Published in final edited form as:

Vaccine. 2014 June 12; 32(28): 3525–3532. doi:10.1016/j.vaccine.2014.04.026.

A synthetic peptide from *Trypanosoma cruzi* mucin-like associated surface protein as candidate for a vaccine against Chagas disease

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Abstract

Chagas disease, caused by *Trypanosoma cruzi*, is responsible for producing significant morbidity and mortality throughout Latin America. The disease has recently become a public health concern to nonendemic regions like the U.S. and Europe. Currently there are no fully effective drugs or vaccine available to treat the disease. The mucin-associated surface proteins (MASPs) are glycosylphosphatidylinositol (GPI)-anchored glycoproteins encoded by a multigene family with hundreds of members. MASPs are among the most abundant antigens found on the surface of the infective trypomastigote stage of *T. cruzi*, thus representing an attractive target for vaccine development. Here we used immunoinformatics to select a 20-mer peptide with several predicted overlapping B-cell, MHC-I, and MHC-II epitopes, from a MASP family member expressed on mammal-dwelling stages of *T. cruzi*. The synthetic MASP peptide conjugated to keyhole limpet hemocyanin (MASPpep-KLH) was tested in presence or not of an adjuvant (alum, Al) as a vaccine candidate in the C3H/HeNsd murine model of *T. cruzi* infection. In considerable contrast to the control groups receiving placebo, Al, or KLH alone or the group immunized with MASPpep-KLH/Al, the group immunized with MASPpep-KLH showed 86% survival rate after challenge with a highly lethal dose of trypomastigotes. As evaluated by quantitative real-time polymerase chain reaction, MASPpep-KLH-immunized animals had much lower parasite load in the heart, liver, and spleen than control animals. Moreover, protected animals produced trypanolytic, protective antibodies, and a cytokine profile conducive to resistance against parasite infection. Finally, *in vivo* depletion of either CD4+ or CD8+T cells indicated that the latter are critical for protection in mice immunized with MASPpep-KLH. In summary, this new peptide-based vaccine with overlapping B- and T-cell epitopes is able to control *T. cruzi* infection in mice by priming both humoral and cellular immunity.

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Keywords

Chagas disease; immunoinformatics; proteomics; *Trypanosoma cruzi*; vaccine

1. Introduction

Trypanosoma cruzi is the causative agent of Chagas disease (ChD), which affects 8-10 million people in Latin America. Lately, ChD has also become a major concern to the United States and other nonendemic countries [1]. ChD is the major cause of stroke in the American continent, causing thousands of death every year. Currently, the available drugs are rather toxic and less effective in the chronic stage of ChD. Moreover, there is no human vaccine for ChD, despite numerous experimental efforts [2]. ChD represents a tremendous economic and social burden, thus a preventive and/or therapeutic vaccine would be very beneficial to endemic and nonendemic countries [3].

T. cruzi is coated by a thick layer of glycosylphosphatidylinositol (GPI)-anchored glycoproteins such as mucins, mucin-associated surface proteins (MASP), and *trans*-sialidase (TS)/gp85 glycoproteins [4]. MASP is the second largest gene family (1,377 genes and 433 pseudogenes), representing ~6% of *T. cruzi* genome [5, 6]. Akin to other major surface glycoproteins, MASP expression is upregulated in the infective trypomastigote stage and some members of this multigene family have been implicated in host-cell invasion [6]. Using proteomics and immunoinformatics, we recently showed that many MASP family members expressed on trypomastigotes have several predicted MHC-I and MHC-II epitopes, making them valuable as targets for vaccine development [7]. Here, we use immunoinformatics to select a potential highly immunogenic 20-mer peptide from a MASP member expressed in mammal-dwelling trypomastigote stage. The synthetic MASP-derived peptide (MASPpep) predicted to contain partially overlapping B- and T-cell (MHC-I and MHC-II) epitopes, was conjugated to keyhole limpet hemocyanin (MASPpep-KLH) and tested as vaccine candidate in the murine model of ChD.

2. Materials and Methods

2.1 *Trypanosoma cruzi* and mice

Mammalian cell (LLC-MK2) culture-derived trypomastigote forms (TCT) of *T. cruzi* were obtained as described [8]. Extracellular vesicles secreted by TCTs (TCT_{EV}) were obtained as described [9]. Intracellular amastigote forms were purified from infected LLC-MK2 cells [10]. Epimastigote forms were obtained as described [4]. All *T. cruzi* stages were from the Y strain. Female C3H/HeNsd and BALB/c mice (6-8 weeks old) were acquired from Harlan Laboratory (Indianapolis, IN). Animal procedures were performed according to NIH guidelines and the protocol approved by UTEP's Institutional Animal Care and Use Committee.

2.2. Immunoinformatics

B-cell epitope prediction was performed using Chou & Fasman beta-turn, Emini surface accessibility, Karplus & Schulz flexibility, and Parker hydrophilicity prediction tools

available at Immune Epitope Database and Analysis Resource (IEDB; http://tools.immuneepitope.org/tools/bcell/iedb_input). For MHC class I epitope prediction, the ProPred I algorithm (<http://www.imtech.res.in/raghava/propred1/>) [11] was employed. For MHC class II epitope prediction, the ProPred MHC Class-II Binding Peptide Prediction Server (<http://www.imtech.res.in/raghava/propred/>) and IEDB MHC-II Binding Predictions tool (http://tools.immuneepitope.org/analyze/html/mhc_II_binding.html) were used. Based on the results of B-cell, MHC-I, and MHC-II epitope predictions a 20-mer peptide (DAENPGGEVFNDNKKGLSRV) (MASPpep), derived from a MASP family member (accession number XP_820771.1, EAN98920.1, TriTryp DB TcCLB.511603.380), was synthesized and conjugated to KLH (MASPpep-KLH) (thinkpeptides, ProImmune, Sarasota, FL).

