in the vaccinated mice. Each of the delivery modes had several advantages and drawbacks. For example, DNA vaccine was the simplest in design, and delivery of DNA vaccine via intradermal/electroporation route (vs. intramuscular route) was most effective in generating protective immunity to challenge infection (Hegazy-Hassan et al., 2019). Further, Hegazy-Hassan et al found that the use of a single intradermal electroporation dose of 10 μg of TcG2 and TcG4 DNA vaccine without adjuvants, induced a protective immune response reducing the amount of DNA and eliminating the need for boost immunizations. DNA-protein vaccine was very complex-requiring two doses of six DNA-encoding plasmids and two doses of three recombinant proteins (Gupta and Garg, 2015). In contrast, MVA can accommodate multiple foreign genes in its genome, can be administered by a variety of routes, has an excellent safety record, and was shown to generate immune responses to a variety of foreign antigens (Meseda et al., 2002). MVA itself can act as an adjuvant to signal the innate immune system and boost T cells (Climent et al., 2011).

As in mice, dogs immunized with TcG1, TcG2, and TcG4-based vaccine also showed significant protection from challenge infection and acute myocarditis (Bhatia et al., 2004; Bhatia and Garg, 2008; Aparicio-Burgos et al., 2011). Others have shown the TcSP and TcSSP4 encoding DNA vaccine induced anti-parasite IgG2 and IFN-γ-producing lymphocytic cell proliferation in dogs. Upon challenge infection, *TcSP*- and *TcSSP4*-immuninzed dogs exhibited a moderate control of tissue parasites and electrocardiographic aberrations (Arce-Fonseca et al., 2013).

3.4. Therapeutic vaccines against Chagas disease

The overall basis for therapeutic vaccines is to modulate or enhance the multiple effector mechanisms against *T. cruzi* so as to clear the parasite's persistence in the infected host. Several candidate antigens (e.g. TSA-1, TS, Tc52, Tc24 and ASP-2) all belonging to trans-sialidase family, have been tested for therapeutic efficacy in acutely or chronically infected mice. When tested in mice immediately after infection or within two weeks post-infection,

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Tc52, TSA-1 and Tc24 DNA vaccine decreased the parasitemia and mortality from infection (Sanchez-Burgos et al., 2007; Dumonteil et al., 2004) that was associated with a vaccine-induced rapid increase in the number of $CD4^+$ and $CD8^+$ T cells (Zapata-Estrella et al., 2006). However these same candidates did not arrest cardiomyopathy in chronically infected mice or infected dogs (reviewed in Quijano-Hernandez et al., 2008). Others showed that therapeutic delivery of ASP2 and TS (individually or in combination as DNA vaccine) did not limit parasitemia or improve the survival rate in infected mice (Sanchez-Burgos et al., 2007; Dumonteil, 2007) despite the fact that these antigens have exhibited excellent efficacy as a prophylactic vaccine. Some studies also observed an increase in myocarditis in infected mice inoculated with TSA1 DNA vaccine (Zapata-Estrella et al., 2006).

In a recent study, Barry et al. (2016) have explored the potential for a nanoparticle, therapeutic vaccine. Authors encapsulated Tc24 protein in poly(lactic-co-glycolic acid) nanoparticle and used the nanoparticles to immunize mice that were previously infected with a highly lethal *T. cruzi* H1 strain (5×10^4 trypomastigotes inoculum). Mice immunized with Tc24 nanoparticles exhibited a Th1 immune response with an increase in splenic antigen-specific IFN-γ, IgG2A, and $CD8^+$ T cells. There was also a visible reduction in inflammatory cell infiltrate and parasite load in tissues (Barry et al., 2016). In another study, the same group utilized Tc24 recombinant protein along with E6020 adjuvant to immunize mice chronically infected with *T. cruzi* H1 strain (500 trypomastigotes inoculum) and showed that 60% of therapeutically vaccinated mice had untraceable parasitic levels, accompanied with a decrease in cardiac fibrosis (Barry et al., 2019).

In our experience, a therapeutic approach focused on the control of the parasite burden is not sufficient to arrest the progression of chronic disease, and immune therapy against parasite persistence should be adjuvanted with other agents to prevent cardiac damage. It is shown that repeat challenge infection of mice enhanced the immune responses but did not provide protection from parasite persistence and cardiac damage in chronically infected mice (Rosas-Jorquera et al., 2013). We noted that infected mice and rats, treated with chemotherapeutic drug (benznidazole) during the indeterminate phase-controlled parasite persistence; however, failed to avert cardiac remodeling and deterioration of ventricular contractility. Instead, maximal benefits were obtained when infected mice and rats were treated with benznidazole, in conjunction with PBN (phenyl-alpha-tert-butyl nitrone) antioxidant to prevent free radical-mediated oxidative insult and mitochondrial deficiencies. This resulted in the preservation of oxidative phosphorylation and left ventricular contractile activity in Chagas hearts (Wen et al., 2010). Likewise, we noted a better efficacy of a TG2/TcG4-based therapeutic vaccine in infected mice that overexpressed glutathione peroxidase and were capable of controlling the oxidative damage caused by mitochondrial deficiencies of electron transport chain of antioxidant response (Gupta et al., 2015). In comparison, wild type infected mice continued to mount cardiac damage after TG2/TcG4 therapeutic vaccine (Gupta et al., 2015). We, therefore, propose that therapeutic vaccines designed to achieve a rapid, short-lived stimulation of type 1 cellular immunity to attack persistent parasites, but prevent cellular injury, along with adjunct therapies capable of controlling the onset of oxidative insult and mitochondrial deficiencies, would prove to be maximally beneficial in preserving cardiac structure and function in Chagas disease.

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4. Summary and future directions

An immune therapy that will control the *T. cruzi* transmission and chronic Chagas cardiomyopathy is urgently needed. In last few decades, significant research efforts have led to the development of several experimental vaccines that have shown promising results in small animal models of *T. cruzi* infection and Chagas disease. We call for significant level of academic-public-private partnership to support future scientists who will test the current and new vaccines against *T. cruzi* in large animal models and seek further development of the best possible immune therapy to prevent human Chagas cardiomyopathy.

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References

Abrahamsohn IA, Coffman RL, 1996 *Trypanosoma cruzi*: IL-10, TNF-alpha, IFN-gamma, and IL-12 regulate innate and acquired immunity to infection. Exp. Parasitol. 84, 231–244. [PubMed: 8932773]

Acevedo GR, Girard MC, Gómez KA, 2018 The unsolved jigsaw puzzle of the immune response in Chagas disease. Front. Immunol. 9, 1929. [PubMed: 30197647]

Acosta-Serrano A, Almeida IC, Freitas-Junior LH, Yoshida N, Schenkman S, 2001 The mucin-like glycoprotein super-family of *Trypanosoma cruzi*: structure and biological roles. Mol. Biochem. Parasitol. 114, 143–150. [PubMed: 11378194]

Aliberti JC, Souto JT, Marino AP, Lannes-Vieira J, Teixeira MM, Farber J, Gazzinelli RT, Silva JS, 2001 Modulation of chemokine production and inflammatory responses in interferon-gamma- and tumor necrosis factor-R1-deficient mice during *Trypanosoma cruzi* infection. Am. J. Pathol. 158, 1433–1440. [PubMed: 11290561]

Almeida IC, Camargo MM, Procópio DO, Silva LS, Mehlert A, Travassos LR, Gazzinelli RT, Ferguson MA, 2000 Highly purified glycosylphosphatidylinositols from *Trypanosoma cruzi* are potent proinflammatory agents. EMBO J. 1476–1485. [PubMed: 10747016]

Almeida IC, Gazzinelli RT, 2001 Proinflammatory activity of glycosylphosphatidylinositol anchors derived from *Trypanosoma cruzi*: structural and functional analyses. J. Leukoc. Biol. 70, 467–477. [PubMed: 11590183]

Angheben A, Boix L, Buonfrate D, Gobbi F, Bisoffi Z, Pupella S, Gandini G, Aprili G, 2015 Chagas disease and transfusion medicine: a perspective from non-endemic countries. Blood Transfus. 13, 540–550. [PubMed: 26513769]

Antunes D, Marins-Dos-Santos A, Ramos MT, Mascarenhas BAS, Moreira C.J.d.C., Farias-de-Oliveira DA, Savino W, Monteiro RQ, de Meis J, 2019 Oral route driven acute *Trypanosoma cruzi* infection unravels an IL-6 dependent hemostatic derangement. Front. Immunol. 10, 1073. [PubMed: 31139194]

Aparicio-Burgos JE, Ochoa-Garcia L, Zepeda-Escobar JA, Gupta S, Dhiman M, Martinez JS, de Oca-Jimenez RM, Val Arreola M, Barbabosa-Pliego A, Vazquez-Chagoyan JC, Garg NJ, 2011 Testing the efficacy of a multi-component DNA-prime/DNA-boost vaccine against *Trypanosoma cruzi* infection in dogs. PLoS Negl. Trop. Dis. 5, e1050.