2.3. Immunization

C3H/HeNsd female mice were immunized (i.p.) with MASPpep-KLH alone or combined with the adjuvant 0.9% Al(OH)₃ (Al) (MASPpep-KLH/Al) (20 µg in 200 µl PBS/animal/immunization). Control groups were treated with phosphate-buffered saline (PBS, placebo), Al, or 20 µg KLH alone. All animals were injected three times at 10-15-day intervals.

2.4. Evaluation of the humoral immune response

Ten days after the last immunization, blood was collected by tail bleeding [12] and serum was separated from blood by centrifugation (1,000 g, 10 min, 4°C). Antibody titers were determined by chemiluminescent enzyme-linked immunosorbent assay (CL-ELISA) [13]. Sera from patients with chronic ChD (ChDS) and healthy individuals (NHS) [14] were kindly provided by Dr Joaquim Gascon (Universitat de Barcelona). Serum pools were generated by mixing ten NHS (NHSP) or ten ChDS (ChDSP). Antibody titers against MASPpep-KLH were also evaluated by CL-ELISA.

2.5. Immunoglobulin isotyping

Immunoglobulin isotyping was performed using the Mouse Immunoglobulin Isotyping ELISA Kit (BD Pharmingen, San Jose, CA), following the manufacturer's instructions.

2.6. Trypomastigote lysis assay

Serum (~50 µl) was collected from non-infected, PBS-, MASPpep-KLH-, and MASPpep-KLH/Al-immunized mice, and lytic assays were performed as described [15]. Positive and negative controls (dead and live parasites, respectively) were included. Three independent experiments, each in triplicate, were performed. For each individual sample, approximately 10,000 events were acquired and analyzed using CXP software (Beckman Coulter, Miami, FL).

2.7. Parasite challenge, parasitemia, and survival

Ten days following the last immunization, mice were inoculated via i.p. with 10⁵ TCTs, and the parasitemia and survival were monitored. Parasitemia was evaluated every day for the first 12 days, and then every third day for a total of 21 days, as described [12].

2.8. Cytokine profile

Four weeks post-infection blood was collected by tail-bleeding and a serum pool from each experimental group was obtained. Cytokines were measured in mouse sera using the Mouse Inflammatory Cytokines Multi-Analyte ELISArray Kit (SABiosciences/Qiagen, Valencia, CA).

2.9. DNA preparation

At the terminal stage of the disease, mice were euthanized and heart, liver, and spleen were collected. This procedure was performed at different endpoints upon the protection offered by the immunization. DNA was extracted using Wizard SV Genomic DNA Purification System (Promega, Madison, WI) and quantified by Nanodrop 1000 Spectrophotometer (Thermo Scientific, Waltham, MA).

2.10. Quantitative Real-Time PCR (qRT-PCR)

To assess parasite load in the heart, liver, and spleen of *T. cruzi*-challenged animals, quantitative real-time PCR (qRT-PCR) was performed as described [16].

2.11. *In vivo* depletion of CD4+ and CD8+ T cells

One week after the last immunization, mice received i.p. injections of 500 µg anti-CD4 or 1,000 µg anti-CD8 monoclonal antibody (mAb)(BD Pharmingen). Unspecific murine IgG was given to the control groups. CD4+ and CD8+T-cell depletion was confirmed by flow cytometry using a Cytomic FC 500 flow cytometer (Beckman Coulter, Miami, FL). For each individual sample, approximately 10,000 events were acquired and analyzed using CXP software (Beckman Coulter). Mice (n=2 per group) were then challenged with 10⁵ TCTs 48 h after the last dose of anti-CD4 or anti-CD8. Parasitemia and survival were followed as described [17].

2.12. Statistical analysis

Statistical significance of comparison of mean values was evaluated by Student's *t*-test or two-way ANOVA with Bonferroni multiple comparisons, using GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA).

3. Results

3.1. Immunoinformatic analysis: selection of a MASP peptide with partially overlapping B-cell, MHC-I, and MHC-II epitopes

Through proteomic analysis of *T. cruzi* extracellular vesicles [9] secreted by TCTs (TCT_{EV}) (Almeida et al., unpublished data), we were able to identify with high confidence a 18-mer peptide (SLLSDAENPGGEVFNDNK) belonging to a MASP family member (XP_820771.1). We then performed immunoinformatic analysis for prediction of human and mouse (C3H/HeNsd, haplotype H^k) B-cell, MHC-I, and MHC-II epitopes in the entire MASP-XP_820771.1 mature protein sequence. Our goal was to find peptide(s) with potential partially overlapping B-cell, and MHC-I and MHC-II epitopes. Coincidentally, our initial immunoinformatic analyses showed that one of the most promising MASP

(XP_820771.1 sequence regions containing several predicted overlapping B- and T-cell epitopes was formed by a 20-mer peptide (DAENPGGEVFNDNKKGLSRV), encompassing most (14 residues, underlined) of the peptide (SLLSDAENPGGEVFNDNK) found by proteomic analysis of TCT_{EV} (data not shown). B-Cell epitope prediction using various IEDB algorithms indicated at least five intermediate-/high-probability B-cell epitopes (Fig. 1). MHC-I Epitope prediction using resulted in the identification of at least six high-probability peptides. Finally, MHC-II epitope prediction led to the identification of at least five intermediate-/high-affinity binders for various DRB1 alleles (Fig. 1 and data not shown). Based on these promising immunoinformatic results, we decided to evaluate the synthetic MASPpep conjugated to KLH (MASPpep-KLH) as potential vaccine candidate for ChD.