Aparicio-Burgos JE, Zepeda-Escobar JA, de Oca-Jimenez RM, Estrada-Franco JG, Barbabosa-Pliego A, Ochoa-Garcia L, Alejandre-Aguilar R, Rivas N, Penuelas-Rivas G, Val-Arreola M, Gupta S, Salazar-Garcia F, Garg NJ, Vazquez-Chagoyan JC, 2015 Immune protection against *Trypanosoma cruzi* induced by TcVac4 in a canine model. PLoS Negl. Trop. Dis. 9, e0003625.

Arce-Fonseca M, Ballinas-Verdugo MA, Zenteno ER, Suarez-Flores D, Carrillo-Sanchez SC, Alejandre-Aguilar R, Rosales-Encina JL, Reyes PA, Rodriguez-Morales O, 2013 Specific humoral

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

and cellular immunity induced by *Trypanosoma cruzi* DNA immunization in a canine model. Vet. Res. 44, 15. [PubMed: 23497041]

Arce-Fonseca M, Rios-Castro M, Carrillo-Sanchez Sdel C, Martinez-Cruz M, Rodriguez-Morales O, 2015 Prophylactic and therapeutic DNA vaccines against Chagas disease. Parasit. Vect. 8, 121.

Ba X, Gupta S, Davidson M, Garg NJ, 2010 *Trypanosoma cruzi* induces the reactive oxygen species-PARP-1-RelA pathway for up-regulation of cytokine expression in cardiomyocytes. J. Biol. Chem. 285, 11596–11606. [PubMed: 20145242]

Barbosa RP, Filho BG, Dos Santos LI, Junior PA, Marques PE, Pereira RV, Cara DC, Bruna-Romero O, Rodrigues MM, Gazzinelli RT, Machado AV, 2013 Vaccination using recombinants influenza and adenoviruses encoding amastigote surface protein-2 are highly effective on protection against *Trypanosoma cruzi* infection. PLoS One 8, e61795.

Barroso A, Gualdrón-López M, Esper L, Brant F, Araújo RRS, Carneiro MBH, Ávila TV, Souza DG, Vieira LQ, Rachid MA, Tanowitz HB, Teixeira MM, Machado FS, 2016 The aryl hydrocarbon receptor modulates production of cytokines and reactive oxygen species and development of myocarditis during *Trypanosoma cruzi* infection. Infect. Immun. 84, 3071–3082. [PubMed: 27481250]

Barry MA, Versteeg L, Wang Q, Pollet J, Zhan B, Gusovsky F, Bottazzi ME, Hotez PJ, Jones KM, 2019 A therapeutic vaccine prototype induces protective immunity and reduces cardiac fibrosis in a mouse model of chronic *Trypanosoma cruzi* infection. PLOS Negl. Trop. Dis. 13, e0007413.

Barry MA, Wang Q, Jones KM, Heffernan MJ, Buhaya MH, Beaumier CM, Keegan BP, Zhan B, Dumonteil E, Bottazzi ME, Hotez PJ, 2016 A therapeutic nanoparticle vaccine against *Trypanosoma cruzi* in a BALB/c mouse model of Chagas disease. Hum. Vaccin. Immunother. 12, 976–987. [PubMed: 26890466]

Basso B, Marini V, 2015 Experimental Chagas disease in Balb/c mice previously vaccinated with *T. rangeli*. II. The innate immune response shows immunological memory: reality or fiction? Immunobiology 220, 428–436. [PubMed: 25454810]

Bhatia V, Garg NJ, 2008 Previously unrecognized vaccine candidates control *Trypanosoma cruzi* infection and immunopathology in mice. Clin. Vaccine Immunol. 15, 1158–1164. [PubMed: 18550728]

Bhatia V, Sinha M, Luxon B, Garg N, 2004 Utility of the *Trypanosoma cruzi* sequence database for identification of potential vaccine candidates by in silico and in vitro screening. Infect. Immun. 72, 6245–6254. [PubMed: 15501750]

Bhatia V, Wen J, Zacks M, Garg N, 2009 American trypanosomiasis and perspectives on vaccine development In: Stanberry LR, Barrett AD (Eds.), Vaccines for Biodefense and Emerging and Neglected Diseases. Academic Press, New York, pp. 1407–1434.

Bivona AE, Sánchez Alberti A, Matos MN, Cerny N, Cardoso AC, Morales C, González G, Cazorla SI, Malchiodi EL, 2018 Trypanosoma cruzi 80 kDa prolyl oligopeptidase (Tc80) as a novel immunogen for Chagas disease vaccine. PLOS Negl. Trop. Dis. 12, e0006384.

Boscardin SB, Kinoshita SS, Fujimura AE, Rodrigues MM, 2003 Immunization with cDNA expressed by amastigotes of *Trypanosoma cruzi* elicits protective immune response against experimental infection. Infect. Immun. 2744–2757. [PubMed: 12704149]

Bregano JW, Picao RC, Graca VK, Menolli RA, Itow Jankevicius S, Pinge Filho P, Jankevicius JV, 2003 *Phytomonas serpens*, a tomato parasite, shares antigens with *Trypanosoma cruzi* that are recognized by human sera and induce protective immunity in mice. FEMS Immunol. Med. Microbiol. 39, 257–264. [PubMed: 14642311]

Brener Z, Gazzinelli RT, 1997 Immunological control of *Trypanosoma cruzi* infection and pathogenesis of Chagas' disease. Int. Arch. Allergy Immunol. 114, 103–110. [PubMed: 9338602]

Buscaglia CA, Campo VA, Di Noia JM, Torrecilhas AC, De Marchi CR, Ferguson MA, Frasch AC, Almeida IC, 2004 The surface coat of the mammal-dwelling infective trypomastigote stage of *Trypanosoma cruzi* is formed by highly diverse immunogenic mucins. J. Biol. Chem. 279, 15860–15869. [PubMed: 14749325]

Bustos PL, Milduberger N, Volta BJ, Perrone AE, Laucella SA, Bua J, 2019 *Trypanosoma cruzi* Infection at the maternal-fetal interface: Implications of parasite load in the congenital

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transmission and challenges in the diagnosis of infected newborns. Front. Microbiol. 10, 1250. [PubMed: 31231337]

Campos SV, Strabelli TM, Amato Neto V, Silva CP, Bacal F, Bocchi EA, Stolf NA, 2008 Risk factors for Chagas' disease reactivation after heart transplantation. J. Heart Lung Transplant. 27, 597–602. [PubMed: 18503957]

Cardoso MS, Junqueira C, Trigueiro RC, Shams-Eldin H, Macedo CS, Araújo PR, Gomes DA, Martinelli PM, Kimmel J, Stahl P, Niehus S, Schwarz RT, Previato JO, Mendonça-Previato L, Gazzinelli RT, Teixeira SMR, 2013 Identification and functional analysis of *Trypanosoma cruzi* genes that encode proteins of the glycosylphosphatidylinositol biosynthetic pathway. PLoS Negl. Trop Dis. 7, e2369.

Cazorla SI, Becker PD, Frank FM, Ebensen T, Sartori MJ, Corral RS, Malchiodi EL, Guzmán CA, 2008 Oral vaccination with *Salmonella enterica* as a cruzipain-DNA delivery system confers protective immunity against *Trypanosoma cruzi*. Infect. Immun. 76, 324–333. [PubMed: 17967857]

CDC, 2007 Blood donor screening for Chagas disease–United States, 2006–2007. MMWR Morb. Mortal. Wkly. Rep. 56, 141–143. [PubMed: 17318113]

CDC, 2019 Centers for Disease Control and Prevention, Antiparasitic Treatment. https://www.cdc.gov/parasites/chagas/health_professionals/tx.html.

Chevillard C, Nunes JPS, Frade AF, Almeida RR, Pandey RP, Nascimento MS, Kalil J, Cunha-Neto E, 2018 Disease tolerance and pathogen resistance genes may underlie *Trypanosoma cruzi* persistence and differential progression to chagas disease cardiomyopathy. Front. Immunol. 9, 2791. [PubMed: 30559742]

Climent N, Guerra S, Garcia F, Rovira C, Miralles L, Gomez CE, Pique N, Gil C, Gatell JM, Esteban M, Gallart T, 2011 Dendritic cells exposed to MVA-based HIV-1 vaccine induce highly functional HIV-1-specific CD8(+) T cell responses in HIV-1-infected individuals. PLoS One 6, e19644.

Cordova E, Boschi A, Ambrosioni J, Cudos C, Corti M, 2008 Reactivation of Chagas disease with central nervous system involvement in HIV-infected patients in Argentina, 1992–2007. Int. J. Infect. Dis. 12, 587–592. [PubMed: 18337139]

Corral RS, Petray PB, 2000 CpG DNA as a Th1-promoting adjuvant in immunization against *Trypanosoma cruzi*. Vaccine 19, 234–242. [PubMed: 10930678]

Coura JR, 2015 The main sceneries of Chagas disease transmission. The vectors, blood and oral transmissions - A comprehensive review. Mem. Inst. Oswaldo Cruz. 110, 277–282. [PubMed: 25466622]

Cuevas IC, Cazzulo JJ, Sanchez DO, 2003 gp63 homologues in *Trypanosoma cruzi*: surface antigens with metalloprotease activity and a possible role in host cell infection. Infect. Immun. 71, 5739–5749. [PubMed: 14500495]

D'Almeida P, Keitel E, Bittar A, Goldani J, Santos A, Neumann J, Garcia V, 1996 Long-term evaluation of kidney donors. Transplant. Proc. 28, 93–94. [PubMed: 8644353]

D'Ávila DA, Galvão LMC, Sousa GR, Britto C, Moreira OC, Chiari E, 2018 Monitoring the parasite load in chronic Chagas disease patients: comparison between blood culture and quantitative real time PCR. PLoS One 13, e0208133.

de Oliveira TB, Pedrosa RC, Filho DW, 2007 Oxidative stress in chronic cardiopathy associated with Chagas disease. Int. J. Cardiol. 116, 357–363. [PubMed: 16859784]

De Pablos LM, Osuna A, 2012 Multigene Families in *Trypanosoma cruzi* and Their Role in Infectivity. Infect. Immunity 80, 2258–2264. [PubMed: 22431647]

de Paulo Martins V, Galizzi M, Salto ML, Docampo R, Moreno SNJ, 2010 Developmental expression of a *Trypanosoma cruzi* phosphoinositide-specific phospholipase C in amastigotes and stimulation of host phosphoinositide hydrolysis. Infect. Immun. 78, 4206–4212. [PubMed: 20643853]

Dhiman M, Estrada-Franco JG, Pando JM, Ramirez-Aguilar FJ, Spratt H, Vazquez-Corzo S, Perez-Molina G, Gallegos-Sandoval R, Moreno R, Garg NJ, 2009 Increased myeloperoxidase activity and protein nitration are indicators of inflammation in patients with Chagas' disease. Clin. Vaccine Immunol. 16, 660–666. [PubMed: 19297613]

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Dhiman M, Nakayasu ES, Madaiah YH, Reynolds BK, Wen JJ, Almeida IC, Garg NJ, 2008 Enhanced nitrosative stress during *Trypanosoma cruzi* infection causes nitrotyrosine modification of host proteins: implications in Chagas' disease. Am. J. Pathol. 173, 728–740. [PubMed: 18688021]

Dhiman M, Wan X-X, PL V, Vargas G, Garg NJ, 2013 MnSODtg mice control myocardial inflammatory and oxidative stress and remodeling responses elicited in chronic Chagas disease. J. Am. Heart Assoc. 2, e000302.

Dhiman M, Zago MP, Nunez S, Nunez-Burgio F, Garg NJ, 2012 Cardiac oxidized antigens are targets of immune recognition by antibodies and potential molecular determinants in Chagas disease pathogenesis. PLoS One 7, e28449.

dos Santos Virgilio F, Pontes C, Dominguez MR, Ersching J, Rodrigues MM, Vasconcelos JR, 2014 CD8+ T Cell-mediated immunity during Trypanosoma cruzi infection: A path for vaccine development? Mediators Inflamm. 2014, 243786.

DosReis GA, 1997 Cell-mediated immunity in experimental *Trypanosoma cruzi* infection. Parasitol. Today 13, 335–342. [PubMed: 15275046]

Dumonteil E, 2007 DNA vaccines against protozoan parasites: advances and challenges. J. Biomed. Biotechnol. 2007, 90520. [PubMed: 17710244]

Dumonteil E, Escobedo-Ortegon J, Reyes-Rodriguez N, Arjona-Torres A, Ramirez-Sierra MJ, 2004 Immunotherapy of *Trypanosoma cruzi* infection with DNA vaccines in mice. Infect. Immun. 72, 46–53. [PubMed: 14688079]

Dumonteil E, Herrera C, Buekens P, 2019 A therapeutic preconceptional vaccine against Chagas disease: A novel indication that could reduce congenital transmission and accelerate vaccine development. PLoS Negl. Trop. Dis. 13, e0006985.

Durante IM, La Spina PE, Carmona SJ, Agüero F, Buscaglia CA, 2017 High-resolution profiling of linear B-cell epitopes from mucin-associated surface proteins (MASPs) of Trypanosoma cruzi during human infections. PLoS Negl. Trop. Dis. 11, e0005986.

Egui A, Thomas MC, Morell M, Maranon C, Carrilero B, Segovia M, Puerta CJ, Pinazo MJ, Rosas F, Gascon J, Lopez MC, 2012 Trypanosoma cruzi paraflagellar rod proteins 2 and 3 contain immunodominant CD8(+) T-cell epitopes that are recognized by cytotoxic T cells from Chagas disease patients. Mol. Immunol. 52, 289–298. [PubMed: 22750229]

El-Sayed NM, Myler PJ, Bartholomeu DC, Nilsson D, Aggarwal G, Tran AN, Ghedin E, Worthey EA, Delcher AL, Blandin G, Westenberger SJ, Caler E, Cerqueira GC, Branche C, Haas B, Anupama A, Arner E, Aslund L, Attipoe P, Bontempi E, Bringaud F, Burton P, Cadag E, Campbell DA, Carrington M, Crabtree J, Darban H, da Silveira JF, de Jong P, Edwards K, Englund PT, Fazelina G, Feldblyum T, Ferella M, Frasch AC, Gull K, Horn D, Hou L, Huang Y, Kindlund E, Klingbeil M, Kluge S, Koo H, Lacerda D, Levin MJ, Lorenzi H, Louie T, Machado CR, McCulloch R, McKenna A, Mizuno Y, Mottram JC, Nelson S, Ochaya S, Osoegawa K, Pai G, Parsons M, Pentony M, Pettersson U, Pop M, Ramirez JL, Rinta J, Robertson L, Salzberg SL, Sanchez DO, Seyler A, Sharma R, Shetty J, Simpson AJ, Sisk E, Tammi MT, Tarleton R, Teixeira S, Van Aken S, Vogt C, Ward PN, Wickstead B, Wortman J, White O, Fraser CM, Stuart KD, Andersson B, 2005 The genome sequence of *Trypanosoma cruzi*, etiologic agent of Chagas disease. Science 309, 409–415. [PubMed: 16020725]

Fernandez-Gomez R, Esteban S, Gomez-Corvera R, Zoulika K, Ouaissi A, 1998 *Trypanosoma cruzi*: Tc52 released protein-induced increased expression of nitric oxide synthase and nitric oxide production by macrophages. J. Immunol. 160, 3471–3479. [PubMed: 9531308]

Ferreira V, Molina C, Tapia V, Nieto V, Galdames P, Cortes C, Ramirez-Toloza G, 2014 Factor H binds to *Trypanosoma cruzi* trypomastigotes via its C-terminus and contributes to parasite resistance against the alternative pathway of complement (MPF2P.809). J. Immunol. 192 (67), 67–68.