3.2. Humoral immune response to MASPpep-KLH

Pooled sera were collected from mice immunized with MASPpep-KLH, MASPpep-KLH/Al, KLH, or Al, and from placebo group (PBS) 12 days after last immunization to determine specific IgG levels by CL-ELISA. To assess the production of antibodies against the MASPpep in human infection, serum pools from ChDSP and NHSP were used as positive and negative controls, respectively. In contrast to the controls (KLH, Al, and PBS), mice immunized with MASPpep-KLH or MASPpep-KLH/Al showed MASPpep-specific antibodies (Fig. 2A). Antiserum from animals immunized with MASPpep-KLH/Al showed a slightly higher but not significant reactivity to MASPpep-KLH than that of MASPpep-KLH group. In contrast to NHSP, ChDSP strongly reacted to MASPpep-KLH, indicating that the 20-mer MASPpep contains one or more B-cell epitopes that induce antibodies in *T. cruzi*-infected humans (Fig. 2A). Altogether, these results experimentally validate the B-cell epitope prediction for MASPpep.

3.3. Immunoglobulin isotyping

Immunoglobulin isotyping was also of interest since the acute phase of the disease is characterized for hypergammaglobulinemia, especially higher titers of IgA, IgM, and IgG, in particular IgG1, IgG2a, and IgG2b isotypes, which are considered to have antiparasitic (trypanolytic) properties [17]. We observed that higher levels of IgG2a ($p<0.05$), IgA ($p<0.05$), and IgM ($p<0.01$) were detected in MASPpep-KLH- and MASPpep-KLH/Al-immunized, non-infected mice as compared to control animals (data not shown).

3.4. Trypomastigote lysis assay

It is well established that patients with chronic ChD as well as chronically infected mice produce lytic (IgG) antibodies that are able to induce lysis in trypomastigotes, thus representing a key mechanism for controlling *T. cruzi* infection [18-21]. Here, we observed that immunization with MASPpep-KLH induces antibodies capable of specifically recognizing MASP in different parasite stages, namely TCTs, epimastigotes and intracellular amastigotes, as well as a MASP molecule secreted in TCT_{EV} (Fig. 2B). In this regard, the trypomastigote lysis assay was performed to address the possible lytic activity of anti-MASPpep-KLH antibodies produced by immunize mice. Fig. 2C shows that MASPpep-KLH antiserum is able to lyse trypomastigotes 3.4-fold higher than the placebo antiserum.

No lytic activity was observed with sera from mice treated with KLH alone (data not shown). Interestingly, the lytic activity of MASPpep-KLH antiserum seemed to be independent on complement, as previously reported for lytic anti- α -galactosyl antibodies against *T. cruzi* mucins [19, 21].

3.5. Parasitemia and survival rate

Next, we examined whether immunization with MASPpep-KLH, in the presence or not of an adjuvant, could induce protection against *T. cruzi* infection in mice. Female mice (C3H/HeNsd) were immunized (i.p.) with MASPpep-KLH or MASPpep-KLH/Al, and then infected with a lethal dose of *T. cruzi* Y strain TCTs (1×10^6), and the parasitemia was followed for 20 days post-infection (dpi). MASPpep-KLH- or MASPpep-KLH/Al-immunized mice did not show any significant decrease in parasitemia upon vaccination (Fig. 3A). However, when the survival rate was evaluated up to a year (experiment endpoint), ~86% of MASPpep-KLH-immunized mice were alive (Fig. 3B). Intriguingly, mice immunized with MASPpep-KLH/Al showed much lower survival rate, similar to that of control groups. In these, all mice died between 15 and 30 dpi. We speculate that early death of mice immunized with MASPpep-KLH/Al could be due to strong inflammatory response triggered by both the adjuvant and parasite.

To test whether the MASPpep-KLH vaccine could produce sterile protection to the immunized mice, qRT-PCR was performed. Heart, liver, and spleen were removed from all animals at the humane endpoint (Fig. 3C). Animals immunized with MASPpep-KLH/Al and, particularly, with MASPpep-KLH showed significantly much lower amount of parasite equivalents in the three organs analyzed. More remarkably, mice immunized with MASPpep-KLH survived past a year and showed a substantial decrease in parasite load in the heart ($\downarrow 97\%$), liver ($\downarrow 85\%$), and spleen ($\downarrow 92\%$) as compared to the control KLH-treated group (Fig. 3C).

3.7. Evaluation of cytokines

Stimulation of selected proinflammatory and anti-inflammatory cytokines plays a crucial role in resistance against *T. cruzi* [22-27]. Therefore, we analyzed the cytokine profile of mice immunized with MASPpep-KLH or MASPpep-KLH/Al, aiming to understand why mice immunized with MASPpep-KLH/Al had much lower survival rate than animals immunized with MASPpep-KLH (Fig. 3B), despite that both groups showed significantly reduced parasite load in heart, liver, and spleen. Four weeks after the last immunization, serum was collected and cytokines (IL-1 α , IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12, IL-17, IFN- γ , and TNF- α) were evaluated by ELISA. We observed that IL-4, IL-10, IFN- γ , IL-12, and IL-17 were significantly increased in both MASPpep-KLH and MASPpep-KLH/Al when compared to control animals (PBS, KLH, and Al) (Fig. 4). Interestingly, IL-4 was considerably augmented in MASPpep-KLH/Al group, whereas IL-17 level was not significantly altered in this group in comparison to MASPpep-KLH group. On the other hand, immunization with MASPpep-KLH induced higher levels of proinflammatory cytokines, such as IFN- γ , IL-12, and IL-17, than those elicited by MASPpep-KLH/Al. Taken together, our results indicate that the MASP-pep-KLH vaccine induces a more

balanced profile of anti-inflammatory and proinflammatory cytokines that are known to play an important role in protection against *T. cruzi* infection [22-28].

3.8. *In vivo* depletion of CD4⁺ and CD8⁺ T cells

To test whether T cells might play a role in the protection elicited by MASP-pep-KLH, we carried out *in vivo* depletion of CD4⁺ or CD8⁺ T cells using specific mAbs, one week after the last immunization dose and 48 h prior to the challenge with trypomastigotes. Control (KLH-treated) animals depleted of either CD4⁺ or CD8⁺ T cells showed high parasitemia (peaking at day 5) and died around day 15 post-infection (Fig. 5A-D). Conversely, mice immunized with MASPpep-KLH and treated with anti-CD4 mAbs before the challenge showed 100% survival rate and much lower parasitemia than KLH-treated controls, indicating that most likely CD4 are not essential for the protection induced by the MASPpep-KLH vaccine (Fig. 5A, B). On the other hand, animals immunized with MASPpep-KLH and treated with anti-CD8 mAbs prior to the challenge showed a parasitemia peak 17 dpi and died 45 dpi. This result strongly indicates that CD8⁺ T cells play a critical role in the protection against *T. cruzi* elicited by MASPpep-KLH.

4. Discussion

In this study, we showed that a synthetic 20-mer peptide (MASPpep) containing potential overlapping B-cell, and CD4 and CD8 T-cell epitopes could induce both protective B cell- and T cell-mediated immunity against *T. cruzi* infection in mice. Previous studies have demonstrated that longer synthetic peptides (15-mer) might contain overlapping B- and T-cell epitopes that can effectively prime both humoral and T cell-mediated immune responses [29-32]. The mechanisms by which these events occur in the case of MASPpep-KLH were not defined here. However, we believe that most likely the activation of B cells occurs following the classical recognition of the B-cell epitope (s) on MASPpep-KLH by the B-cell receptor [33]. On the other hand, the activation of CD8⁺ T cells by MASPpep-KLH could occur through the conventional cross-presentation by dendritic cells (DCs), following internalization of the antigenic complex, processing, and loading onto MHC I [34], or even through the non-conventional IgG-mediated cross-presentation by DCs [35].

The synthetic MASPpep used in this study was derived from a MASP family member (XP_820771.1), which like several other MASP molecules [9] was found to be secreted to the extracellular milieu by infective parasite forms. A recent study has shown that MASP52, another MASP family member, is also secreted by metacyclic trypomastigotes and seems to play a role in host-cell invasion [36]. In another study, it was observed that antibodies against a conserved motif of MASPs reacted to the supernatant of parasites, further corroborating the observations that MASP molecules are constitutively shed by the parasite [37]. Furthermore, proteomic analyses performed by our group and others clearly showed that MASP are major surface molecules in TCTs [7, 38, 39]. Interestingly, Murta *et al.* [40] have shown that MASP expression is upregulated in drug-resistant *T. cruzi* strains. Together, these findings strongly indicate that MASP molecules are very attractive targets for development of a ChD vaccine.

Protective trypanolytic antibodies play an importance role in both the acute and chronic stage of ChD [18-20, 41, 42]. Serum transferred from chronically infected to naïve mice reduces parasitemia and prolongs survival after challenge [41, 43]. In addition, in the acute phase of the disease B cells play a valuable role in recruiting CD4⁺ and CD8⁺ T cells and maintaining memory and effector T cells [44]. Here, we showed that mice immunized with MASPpep-KLH produced protective antibodies capable of killing trypomastigote in absence of complement, in a fashion similar to lytic anti- α -galactosyl antibodies elicited against *T. cruzi* trypomastigote GPI-mucins [19, 21]. Our further examination of the Ig isotype revealed that IgM, IgG2A, and IgA had significant high titers in animals immunized with MASPpep-KLH (data not shown), which after challenge showed 86% survival rate. These results are supported by the literature describing the anti-parasitic effects particularly of IgM and IgG in murine and human ChD [17, 19, 20, 42].

Here, we have also analyzed the profile of cytokines relevant to the control of *T. cruzi* infection. We showed that IL-4 was produced at higher levels in the serum of mice immunized with MASPpep-KLH and MASPpep-KLH/Al when compared to the control groups. IL-4 is a stimulating factor for the differentiation and proliferation of B cells [45], supporting the higher antibody production observed in the two MASPpep-KLH-immunized groups. Although IL-4 per se is not a major determinant of resistance to *T. cruzi* infection or disease outcome, when expressed together with IL-10 it is able to control myocarditis [22]. It has also been shown that when IL-4 is more dominant than IL-10, parasitemia is enhanced [23]. This observation is very appropriate in the context of our MASPpep-KLH vaccine, which was able to elicit much higher levels IL-10 (2,854 pg/ml serum) than IL-4 (594 pg/ml serum). During infection, IL-10 can actually increase parasitemia; however, this cytokine is essential for host survival. Mice depleted of IL-10 show lower parasitemia, but higher mortality [28]. IL-10 is also able to prevent inflammatory damage and, thus, mortality [24].

Both IL-12 and IFN- γ are critical for controlling parasitemia, inflammatory response, and mortality in experimental ChD [25, 28]. IL-12-knockout mice show higher parasitemia and mortality [23, 46]. Administration of recombinant IFN- γ to mice increases resistance to *T. cruzi*, whereas neutralization of it results in higher susceptibility [47]. In this study, both IL-12 and IFN- γ were produced at higher levels in mice immunized with MASPpep-KLH as compared to the control animals. Likewise, IL-17 levels were significantly higher in MASPpep-KLH-immunized mice than in the controls. This result is relevant, because IL-17 helps to reduce the strong inflammatory response and mortality in *T. cruzi* infection [26]. Furthermore, IL-17 plays an important role in regulating parasite-induced myocarditis, parasitemia, and survival by modulating the Th1 response against the parasite [49, 50].