Flechas ID, Cuellar A, Cucunubá ZM, Rosas F, Velasco V, Steindel M, Thomas Mdel C, López MC, González JM, Puerta CJ, 2009 Characterising the KMP-11 and HSP-70 recombinant antigens' humoral immune response profile in chagasic patients. BMC Infect. Dis. 9, 186. [PubMed: 19939275]

Fonseca L.M.d., da Costa KM, Chaves V.d.S., Freire-de-Lima CG, Morrot A, Mendonça-Previato L, Previato JO, Freire-de-Lima L, 2019 Theft and reception of host cell's sialic acid: dynamics of Trypanosoma cruzi trans-sialidases and mucin-like molecules on Chagas' Disease immunomodulation. Front. Immunol. 10, 164. [PubMed: 30787935]

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Fonseca R, Salgado RM, Borges da Silva H, Nascimento R.S.d., D'Império-Lima MR, Alvarez JM, 2018 Programmed cell death protein 1–PDL1 interaction prevents heart damage in chronic Trypanosoma cruzi infection. Front. Immunol. 9, 997. [PubMed: 29867974]

Fonseca SG, Moins-Teisserenc H, Clave E, Ianni B, Nunes VL, Mady C, Iwai LK, Sette A, Sidney J, Marin ML, Goldberg AC, Guilherme L, Charron D, Toubert A, Kalil J, Cunha-Neto E, 2005 Identification of multiple HLA-A*0201-restricted cruzipain and FL-160 CD8+ epitopes recognized by T cells from chronically *Trypanosoma cruzi*-infected patients. Microbes Infect. 7, 688–697. [PubMed: 15848276]

Fralish BH, Tarleton RL, 2003 Genetic immunization with LYT1 or a pool of transsialidase genes protects mice from lethal *Trypanosoma cruzi* infection. Vaccine 21, 3070–3080. [PubMed: 12798651]

Frevert U, Schenkman S, Nussenzweig V, 1992 Stage-specific expression and intracellular shedding of the cell surface trans-sialidase of *Trypanosoma cruzi*. Infect. Immun. 60, 2349–2360. [PubMed: 1375197]

Garg N, 2005 Mitochondrial disorders in chagasic cardiomyopathy. Front. Biosci. 10, 1341–1354. [PubMed: 15769630]

Garg N, Bhatia V, 2005 Current status and future prospects for a vaccine against American trypanosomiasis. Expert Rev. Vaccines 4, 867–880. [PubMed: 16372882]

Garg N, Nunes MP, Tarleton RL, 1997 Delivery by *Trypanosoma cruzi* of proteins into the MHC class I antigen processing and presentation pathway. J. Immunol. 158, 3293–3302. [PubMed: 9120286]

Garg N, Tarleton R, 1998 Elicitation of protective cellular and humoral immune responses to Trypanosoma cruzi infection using DNA vaccines can be augmented with cytokines. In: Proceedings 10th International Congress of Immunology New Delhi, India pp. 1421–1426.

Garg N, Tarleton RL, 2002 Genetic immunization elicits antigen-specific protective immune responses and decreases disease severity in *Trypanosoma cruzi* infection. Infect. Immun. 70, 5547–5555. [PubMed: 12228281]

Godsel LM, Engman DM, 1999 Flagellar protein localization mediated by a calcium-myristoyl/palmitoyl switch mechanism. EMBO J. 18, 2057–2065. [PubMed: 10205160]

Gorosito Serrán M, Tosello Boari J, Fiocca Vernengo F, Beccaría CG, Ramello MC, Bermejo DA, Cook AG, Vinuesa CG, Montes CL, Acosta Rodriguez EV, Gruppi A, 2017 Unconventional pro-inflammatory CD4+ T cell response in B cell-deficient mice infected with *Trypanosoma cruzi*. Front. Immunol. 8, 1548. [PubMed: 29209313]

Gray EB, La Hoz RM, Green JS, Vikram HR, Benedict T, Rivera H, Montgomery SP, 2018 Reactivation of Chagas disease among heart transplant recipients in the United States, 2012–2016. Transpl. Infect. Dis. 20, e12996.

Guiñazú N, Pellegrini A, Giordanengo L, Aoki MP, Rivarola HW, Cano R, Rodrigues MM, Gea S, 2004 Immune response to a major *Trypanosoma cruzi* antigen, cruzipain, is differentially modulated in C57BL/6 and BALB/c mice. Microbes Infect. 6, 1250–1258. [PubMed: 15555530]

Gupta S, Bhatia V, Wen J-J, Wu Y, Huang M-H, Garg NJ, 2009 *Trypanosoma cruzi* infection disturbs mitochondrial membrane potential and ROS production rate in cardiomyocytes. Free Radic. Biol. Med. 47, 1414–1421. [PubMed: 19686837]

Gupta S, Garg NJ, 2010 Prophylactic efficacy of TcVac2R against *Trypanosoma cruzi* in mice. PLoS Negl. Trop. Dis. 4, e797.

Gupta S, Garg NJ, 2015 A two-component DNA-prime/protein-boost vaccination strategy for eliciting long-term, protective T cell immunity against *Trypanosoma cruzi*. PLoS Pathog. 11, e1004828.

Gupta S, Salgado-Jiménez B, Lokugamage N, Vázquez-Chagoyán JC, Garg NJ, 2019 TcG2/TcG4 DNA vaccine induces Th1 immunity against acute Trypanosoma cruzi Infection: adjuvant and antigenic effects of heterologous T. rangeli booster immunization. Front. Immunol. 10, 1456. [PubMed: 31293599]

Gupta S, Smith C, Auclair S, Delgadillo Ade J, Garg NJ, 2015 Therapeutic efficacy of a subunit vaccine in controlling chronic *Trypanosoma cruzi* infection and Chagas disease is enhanced by glutathione peroxidase over-expression. PLoS One 10, e0130562.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Gupta S, Wan X, Zago MP, Sellers VC, Silva TS, Assiah D, Dhiman M, Nunez S, Petersen JR, Vazquez-Chagoyan JC, Estrada-Franco JG, Garg NJ, 2013 Antigenicity and diagnostic potential of vaccine candidates in human Chagas disease. PLoS Negl. Trop. Dis. 7, e2018.

Gutierrez FR, Guedes PM, Gazzinelli RT, Silva JS, 2009 The role of parasite persistence in pathogenesis of Chagas heart disease. Parasite Immunol. 31, 673–685. [PubMed: 19825107]

Hegazy-Hassan W, Zepeda-Escobar JA, Ochoa-García L, Contreras-Ortíz JME, Tenorio-Borroto E, Barbabosa-Pliego A, Aparicio-Burgos JE, Oros-Pantoja R, Rivas-Santiago B, Díaz-Albiter H, Garg NJ, Vázquez-Chagoyán JC, 2019 TcVac1 vaccine delivery by intradermal electroporation enhances vaccine induced immune protection against *Trypanosoma cruzi* infection in mice. Vaccine 37, 248–257. [PubMed: 30497833]

Hernández D, Teherán A, Flórez A, Ramírez JD, 2018 Comparison of parasite loads in serum and blood samples from patients in acute and chronic phases of Chagas disease. Parasitology 145, 1–7. [PubMed: 29144211]

Introini MV, Basso B, Moretti E, 1998 Experimental Chagas' disease: I. Study of different immunization conditions in the infection course. Bol. Chil. Parasitol. 53, 45–51. [PubMed: 10413878]

Koo S., Garg NJ, 2019 Metabolic programming of macrophage functions and pathogens control. Redox Biol. 24, 101198.

Krautz GM, Kissinger JC, Krettli AU, 2000 The targets of the lytic antibody response against *Trypanosoma cruzi*. Parasitol. Today 16, 31–34. [PubMed: 10637586]

Krautz GM, Peterson JD, Godsel LM, Krettli AU, Engman DM, 1998 Human antibody responses to *Trypanosoma cruzi* 70-kD heat-shock proteins. Am. J. Trop. Med. Hyg. 58, 137–143. [PubMed: 9502594]

Lattes R, Lasala MB, 2014 Chagas disease in the immunosuppressed patient. Clin. Microbiol. Infect. 20, 300–309. [PubMed: 24602129]

Lee BY, Bacon KM, Bottazzi ME, Hotez PJ, 2013 Global economic burden of Chagas disease: a computational simulation model. Lancet Infect. Dis. 13, 342–348. [PubMed: 23395248]

Lee BY, Bacon KM, Connor DL, Willig AM, Bailey RR, 2010 The potential economic value of a *Trypanosoma* cruzi (Chagas disease) vaccine in Latin America. PLoS Negl. Trop. Dis. 4, e916.

Lee BY, Bacon KM, Wateska AR, Bottazzi ME, Dumonteil E, Hotez PJ, 2012 Modeling the economic value of a Chagas' disease therapeutic vaccine. Hum. Vaccin. Immunother. 8, 1293–1301. [PubMed: 22894964]

Leiby DA, Rentas FJ, Nelson KE, Stambolis VA, Ness PM, Parnis C, McAllister HA Jr., Yawn DH, Stumpf RJ, Kirchhoff LV, 2000 Evidence of Trypanosoma cruzi infection (Chagas' disease) among patients undergoing cardiac surgery. Circulation 102, 2978–2982. [PubMed: 11113049]

Lidani KCF, Bavia L, Ambrosio AR, de Messias-Reason IJ, 2017 The complement system: a prey of *Trypanosoma cruzi*. Front. Microbiol. 8, 607. [PubMed: 28473804]

Lopez M, Tanowitz HB, Garg NJ, 2018 Pathogenesis of chronic Chagas disease: macrophages, mitochondria, and oxidative stress. Curr. Clin. Microbiol. Rep. 5, 45–54. [PubMed: 29868332]

Low HP, Santos MA, Wizel B, Tarleton RL, 1998 Amastigote surface proteins of *Trypanosoma cruzi* are targets for CD8+ CTL. J. Immunol. 160, 1817–1823. [PubMed: 9469442]

Low HP, Tarleton RL, 1997 Molecular cloning of the gene encoding the 83 kDa amastigote surface protein and its identification as a member of the *Trypanosoma cruzi* sialidase superfamily. Mol. Biochem. Parasitol. 88, 137–149. [PubMed: 9274875]

Ma Y, Weiss LM, Huang H, 2015 Strategy for the Development of vaccines against Chagas disease. HSOA J. Vaccin. Res. Vaccin. 1, 001.

Maranon C, Thomas MC, Planelles L, Lopez MC, 2001 The immunization of A2/K (b) transgenic mice with the KMP11-HSP70 fusion protein induces CTL response against human cells expressing the T. cruzi KMP11 antigen: identification of A2-restricted epitopes. Mol. Immunol. 38, 279–287. [PubMed: 11566321]

Marin-Neto JA, Cunha-Neto E, Maciel BC, Simoes MV, 2007 Pathogenesis of chronic Chagas heart disease. Circulation 115, 1109–1123. [PubMed: 17339569]

Marshall JS, Warrington R, Watson W, Kim HL, 2018 An introduction to immunology and immunopathology. Allergy Asthma Clin. Immunol. 14, 49. [PubMed: 30263032]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Martinez-Campos V, Martinez-Vega P, Ramirez-Sierra MJ, Rosado-Vallado M, Seid CA, Hudspeth EM, Wei J, Liu Z, Kwityn C, Hammond M, Ortega-López J, Zhan B, Hotez PJ, Bottazzi ME, Dumonteil E, 2015 Expression, purification, immunogenicity, and protective efficacy of a recombinant Tc24 antigen as a vaccine against *Trypanosoma cruzi* infection in mice. Vaccine 33, 4505–4512. [PubMed: 26192358]

Martins GA, Cardoso MA, Aliberti JC, Silva JS, 1998 Nitric oxide-induced apoptotic cell death in the acute phase of *Trypanosoma cruzi* infection in mice. Immunol. Lett. 63, 113–120. [PubMed: 9761373]

Mateus J, Guerrero P, Lasso P, Cuervo C, González JM, Puerta CJ, Cuéllar A, 2019 An animal model of acute and chronic Chagas disease with the reticulotropic Y strain of *Trypanosoma cruzi* that depicts the multifunctionality and dysfunctionality of T cells. Front. Immunol. 10, 918. [PubMed: 31105709]

Medina TS, Oliveira GG, Silva MC, David BA, Silva GK, Fonseca DM, Sesti-Costa R, Frade AF, Baron MA, Ianni B, Pereira AC, Chevillard C, Cunha-Neto E, Marin-Neto JA, Silva JS, 2017 Ebi3 prevents *Trypanosoma cruzi*-induced myocarditis by dampening IFN-γ-driven inflammation. Front. Immunol. 8, 1213. [PubMed: 29033934]

Meseda CA, Elkins KL, Merchlinsky MJ, Weir JP, 2002 Prime-boost immunization with DNA and modified vaccinia virus ankara vectors expressing herpes simplex virus-2 glycoprotein D elicits greater specific antibody and cytokine responses than DNA vaccine alone. J. Infect. Dis. 186, 1065–1073. [PubMed: 12355355]

Messenger LA, Bern C, 2018 Congenital Chagas disease: current diagnostics, limitations and future perspectives. Curr. Opin. Infect. Dis. 31, 415–421. [PubMed: 30095485]

Meymandi S, Hernandez S, Park S, Sanchez DR, Forsyth C, 2018 Treatment of Chagas disease in the United States. Curr. Treat. Options Infect. Dis. 10, 373–388. [PubMed: 30220883]

Michailowsky V, Luhrs K, Rocha MO, Fouts D, Gazzinelli RT, Manning JE, 2003 Humoral and cellular immune responses to *Trypanosoma cruzi*-derived paraflagellar rod proteins in patients with Chagas' disease. Infect. Immun. 71, 3165–3171. [PubMed: 12761095]

Morell M, Thomas MC, Caballero T, Alonso C, Lopez MC, 2006 The genetic immunization with paraflagellar rod protein-2 fused to the HSP70 confers protection against late *Trypanosoma cruzi* infection. Vaccine 24, 7046–7055. [PubMed: 16901590]

Norris KA, Galvao LM, Schrimpf JE, Cancado JR, Krettli AU, 1994 Humoral immune response to the *Trypanosoma cruzi* complement regulatory protein as an indicator of parasitologic clearance in human Chagas' disease. Infect. Immun. 62, 4072–4074. [PubMed: 8063427]

Nunes MCP, Beaton A, Acquatella H, Bern C, Bolger AF, Echeverría LE, Dutra WO, Gascon J, Morillo CA, Oliveira-Filho J, Ribeiro ALP, Marin-Neto JA, 2018 Chagas cardiomyopathy: an update of current clinical knowledge and management: a scientific statement from the American Heart Association. Circulation 138, e169–e209. [PubMed: 30354432]

Pack AD, Collins MH, Rosenberg CS, Tarleton RL, 2018 Highly competent, non-exhausted CD8+ T cells continue to tightly control pathogen load throughout chronic *Trypanosoma cruzi* infection. PLOS Pathogens 14, e1007410.

Paiva CN, Medei E, Bozza MT, 2018 ROS and *Trypanosoma cruzi*: fuel to infection, poison to the heart. PLoS Pathog. 14, e1006928.

Pech-Canul AC, Monteon V, Solis-Oviedo RL, 2017 A brief view of the surface membrane proteins from *Trypanosoma cruzi*. J. Parasitol. Res. 2017, 13.

Pereira IR, Vilar-Pereira G, Marques V, da Silva AA, Caetano B, Moreira OC, Machado AV, Bruna-Romero O, Rodrigues MM, Gazzinelli RT, Lannes-Vieira J, 2015 A human type 5 adenovirus-based *Trypanosoma cruzi* therapeutic vaccine reprograms immune response and reverses chronic cardiomyopathy. PLoS Pathog. 11, e1004594.

Pereira-Chioccola VL, Schenkman S, Kloetzel JK, 1994 Sera from chronic Chagasic patients and rodents infected with *Trypanosoma cruzi* inhibit trans-sialidase by recognizing its amino-terminal and catalytic domain. Infect. Immun. 62, 2973–2978. [PubMed: 8005683]

Pérez-Antón E, Egui A, Thomas MC, Puerta CJ, González JM, Cuéllar A, Segovia M, López MC, 2018 Impact of benznidazole treatment on the functional response of *Trypanosoma cruzi* antigen-

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

specific CD4+CD8+ T cells in chronic Chagas disease patients. PLOS Negl. Trop. Dis. 12, e0006480.

Perez-Fuentes R, Guegan JF, Barnabe C, Lopez-Colombo A, Salgado-Rosas H, Torres-Rasgado E, Briones B, Romero-Diaz M, Ramos-Jimenez J, Sanchez-Guillen Mdel C, 2003 Severity of chronic Chagas disease is associated with cytokine/antioxidant imbalance in chronically infected individuals. Int. J. Parasitol. 33, 293–299. [PubMed: 12670514]

Pinazo MJ, Torrico F, Gascón J, 2015 Pathogenesis of Chagas disease in humans. Hum. Emerg. Re-emerg. Infect. 349–369.