Both CD8⁺ and CD4⁺ T lymphocytes have been established to be relevant for inducing a protective immune response against *T. cruzi* [51-57]. Our data indicate that the MASPpep-KLH vaccine is probably protecting through stimulation of CD8⁺ T cells, since mice immunized with MASPpep-KLH and then depleted of CD8⁺ T cells were very susceptible to *T. cruzi* infection and died 45 days after the challenge. Conversely, mice immunized with MASPpep-KLH and depleted of CD4⁺ T cells survived the lethal challenge with *T. cruzi*.

In conclusion, here we have provided evidence that a synthetic peptide-based vaccine, MASPpep-KLH, is capable to effectively controlling *T. cruzi* infection, prolonging survival, and possibly reducing disease progression. The MASPpep-KLH vaccine was shown to elicit an ideal immune stimulation, engaging both humoral and cellular responses. Therefore, we foresee that efforts in the optimization of this vaccine candidate by improving the delivery system, immunization protocols, adjuvant and carrier molecules, might eventually provide a fully protective, sterile vaccine against ChD.

Acknowledgments

We are very grateful to Dr. Sid Das (UTEP) for continuous support and manuscript revision, and Dr. Joaquim Gascon and Dr. Montserrat Gállego (CRESIB/Hospital Clinic/Universitat de Barcelona) for kindly providing the human sera. This study was supported by NIH grants # 2S06GM00812-37 (to ICA and RAM), R01AI070655-A5 and R01AI070655-A5S1 (to ICA), and 5G12MD007592 (to BBRC/UTEP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (to AFM), and by UTEP's University Research Incentive grant. CS was supported by the Howard Hughes Medical Institute Mentor Fellowship. We are thankful to the Biomolecule Analysis and Cell Biology, Screening and Imaging Core Facilities, and Statistical Consulting Laboratory (SCL) at the BBRC/UTEP (NIH/NIMHD grant 5G12MD007592).

References

1. Rassi A Jr, Rassi A, Marcondes de Rezende J. American trypanosomiasis (Chagas disease). Infectious disease clinics of North America. 2012; 26:275–91. [PubMed: 22632639]
2. Vazquez-Chagoyan JC, Gupta S, Garg NJ. Vaccine development against *Trypanosoma cruzi* and Chagas disease. Advances in parasitology. 2011; 75:121–46. [PubMed: 21820554]
3. Lee BY, Bacon KM, Wateska AR, Bottazzi ME, Dumonteil E, Hotez PJ. Modeling the economic value of a Chagas' disease therapeutic vaccine. Human vaccines & immunotherapeutics. 2012; 8:1293–301. [PubMed: 22894964]
4. Acosta-Serrano, A.; Hutchinson, C.; Nakayasu, ES.; Almeida, IC.; Carrington, M. Comparison and evolution of the surface architecture of trypanosomatid parasites. In: Barry, JD.; Mottram, JC.; McCulloch, R.; Acosta-Serrano, A., editors. Trypanosomes: After the genome. Norwich, UK: Horizon Scientific Press; 2007. p. 319-37.
5. El-Sayed NM, Myler PJ, Bartholomeu DC, Nilsson D, Aggarwal G, Tran AN, et al. The genome sequence of *Trypanosoma cruzi*, etiologic agent of Chagas disease. Science. 2005; 309:409–15. [PubMed: 16020725]
6. De Pablos LM, Osuna A. Multigene families in *Trypanosoma cruzi* and their role in infectivity. Infection and immunity. 2012; 80:2258–64. [PubMed: 22431647]
7. Nakayasu ES, Sobreira TJ, Torres R Jr, Ganiko L, Oliveira PS, Marques AF, et al. Improved proteomic approach for the discovery of potential vaccine targets in *Trypanosoma cruzi*. Journal of proteome research. 2012; 11:237–46. [PubMed: 22115061]
8. Andrews NW, Colli W. Adhesion and interiorization of *Trypanosoma cruzi* in mammalian cells. J Protozool. 1982; 29:264–9. [PubMed: 7047731]
9. Bayer-Santos E, Aguilar-Bonavides C, Rodrigues SP, Cordero EM, Marques AF, Varela-Ramirez A, et al. Proteomic analysis of *Trypanosoma cruzi* secretome: characterization of two populations of extracellular vesicles and soluble proteins. Journal of proteome research. 2013; 12:883–97. [PubMed: 23214914]
10. Marques, AF.; Nakayasu, ES.; Almeida, IC. Purification of extracellular and intracellular amastigotes of *Trypanosoma cruzi* from mammalian host-infected cells. Protocol Exchange. <http://www.nature.com/protocolexchange/protocols/22402011>
11. Singh H, Raghava GP. ProPred1: prediction of promiscuous MHC Class-I binding sites. Bioinformatics. 2003; 19:1009–14. [PubMed: 12761064]
12. Brener Z. Therapeutic activity and criterion of cure on mice experimentally infected with *Trypanosoma cruzi*. Revista do Instituto de Medicina Tropical de Sao Paulo. 1962; 4:389–96. [PubMed: 14015230]