Portillo S, Zepeda BG, Iniguez E, Olivas JJ, Karimi NH, Moreira OC, Marques AF, Michael K, Maldonado RA, Almeida IC, 2019 A prophylactic α-Gal-based glycovaccine effectively protects against murine acute Chagas disease. NPJ Vaccines 4, 13. [PubMed: 30911415]

Quijano-Hernandez I, Dumonteil E, 2011 Advances and challenges towards a vaccine against Chagas disease. Hum Vaccin. 7, 1184–1191. [PubMed: 22048121]

Quijano-Hernandez IA, Bolio-Gonzalez ME, Rodriguez-Buenfil JC, Ramirez-Sierra MJ, Dumonteil E, 2008 Therapeutic DNA vaccine against *Trypanosoma cruzi* infection in dogs. Ann. NY Acad. Sci. 1149, 343–346. [PubMed: 19120245]

Rassi A, Marin JA, 2017 Chronic Chagas cardiomyopathy: a review of the main pathogenic mechanisms and the efficacy of aetiological treatment following the BENznidazole Evaluation for Interrupting Trypanosomiasis (BENEFIT) trial. Mem. Inst. Oswaldo Cruz. 112, 224–235. [PubMed: 28225900]

Ribas SE, Maia CH, Gutierrez PS, Issa VS, Strabelli TM, Ayub-Ferreira SM, 2016 *Trypanosoma cruzi* myocardial infection reactivation after heart transplant: a case report. Ann. Clin. Cytol. Pathol. 2, 1037.

Ribeirao M, Pereira-Chioccola VL, Renia L, Augusto Fragata Filho A, Schenkman S, Rodrigues MM, 2000 Chagasic patients develop a type 1 immune response to *Trypanosoma cruzi* trans-sialidase. Parasite Immunol. 22, 49–53. [PubMed: 10607290]

Ribeiro FAP, Pontes C, Gazzinelli RT, Romero OB, Lazzarin MC, Dos Santos JF, de Oliveira F, Pisani LP, de Vasconcelos JRC, Ribeiro DA, 2019 Therapeutic effects of vaccine derived from amastigote surface protein-2 (ASP-2) against Chagas disease in mouse liver. Cytokine 113, 285–290. [PubMed: 30037707]

Rodrigues-dos-Santos Í, Melo MF, de Castro L, Hasslocher-Moreno AM, do Brasil PEAA, Silvestre de Sousa A, Britto C, Moreira OC, 2018 Exploring the parasite load and molecular diversity of *Trypanosoma cruzi* in patients with chronic Chagas disease from different regions of Brazil. PLOS Negl. Trop. Dis. 12, e0006939.

Rodriguez-Morales O, Monteon-Padilla V, Carrillo-Sanchez SC, Rios-Castro M, Martinez-Cruz M, Carabarin-Lima A, Arce-Fonseca M, 2015 Experimental vaccines against Chagas disease: a journey through history. J. Immunol. Res. 2015, 489758.

Rosas-Jorquera CE, Sardinha LR, Pretel FD, Bombeiro AL, D'Império Lima MR, Alvarez JM, 2013 Challenge of chronically infected mice with homologous *Trypanosoma cruzi* parasites enhances the immune response but does not modify cardiopathy: implications for the design of a therapeutic vaccine. Clin. Vaccine Immunol. 20, 248–254. [PubMed: 23254299]

Sanchez-Burgos G, Mezquita-Vega RG, Escobedo-Ortegon J, Ramirez-Sierra MJ, Arjona-Torres A, Ouaissi A, Rodrigues MM, Dumonteil E, 2007 Comparative evaluation of therapeutic DNA vaccines against *Trypanosoma cruzi* in mice. FEMS Immunol. Med. Microbiol. 50, 333–341. [PubMed: 17521394]

Sanchez-Valdez FJ, Perez Brandan C, Ferreira A, Basombrio MA, 2015 Gene-deleted live-attenuated *Trypanosoma cruzi* parasites as vaccines to protect against Chagas disease. Expert Rev. Vaccines 14, 681–697. [PubMed: 25496192]

Santos MA, Garg NJ, Tarleton RL, 1997 The identification and molecular characterization of *Trypanosoma cruzi* amastigote surface protein-1, a member of the trans-sialidase gene super-family. Mol. Biochem. Parasitol. 86, 1–11. [PubMed: 9178263]

Schenkman S, Ferguson MA, Heise N, de Almeida ML, Mortara RA, Yoshida N, 1993 Mucin-like glycoproteins linked to the membrane by glycosylphosphatidylinositol anchor are the major

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

acceptors of sialic acid in a reaction catalyzed by trans-sialidase in metacyclic forms of *Trypanosoma cruzi*. Mol. Biochem. Parasitol. 59, 293–303. [PubMed: 8341326]

Schmunis GA, Cruz JR, 2005 Safety of the blood supply in Latin America. Clin. Microbiol. Rev. 18, 12–29. [PubMed: 15653816]

Sepulveda P, Hontebeyrie M, Liegeard P, Mascilli A, Norris KA, 2000 DNA-based immunization with *Trypanosoma cruzi* complement regulatory protein elicits complement lytic antibodies and confers protection against *Trypanosoma cruzi* infection. Infect. Immun. 68, 4986–4991. [PubMed: 10948115]

Serna C, Lara JA, Rodrigues SP, Marques AF, Almeida IC, Maldonado RA, 2014 A synthetic peptide from *Trypanosoma cruzi* mucin-like associated surface protein as candidate for a vaccine against Chagas disease. Vaccine 32, 3525–3532. [PubMed: 24793944]

Silva RR, Mariante RM, Silva AA, dos Santos ALB, Roffê E, Santiago H, Gazzinelli RT, Lannes-Vieira J, 2015 Interferon-gamma promotes infection of astrocytes by *Trypanosoma cruzi*. PLoS One 10, e0118600.

Taibi A, Plumas-Marty B, Guevara-Espinoza A, Schöneck R, Pessoa H, Loyens M, Piras R, Aguirre T, Gras-Masse H, Bossus M, 1993 *Trypanosoma cruzi*: immunity-induced in mice and rats by trypomastigote excretory-secretory antigens and identification of a peptide sequence containing a T cell epitope with protective activity. J. Immunol. 151, 2676–2689. [PubMed: 7689612]

Trujillo C, Ramirez R, Velez ID, Berberich C, 1999 The humoral immune response to the kinetoplastid membrane protein-11 in patients with American leishmaniasis and Chagas disease: prevalence of IgG subclasses and mapping of epitopes. Immunol. Lett. 70, 203–209. [PubMed: 10656675]

Vasconcelos JRC, Boscardin SB, Hiyane MI, Kinoshita SS, Fujimura AE, Rodrigues MM, 2003 A DNA-priming protein-boosting regimen significantly improves type 1 immune response but not protective immunity to *Trypanosoma cruzi* infection in a highly susceptible mouse strain. Immunol. Cell Biol. 81, 121–129. [PubMed: 12631235]

Vazquez-Chagoyan JC, Gupta S, Garg NJ, 2011 Vaccine development against *Trypanosoma cruzi* and Chagas disease. Adv. Parasitol. 75, 121–146. [PubMed: 21820554]

Villanueva-Lizama LE, Cruz-Chan JV, Aguilar-Cetina A.d.C., Herrera-Sanchez LF, Rodriguez-Perez JM, Rosado-Vallado ME Ramirez-Sierra MJ, Ortega-Lopez J, Jones K, Hotez P, Bottazzi ME, Dumonteil E, 2018 Trypanosoma cruzi vaccine candidate antigens Tc24 and TSA-1 recall memory immune response associated with HLA-A and -B supertypes in Chagasic chronic patients from Mexico. PLOS Negl. Trop. Dis. 12, e0006240.

Wan X, Wen JJ, Koo SJ, Liang LY, Garg NJ, 2016 SIRT1-PGC1alpha-NFkappaB pathway of oxidative and inflammatory stress during *Trypanosoma cruzi* infection: benefits of SIRT1-targeted therapy in tmproving heart function in Chagas disease. PLoS Pathog. 12, e1005954.

Wen J-J, Bhatia V, Popov VL, Garg NJ, 2006 Phenyl-alpha-tert-butyl nitrone reverses mitochondrial decay in acute Chagas disease. Am. J. Pathol. 169, 1953–1964. [PubMed: 17148660]

Wen JJ, Dhiman M, Whorton EB, Garg NJ, 2008 Tissue-specific oxidative imbalance and mitochondrial dysfunction during *Trypanosoma cruzi* infection in mice. Microbes Infect. 10, 1201–1209. [PubMed: 18675934]

Wen JJ, Garg NJ, 2008 Mitochondrial generation of reactive oxygen species is enhanced at the Q(o) site of the complex III in the myocardium of Trypanosoma cruzi-infected mice: beneficial effects of an antioxidant. J. Bioenerg. Biomembr. 40, 587–598. [PubMed: 19009337]

Wen JJ, Garg NJ, 2010 Mitochondrial complex III defects contribute to inefficient respiration and ATP synthesis in the myocardium of *Trypanosoma cruzi*-infected mice. Antioxid. Redox. Signal 12, 27–37. [PubMed: 19624257]

Wen J-J, Gupta S, Guan Z, Dhiman M, Condon D, Lui CY, Garg NJ, 2010 Phenyl-alpha-tert-butyl-nitrone and benzonidazole treatment controlled the mitochondrial oxidative stress and evolution of cardiomyopathy in chronic chagasic rats. J. Am. Coll. Cardiol. 55, 2499–2508. [PubMed: 20510218]

Wen JJ, Porter C, Garg NJ, 2017 Inhibition of NFE2L2-antioxidant response element pathway by mitochondrial reactive oxygen species contributes to development of cardiomyopathy and left ventricular dysfunction in Chagas disease. Antioxid. Redox. Signal 27, 550–566. [PubMed: 28132522]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Wen J-J, Vyatkina G, Garg NJ, 2004 Oxidative damage during chagasic cardiomyopathy development: role of mitochondrial oxidant release and inefficient antioxidant defense. Free Radic. Biol. Med. 37, 1821–1833. [PubMed: 15528041]

Wen J-J, Yachelini PC, Sembaj A, Manzur RE, Garg NJ, 2006 Increased oxidative stress is correlated with mitochondrial dysfunction in chagasic patients. Free Rad. Biol. Med. 41, 270–276. [PubMed: 16814107]

WHO: World Health Organization, Chagas disease (American trypanosomiasis). 2019 https://www.who.int/neglected_diseases/news/Chagas-Preventing-mother-to-child-transmission/en/.

Wizel B, Nunes M, Tarleton RL, 1997 Identification of *Trypanosoma cruzi* trans-sialidase family members as targets of protective CD8+ TC1 responses. J. Immunol. 159, 6120–6130. [PubMed: 9550413]

Wizel B, Palmieri M, Mendoza C, Arana B, Sidney J, Sette A, Tarleton R, 1998 Human infection with *Trypanosoma cruzi* induces parasite antigen-specific cytotoxic T lymphocyte responses. J. Clin. Invest. 102, 1062–1071. [PubMed: 9727076]

Wrightsman RA, Luhrs KA, Fouts D, Manning JE, 2002 Paraflagellar rod protein-specific CD8+ cytotoxic T lymphocytes target *Trypanosoma cruzi*-infected host cells. Parasite Immunol. 24, 401–412. [PubMed: 12406194]

Yoshida N, Araya JE, da Silveira JF, Giorgio S, 1993 *Trypanosoma cruzi*: antibody production and T cell response induced by stage-specific surface glycoproteins purified from metacyclic trypomastigotes. Exp. Parasitol. 77, 405–413. [PubMed: 8253154]

Zacks MA, Wen JJ, Vyatkina G, Bhatia V, Garg NJ, 2005 An overview of chagasic cardiomyopathy: pathogenic importance of oxidative stress. An. Acad. Bras. Cienc. 77, 695–715. [PubMed: 16341444]

Zapata-Estrella H, Hummel-Newell C, Sanchez-Burgos G, Escobedo-Ortegon J, Ramirez-Sierra MJ, Arjona-Torres A, Dumonteil E, 2006 Control of *Trypanosoma cruzi* infection and changes in T-cell populations induced by a therapeutic DNA vaccine in mice. Immunol. Lett. 103, 186–191. [PubMed: 16378645]

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Table 1

Properties of some of the *T. cruzi* antigenic targets.

Antigen	Model	Recognition by immune components	References
TSA-1	C57BL/6 mice, Human (PBMC’s)	MHC-I, $CD8^+$ T cell, HLA-A2	Wizel et al. (1997), Wizel et al. (1998)
ASP1	C57BL/6, C3H/HeSnJ mice, Human (PBMC’s)	MHC-I, $CD8^+$ T cell, HLA-A2	Wizel et al. (1998), Santos et al. (1997), Low et al. (1998)
ASP2 (ASP-2-like clone 9)	BALB/c, C3H/HeJ, C57BL/6 mice, Human (PBMC’s)	$CD4^+$ Th1, $CD8^+$ CTL, HLA-A2	Wizel et al. (1998), Boscardin et al. (2003), Barbosa et al. (2013), Ribeiro et al. (2019), Pereira et al. (2015)
MASP	BALB/c mice, Human (sera)	B cells, $CD8^+$ T cell, IgM, IgG2A, IgA	Serna et al. (2014), Durante et al. (2017)
Tc24 (FCaBP)	BALB/c, ICR mice	Th1, IgG2b	Krautz et al. (1998), Sanchez-Burgos et al. (2007), Dumonteil et al. (2004), Taibi et al. (1993), Martinez-Campos et al. (2015)
TSA-1 & Tc24	Human (PBMC's)	HLA-A, HLA-B	Villanueva-Lizama et al. (2018)
CRP (gp160)	BALB/c, C3H/HeJ mice, Human (sera)	Th1, IgG, Complement mediated lysis	Norris et al. (1994), Sepulveda et al. (2000)
Cruzipain	Human (PBMC’s)	HLA-A2, $CD8^+$ T cell	Fonseca et al. (2005)
Cruzipain	BALB/c mice	Th2, $CD19^+$	Guiñazú et al. (2004)
Cruzipain	C57BL/6 mice	Th1, $CD4^+$ $TGF\beta^+$	Guiñazú et al. (2004)
TcG1, TcG2, & TcG4	C57BL/6 mice	Th1, IgG2b/ IgG1	Bhatia and Garg (2008)
Gp63	NA	NA	Cuevas et al. (2003)
Gp35/50	BALB/c mice anti-sera	No response	Yoshida et al. (1993)
Gp82 & gp90	BALB/c mice anti-sera	Complement mediated lysis, $CD4^+$ T cell	Yoshida et al. (1993)
Ssp3	NA	No response	Frevert et al. (1992)
KMP-11	Human (sera)	IgG1, IgG3, IgG4	Flechas et al. (2009)
LYT1	C57BL/6 mice	$CD8^+$ CTL	Fralish and Tarleton (2003)
PFR1, PFR2	C57BL/6, BALB/c mice	$CD8^+$ CTL	Wrightsman et al. (2002)
PFR2 or PFR3	BALB/c mice, Human (PBMC’s)	$CD8^+$ CTL, HLA-A	Egui et al. (2012)
Tc52	ICR mice	NA	Sanchez-Burgos et al. (2007)

Abbreviations: ASP2 - amastigote surface protein 2; CRP - complement regulatory protein; CTL - cytotoxic T lymphocyte; HLA - human leukocyte antigen (type A and type B); Ig - immunoglobulin isotypes; MASP – mucin-associated surface protein; MHC-1 - major histocompatibility complex 1; PBMCs - peripheral blood mononuclear cells; Tc24 - trypomastigote excretory–secretory protein 24; TSA - trypomastigote surface antigen; Th1 - T helper type 1; Ssp3 - stage-specific antigen 3; KMP - kinetoplastid membrane protein; PFR (1, 2 or 3) - paraflagellar rod protein (1, 2 or 3); NA - not available.

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Table 2

Subunit vaccines' efficacy against *T. cruzi* and Chagas disease (modified and adapted from Vazquez-Chagoyan et al., 2011)