13. Almeida IC, Covas DT, Soussumi LM, Travassos LR. A highly sensitive and specific chemiluminescent enzyme-linked immunosorbent assay for diagnosis of active *Trypanosoma cruzi* infection. *Transfusion*. 1997; 37:850–7. [PubMed: 9280332]
14. Pinazo MJ, Munoz J, Posada E, Lopez-Chejade P, Gallego M, Ayala E, et al. Tolerance of benznidazole in treatment of Chagas' disease in adults. *Antimicrobial agents and chemotherapy*. 2010; 54:4896–9. [PubMed: 20823286]
15. Almeida IC, M S, Gorin PA, Travassos LR. Complement-mediated lysis of *Trypanosoma cruzi* trypomastigotes by human anti-alpha-galactosyl antibodies. *J Immunol*. 1991; 146:2394–400. [PubMed: 1706399]
16. Cummings KL, Tarleton RL. Rapid quantitation of *Trypanosoma cruzi* in host tissue by real-time PCR. *Molecular and biochemical parasitology*. 2003; 129:53–9. [PubMed: 12798506]
17. Vasconcelos JR, Hiyane MI, Marinho CR, Claser C, Machado AM, Gazzinelli RT, et al. Protective immunity against *trypanosoma cruzi* infection in a highly susceptible mouse strain after vaccination with genes encoding the amastigote surface protein-2 and trans-sialidase. *Hum Gene Ther*. 2004; 15:878–86. [PubMed: 15353042]
18. Kretzli AU. The utility of anti-trypomastigote lytic antibodies for determining cure of *Trypanosoma cruzi* infections in treated patients: an overview and perspectives. *Memorias do Instituto Oswaldo Cruz*. 2009; 104 Suppl 1:142–51. [PubMed: 19753469]
19. Almeida IC, Ferguson MA, Schenkman S, Travassos LR. Lytic anti-alpha-galactosyl antibodies from patients with chronic Chagas' disease recognize novel O-linked oligosaccharides on mucin-like glycosyl-phosphatidylinositol-anchored glycoproteins of *Trypanosoma cruzi*. *Biochem J*. 1994; 304(Pt 3):793–802. [PubMed: 7818483]
20. Almeida IC, Milani SR, Gorin PA, Travassos LR. Complement-mediated lysis of *Trypanosoma cruzi* trypomastigotes by human anti-alpha-galactosyl antibodies. *J Immunol*. 1991; 146:2394–400. [PubMed: 1706399]
21. Pereira-Chioccia VL, Acosta-Serrano A, Correia de Almeida I, Ferguson MA, Souto-Pradon T, Rodrigues MM, et al. Mucin-like molecules form a negatively charged coat that protects *Trypanosoma cruzi* trypomastigotes from killing by human anti-alpha-galactosyl antibodies. *J Cell Sci*. 2000; 113(Pt 7):1299–307. [PubMed: 10704380]
22. Abrahamsohn IA, da Silva AP, Coffman RL. Effects of interleukin-4 deprivation and treatment on resistance to *Trypanosoma cruzi*. *Infection and immunity*. 2000; 68:1975–9. [PubMed: 10722591]
23. Michailowsky V, Silva NM, Rocha CD, Vieira LQ, Lannes-Vieira J, Gazzinelli RT. Pivotal role of interleukin-12 and interferon-gamma axis in controlling tissue parasitism and inflammation in the heart and central nervous system during *Trypanosoma cruzi* infection. *The American journal of pathology*. 2001; 159:1723–33. [PubMed: 11696433]
24. Hunter CA, Ellis-Neyes LA, Slifer T, Kanaly S, Grunig G, Fort M, et al. IL-10 is required to prevent immune hyperactivity during infection with *Trypanosoma cruzi*. *J Immunol*. 1997; 158:3311–6. [PubMed: 9120288]
25. Aliberti JC, Cardoso MA, Martins GA, Gazzinelli RT, Vieira LQ, Silva JS. Interleukin-12 mediates resistance to *Trypanosoma cruzi* in mice and is produced by murine macrophages in response to live trypomastigotes. *Infection and immunity*. 1996; 64:1961–7. [PubMed: 8675294]
26. Tosello Boari J, Amezcua Vesely MC, Bermejo DA, Ramello MC, Montes CL, Cepas H, et al. IL-17RA signaling reduces inflammation and mortality during *Trypanosoma cruzi* infection by recruiting suppressive IL-10-producing neutrophils. *PLoS pathogens*. 2012; 8:e1002658. [PubMed: 22577359]
27. Magalhaes LM, Villani FN, Nunes Mdo C, Gollob KJ, Rocha MO, Dutra WO. High interleukin 17 expression is correlated with better cardiac function in human Chagas disease. *The Journal of infectious diseases*. 2013; 207:661–5. [PubMed: 23204182]
28. Abrahamsohn IA, Coffman RL. *Trypanosoma cruzi*: IL-10, TNF, IFN-gamma, and IL-12 regulate innate and acquired immunity to infection. *Experimental parasitology*. 1996; 84:231–44. [PubMed: 8932773]
29. Arruda LB, Sim D, Chikhlikar PR, Maciel M Jr, Akasaki K, August JT, et al. Dendritic cell-lysosomal-associated membrane protein (LAMP) and LAMP-1-HIV-1 gag chimeras have distinct

- cellular trafficking pathways and prime T and B cell responses to a diverse repertoire of epitopes. *J Immunol.* 2006; 177:2265–75. [PubMed: 16887987]
30. Singh B, Lee KC, Fraga E, Wilkinson A, Wong M, Barton MA. Minimum peptide sequences necessary for priming and triggering of humoral and cell-mediated immune responses in mice: use of synthetic peptide antigens of defined structure. *J Immunol.* 1980; 124:1336–43. [PubMed: 6153678]
 31. da Silva AN, Nascimento EJ, Cordeiro MT, Gil LH, Abath FG, Montenegro SM, et al. Identification of continuous human B-cell epitopes in the envelope glycoprotein of dengue virus type 3 (DENV-3). *PloS one.* 2009; 4:e7425. [PubMed: 19826631]
 32. Reynolds J, Haxby J, Juggapah JK, Evans DJ, Pusey CD. Identification of a nephritogenic immunodominant B and T cell epitope in experimental autoimmune glomerulonephritis. *Clinical and experimental immunology.* 2009; 155:311–9. [PubMed: 19040602]
 33. Avalos AM, Ploegh HL. Early BCR Events and Antigen Capture, Processing, and Loading on MHC Class II on B Cells. *Frontiers in immunology.* 2014; 5:92. [PubMed: 24653721]
 34. Schuette V, Burgdorf S. The ins-and-outs of endosomal antigens for cross-presentation. *Current opinion in immunology.* 2014; 26C:63–8. [PubMed: 24556402]
 35. Baker K, Rath T, Lencer WI, Fiebiger E, Blumberg RS. Cross-presentation of IgG-containing immune complexes. *Cellular and molecular life sciences : CMLS.* 2013; 70:1319–34. [PubMed: 22847331]
 36. De Pablos LM, Gonzalez GG, Solano Parada J, Seco Hidalgo V, Diaz Lozano IM, Gomez Samblas MM, et al. Differential expression and characterization of a member of the mucin-associated surface protein family secreted by *Trypanosoma cruzi*. *Infection and immunity.* 2011; 79:3993–4001. [PubMed: 21788387]
 37. Bartholomeu DC, Cerqueira GC, Leao AC, daRocha WD, Pais FS, Macedo C, et al. Genomic organization and expression profile of the mucin-associated surface protein (masp) family of the human pathogen *Trypanosoma cruzi*. *Nucleic acids research.* 2009; 37:3407–17. [PubMed: 19336417]
 38. Atwood JA 3rd, Minning T, Ludolf F, Nuccio A, Weatherly DB, Alvarez-Manilla G, et al. Glycoproteomics of *Trypanosoma cruzi* trypomastigotes using subcellular fractionation, lectin affinity, and stable isotope labeling. *Journal of proteome research.* 2006; 5:3376–84. [PubMed: 17137339]
 39. Atwood JA 3rd, Weatherly DB, Minning TA, Bundy B, Cavola C, Oppenheimer FR, et al. The *Trypanosoma cruzi* proteome. *Science.* 2005; 309:473–6. [PubMed: 16020736]
 40. Murta SM, Nogueira FB, Dos Santos PF, Campos FM, Volpe C, Liarte DB, et al. Differential gene expression in *Trypanosoma cruzi* populations susceptible and resistant to benznidazole. *Acta tropica.* 2008; 107:59–65. [PubMed: 18501872]
 41. Kretzli AU, Brener Z. Protective effects of specific antibodies in *Trypanosoma cruzi* infections. *J Immunol.* 1976; 116:755–60. [PubMed: 815433]
 42. Gazzinelli RT, Pereira ME, Romanha A, Gazzinelli G, Brener Z. Direct lysis of *Trypanosoma cruzi*: a novel effector mechanism of protection mediated by human anti-gal antibodies. *Parasite Immunol.* 1991; 13:345–56. [PubMed: 1717927]
 43. Kierszenbaum F. Protection of congenitally athymic mice against *Trypanosoma cruzi* infection by passive antibody transfer. *The Journal of parasitology.* 1980; 66:673–5. [PubMed: 6775071]
 44. Boscardin SB, Torrecilhas AC, Manarin R, Revelli S, Rey EG, Tonelli RR, et al. Chagas' disease: an update on immune mechanisms and therapeutic strategies. *Journal of cellular and molecular medicine.* 2010; 14:1373–84. [PubMed: 20070438]
 45. Wurster AL, Rodgers VL, White MF, Rothstein TL, Grusby MJ. Interleukin-4-mediated protection of primary B cells from apoptosis through Stat6-dependent up-regulation of Bcl-xL. *The Journal of biological chemistry.* 2002; 277:27169–75. [PubMed: 12023955]
 46. Galvao Da Silva AP, Jacysyn JF, De Almeida Abrahamssohn I. Resistant mice lacking interleukin-12 become susceptible to *Trypanosoma cruzi* infection but fail to mount a T helper type 2 response. *Immunology.* 2003; 108:230–7. [PubMed: 12562332]