Antigens	Adjuvant	Prophylactic or Therapeutic	Experimental Model (T. cruzi strain), acute, intermediate or chronic	Parasite burden (location)	% Survival (days post-infection)	References
Recombinant DNA or recombinant protein						
PFR1, PFR3	iFreund	Prophylactic	C57, BALB/c mice (Peru & Y), chronic	Decrease[B] (blood)	100 (17[A])	Wrightsman et al. (2002)
TSA1	IL-12 / GM-CSF	Prophylactic	C3H, C57 mice (Brazil), acute & chronic	Decrease[B] (heart, skm)	60 (140)	Wizel et al.(1998, Garg and Tarleton 2002
ASP1	IL-12 / GM-CSF	Prophylactic		Decrease[B] (heart, skm)	60 (140)	Garg and Tarleton (2002)
ASP2	IL-12 / GM-CSF	Prophylactic	C3H, C57 mice (Brazil), acute & chronic	Decrease[B] (heart, skm)	80 (140)	(Garg and Tarleton, 2002)
ASP1, ASP2, TSA1	IL-12 / GM-CSF	Prophylactic	C3H, C57 mice (Brazil) acute & chronic	Decrease[B] (heart, skm)	80 (126)	Garg and Tarleton (2002)
ASP2 (ASP-2-like clone 9)	Complete Freund	Prophylactic	BALB/c, C57 mice (Y)	Decrease[B] (blood)	100 (60)	Boscardin et al. (2003)
TcG2, TcG4	IL-12 / GM-CSF	Prophylactic	BALB/c mice (Sylvio), acute	Decrease[B] (heart, skm)	66.7 (60)	Hegazy-Hassan et al. (2019)
LYT1	IL-12 / GM-CSF	Prophylactic	C3H, C57 mice (Brazil), chronic	NA	80 (75)	Fralish and Tarleton (2003)
TCβ3	IL-12 / GM-CSF	Prophylactic	C3H, C57 mice (Brazil), chronic	NA	40 (75)	Fralish and Tarleton (2003)
FCaBP (Tc24)	IL-12 / GM-CSF	Prophylactic	C3H, C57 mice (Brazil), chronic	NA	0 (65)	Fralish and Tarleton (2003)
PFR2, PFR3	HSP70	Prophylactic	BALB/c, C57 mice (Y), chronic	NA	100 (40)	Morell et al. (2006)
TcG1	IL-12 / GM-CSF	Prophylactic	C57 mice (Sylvio), acute, intermediate, chronic	66% Decrease[B] (heart)	100 (30–120)	Bhatia et al. (2004), Bhatia and Garg (2008)
TcG2	IL-12 / GM-CSF	Prophylactic	C57 mice (Sylvio), acute, intermediate chronic	50% Decrease[B] (heart)	100 (30–120)	Bhatia et al. (2004), Bhatia and Garg (2008)
TcG4	IL-12 / GM-CSF	Prophylactic	C57mice (Sylvio), acute, intermediate, chronic	90% Decrease[B] (heart)	100 (30–120)	Bhatia et al. (2004), Bhatia and Garg (2008)
CRP (gp160)	iFreund	Prophylactic	BALB/c, C3H mice (Y) acute, chronic	Decrease[B] (blood)	100 (40)	Sepulveda et al. (2000)
Tc24	MPLA	Prophylactic	BALB/c mice (H1), acute	Decrease[B] (blood & heart)	50 (50)	Martinez-Campos et al. (2015)
SCz DNA prime / rCz boost[C]	CpG-ODN, GM-CSF, MALP-2	Prophylactic	C3H mice (RA), acute, chronic	Decrease[B] (blood)	100 (30)[D]	Cazorla et al. (2008)
Galα3LN-HSA[E]	LMPLA	Prophylactic	C57 mice (Y), acute	Decrease[B] (heart, lung, spleen, skm)	80, 100 (32)[F]	Portillo et al. (2019)

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Antigens	Adjuvant	Prophylactic or Therapeutic	Experimental Model (T. cruzi strain), acute, intermediate or chronic	Parasite burden (location)	% Survival (days post-infection)	References
TcG1, TcG2, TcG4 (DNA prime / DNA boost)	IL-12 / GM-CSF	Prophylactic	Canine (Sylvio), acute, chronic	No change[B] in acute heart, Decrease[B] in chronic heart	67 (60–365)	Aparicio-Burgos et al. (2011)
TcG1, TcG2, TcG4 (DNA prime / protein boost)	IL-12 / GM-CSF	Prophylactic	C57 mice (Sylvio), acute, chronic	Decrease[B] (heart, spleen, liver, kidney, skm)	NA	Gupta and Garg (2010)
TcG2, TcG4 (DNA prime / protein boost)	IL-12 / GM-CSF	Prophylactic	C57 mice (Sylvio), acute, chronic	Decrease[B] (blood, spleen, heart, skm)	NA	Gupta and Garg (2015)
TcG1, TcG2, TcG4 (DNA prime / *T. rangeli* boost)	IL-12 / GM-CSF	Prophylactic	Canine (Sylvio), acute, chronic	Decrease[B] (blood)	100 (60–365)	Aparicio-Burgos et al. (2015)
TcSP, TcSSP4		Prophylactic	Canine (Ninoa), acute, chronic	Decrease[B] (blood)	100 (65)	Arce-Fonseca et al. (2013)
TcG2, TcG4 (DNA prime/*T. rangeli*-boost)		Prophylactic	C57 mice (Sylvio), acute	Decrease[B] (heart, skm)	NA	Gupta et al. (2019)
Recombinant virus						
rAd5-Tc80	CpG-ODN	Prophylactic	BALB/c & C3H mice (Tulahuen, RA, K98), acute, chronic	Decrease[B] (heart, skm)	80-STc80, 67-Pboost (23)	Bivona et al. (2018)
Flu-ASP2 (DNA prime / Ad5 boost)[G]		Prophylactic	C57, C3H mice (Y)	Decrease[B] (blood)	80, 0 (50)[H]	Barbosa et al. (2013)
rAdVax[I]		Therapeutic & Prophylactic	C57 mice (Colombian, CL-Brenner), acute, chronic	Decrease[B] (heart)	87 (230)	Pereira et al. (2015)
Ad5ASP-2[J]		Therapeutic	A/Sn mice (Y), acute	Decrease[B] (liver)	NA	Ribeiro et al. (2019)
Immunotherapeutic vaccine						
TSA1		Therapeutic	BALB/c mice (H4), acute	Decrease[B] (blood)	>70 (45)	Dumonteil et al. (2004)
TSA1		Therapeutic	ICR mice (H1), acute, chronic	Decrease[B] (blood, heart)	100 (20–80)	Zapata-Estrella et al. (2006)
Tc24 (FCaBP)		Therapeutic	CD1 mice (H1), chronic	Decrease[B] (blood, heart)	100 (140)	Dumonteil et al. (2004)
TSA1[K]	Alum	Therapeutic	ICR mice (H1), acute	Decrease[B] (blood, heart)	70 (50)	Sanchez-Burgos et al. (2007)
Tc24 (FCaBP)[K]	Alum	Therapeutic	ICR mice (H1), acut	Decrease[B] (blood, heart)	85 (50)	Sanchez-Burgos et al. (2007)
Tc52[K]	Alum	Therapeutic	ICR mice (H1), acute	Decrease[B] (blood, heart)	75 (50)	Sanchez-Burgos et al. (2007)
TcG2, TcG4 (DNA prime / protein boost)	IL-12 / GM-CSF	Therapeutic	CD1×C57 mice (Sylvio), chronic	Decrease[B] (blood, skm, heart)	NA	Gupta et al. (2015)
Tc24	CpG-ODN	Therapeutic	BALB/c mice (H1), acute	ICR mice (H1), acute	NA	Barry et al. (2016)

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Antigens	Adjuvant	Prophylactic or Therapeutic	Experimental Model (T. cruzi strain), acute, intermediate or chronic	Parasite burden (location)	% Survival (days post-infection)	References
Tc24	E6020	Therapeutic	ICR mice (H1), chronic	ICR mice (H1), acut	NA	Barry et al. (2019)

Abbreviations: ASP2 - amastigote surface protein 2; CRP - complement regulatory protein; Tc24 - trypomastigote excretory–secretory protein 24; TSA - trypomastigote surface antigen; PFR (1, 2 or 3) - paraflagellar rod protein (1, 2 or 3); MPLA - monophosphoryl-lipid A; LMPLA - liposomal-monophosphoryl lipid A; TcSSP4 - gene encoding an amastigote-specific surface protein in *T. cruzi*; TcSP - gene encoding a member of the transsialidase family in *T. cruzi*; C57 – C57BL/6; C3H – C3H/HeN; skm – skeletal muscle; NA - not available.

Key:

A = mean survival time >120 days

B = compared to that detected in infected mice that were not vaccinated or were injected with the plasmid vector only

C = Salmonella carrying Cz-DNA priming and recombinant cruzipain (rCz) boosting

D = Salmonella carrying Cz DNA (SCz) only, no adjuvants used

E = Galα1,3Galβ1,4GlcNAc (Galα3LN), also known as the α-Gal epitope, coupled to the carrier protein human serum albumin (Galα3LN-HSA)

F = Galα3LN-HSA+LMPLA (80% survival), Galα3LN-HSA (100% survival)

G = ASP2 medial (Flu-M-ASP2) and carboxi-terminal (Flu-C-ASP2) segments

H = Flu-M-ASP2 (80% survival), Flu-C-ASP2 (0% survival)

I = rAdVax - recombinant adenovirus serotype 5 carrying sequences of ASP2 and TS *T. cruzi* antigens (rAdASP2+rAdTS)

J = AdASP-2 - recombinant human type 5 replication-defective adenoviruses expressing *T. cruzi* Amastigote Surface Protein-2

K = Tc24 was most effective in reducing blood and heart parasite burden compared to TSA1 and Tc52