47. Hoft DF, Schnapp AR, Eickhoff CS, Roodman ST. Involvement of CD4(+) Th1 cells in systemic immunity protective against primary and secondary challenges with *Trypanosoma cruzi*. *Infection and immunity*. 2000; 68:197–204. [PubMed: 10603388]
48. Boari JT, Amezcua Vesely MC, Bemejo DA, Ramello MC, Montes CL, Cejas H, Gruppi A, Acosta Rodriguez EV. IL-17RA Signaling Reduces Inflammation and Mortality During *Trypanosoma cruzi* Infection by Recruiting Suppressive IL-10-Producing Neutrophils. *PLoS pathogens*. 2012; 8:e1002658. [PubMed: 22577359]
49. da Matta Guedes PM, Gutierrez FR, Maia FL, Milanezi CM, Silva GK, Pavanelli WR, et al. IL-17 produced during *Trypanosoma cruzi* infection plays a central role in regulating parasite-induced myocarditis. *PLoS neglected tropical diseases*. 2010; 4:e604. [PubMed: 20169058]
50. Miyazaki Y, Hamano S, Wang S, Shimano Y, Iwakura Y, Yoshida H. IL-17 is necessary for host protection against acute-phase *Trypanosoma cruzi* infection. *J Immunol*. 2010; 185:1150–7. [PubMed: 20562260]
51. Rottenberg ME, Bakhiet M, Olsson T, Kristensson K, Mak T, Wigzell H, et al. Differential susceptibilities of mice genomically deleted of CD4 and CD8 to infections with *Trypanosoma cruzi* or *Trypanosoma brucei*. *Infection and immunity*. 1993; 61:5129–33. [PubMed: 8225589]
52. Tarleton RL, Koller BH, Latour A, Postan M. Susceptibility of beta 2-microglobulin-deficient mice to *Trypanosoma cruzi* infection. *Nature*. 1992; 356:338–40. [PubMed: 1549177]
53. Junqueira C, Caetano B, Bartholomeu DC, Melo MB, Ropert C, Rodrigues MM, et al. The endless race between *Trypanosoma cruzi* and host immunity: lessons for and beyond Chagas disease. *Expert reviews in molecular medicine*. 2010; 12:e29. [PubMed: 20840799]
54. Cunha-Neto E, Teixeira PC, Fonseca SG, Bilate AM, Kalil J. Myocardial gene and protein expression profiles after autoimmune injury in Chagas' disease cardiomyopathy. *Autoimmunity reviews*. 2011; 10:163–5. [PubMed: 20883825]
55. de Araujo FF, Vitelli-Avelar DM, Teixeira-Carvalho A, Antas PR, Assis Silva Gomes J, Sathler-Avelar R, et al. Regulatory T cells phenotype in different clinical forms of Chagas' disease. *PLoS neglected tropical diseases*. 2011; 5:e992. [PubMed: 21655351]
56. Morrot A, Barreto de Albuquerque J, Berbert LR, de Carvalho Pinto CE, de Meis J, Savino W. Dynamics of Lymphocyte Populations during *Trypanosoma cruzi* Infection: From Thymocyte Depletion to Differential Cell Expansion/Contraction in Peripheral Lymphoid Organs. *Journal of tropical medicine*. 2012; 2012:747185. [PubMed: 22505943]
57. Perez AR, Morrot A, Berbert LR, Terra-Granado E, Savino W. Extrathymic CD4+CD8+ lymphocytes in Chagas disease: possible relationship with an immunoendocrine imbalance. *Annals of the New York Academy of Sciences*. 2012; 1262:27–36. [PubMed: 22823432]

Fig. 1. Partially overlapping B-cell, MHC-I and MHC-II epitopes on MASP-XP_820771.1-derived peptide, as predicted by immunoinformatic analyses.

Fig. 2.

Specific antibody responses against MASPpep in mice and humans. (A) Anti-MAASPpep-KLH-specific antibodies were detected by CL-ELISA in sera of MASPpep-KLH-immunized mice and patients with chronic Chagas disease. MASPpep-KLH (10 μ g/well) was incubated with a serum pool (at 1:100 dilution) from the specific immunized mouse group (PBS, KLH, AI, MASPpep-KLH, or MASPpep-KLH/AI) or with a serum pool (at 1:100 dilution) from patients with chronic Chagas disease (ChSP) or healthy individuals (NHSP). RLU, Relative luminescence units. Statistical analysis: Student's *t*-test. *, $p < 0.05$; ***, $p < 0.001$. (B) Western blotting analysis with pooled sera from MASPpep-KLH-immunized animals (MAASPpep-KLH antiserum). TCT, Host cell-derived trypomastigote lysate immunoprecipitated with MASPpep-KLH antiserum; TCT_{EV}, TCT-derived extracellular vesicles purified by differential ultracentrifugation from conditioned culture medium. Epi and Ama, whole-cell lysates from epimastigotes and intracellular amastigotes, respectively. (C) Trypomastigote lysis assay. TCTs (1×10^7 /ml) were incubated with pooled sera from non-immunized (Placebo, PBS) or MASPpep-KLH-immunized mice. The assay was performed in the presence of inactive (iC) or active (aC) complement. Live, untreated TCTs were used as negative control (-C), whereas TCTs treated with 200 μ M H₂O₂ were used as positive controls (+C). All experiments were performed with *T. cruzi* Y strain. The data are representative of three independent experiments.

Fig. 3.

Parasitemia (A), Kaplan-Meier curves for survival (B), and parasite load (C) in mice immunized with MASPpep-KLH or MASPpep-KLH/AI, and then infected with *T. cruzi* trypomastigotes (1×10^6 cells). (A) Parasitemia levels are shown as trypomastigotes/ml. Each point corresponds to the mean parasitemia level. Blood parasitemia was followed in all four groups ($n=4$ animals per group). From the 3rd to the 20th day post-infection, five microliters of blood was taken from the tail and the number of trypomastigotes was determined. (B) Survival was monitored daily in all groups ($n=4$ animals per group). Three independent studies were performed with similar results. (C) Parasite load as measured by qRT-PCR of whole heart, liver, and spleen of mice immunized with MASPpep-KLH or MASPpep-KLH/AI/OH, and infected with *T. cruzi* trypomastigotes (Y strain). Parasite load represents the equivalent of parasites in 50 ng gDNA obtained from each specific organ. Each determination was done in duplicate. Statistical analysis: two-way Anova with Bonferroni multiple comparisons. *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.0001$.

Fig. 4.

Cytokine profile of immunized mice following parasite challenge. Anti-inflammatory (IL-4 and IL-10) and proinflammatory (IFN- γ , IL-12, and IL-17) cytokines were assayed in triplicate by ELISA four weeks after the last immunization and approx. two weeks after the challenge. Each bar represents the mean value $\pm$ SEM ($n=4$ animals per group) for each treatment. Statistical analysis: Student's t-test. *, $p < 0.05$; ns, not significant.

Fig. 5.

Effect of in vivo depletion of CD4⁺ or CD8⁺ T cells in mice immunized with KLH or MASPpep-KLH. Kaplan-Meier curves for survival and parasitemia of mice immunized with MASPpep-KLH or KLH, and then treated with anti-CD4 (A and B) or anti-CD8 mAb (C and D). N=2 animals per group.