Previously Unrecognized Vaccine Candidates Control *Trypanosoma cruzi* Infection and Immunopathology in Mice

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*Trypanosoma cruzi* is the etiologic agent of Chagas’ disease, a major health problem in Latin America and an emerging infectious disease in the United States. Previously, we screened a *T. cruzi* sequence database by a computational-bioinformatic approach and identified antigens that exhibited the characteristics of good vaccine candidates. In this study, we tested the vaccine efficacy of three of the putative candidate antigens against *T. cruzi* infection and disease in a mouse model. C57BL/6 mice vaccinated with *T. cruzi* G1 (TcG1)-, TcG2-, or TcG4-encoding plasmids and cytokine (interleukin-12 and granulocyte-macrophage colony-stimulating factor) expression plasmids elicited a strong Th1-type antibody response dominated by immunoglobulin G2b (IgG2b)/IgG1 isotypes. The dominant IgG2b/IgG1 antibody response was maintained after a challenge infection and was associated with 50 to 90% control of the acute-phase tissue parasite burden and an almost undetectable level of tissue parasites during the chronic phase, as determined by a sensitive *T. cruzi* 18S rRNA gene-specific real-time PCR approach. Splenocytes from vaccinated-and-infected mice, compared to unvaccinated-and-infected mice, exhibited decreased (−50% lower) proliferation and gamma interferon (IFN-\(\gamma\)) production when stimulated in vitro with *T. cruzi* antigens, thus suggesting that protection from challenge infection was not provided by an active T-cell response. Subsequently, the serum and cardiac levels of IFN-\(\gamma\) and tumor necrosis factor alpha and infiltration of inflammatory infiltrate in the heart were decreased in vaccinated mice during the course of infection and chronic disease development. Taken together, these results demonstrate the identification of novel vaccine candidates that provided protection from *T. cruzi*-induced immunopathology in experimental mice.

*Trypanosoma cruzi* is the causative agent of Chagas’ disease in humans, which is a major health problem in Latin America and is considered an emerging infectious disease in the United States (19, 20). Infection with *T. cruzi* results in an acute parasitemia that is generally associated with mild illness and followed by an intermediate phase wherein infected individuals develop the clinical form of Chagas’ disease, which results in congestive heart failure-related deaths of young adults in areas of high endemicity every year (19, 20). No vaccines are available.

A variety of experimental animal models have been used to identify the effector mechanisms required for the control of *T. cruzi* infection (reviewed in reference 35). These studies attributed potential roles to all of the components of the immune system, i.e., granulocytes, natural killer cells, the action of lytic antibodies, and CD4\(^+\) and CD8\(^+\) T-cell subsets, in the control of *T. cruzi* infection. Others have suggested that the persistent asitemia that is generally associated with mild illness and followed by an intermediate phase wherein infected individuals develop the clinical form of Chagas’ disease, which results in congestive heart failure-related deaths of young adults in areas of endemicity every year (19, 20). No vaccines are available.

From these studies that a finely tuned, regulated activation of the immune system capable of controlling *T. cruzi* infection and not having adverse effects on the host cellular components would be essential to prevent the progression of chronic Chagas’ disease.

Efforts toward subunit vaccine development against *T. cruzi* have mainly focused on antigens that are expressed on the plasma membrane of the parasite, attached by a glycosylphosphatidylinositol (GPI) anchor. GPI proteins are considered good antigenic targets because they are abundantly expressed in the infective and intracellular stages of *T. cruzi* (36) and were shown to be recognized by both the humoral and cellular arms of the immune system in infected hosts (14, 22). Subsequently, several defined *T. cruzi* GPI-anchored proteins were tested as vaccine candidates. Recombinant GPI proteins, e.g., GP90, GP56, and GP82 (18, 29, 30), and DNA expression plasmids encoding GPI proteins, e.g., ASP-1, ASP-2, TSA-1, and trans-sialidase (TS) (8, 11, 13), were demonstrated to elicit various degrees of resistance to *T. cruzi* infection in different animal models.

A majority of the protective candidate antigens identified so far belong to the TS gene family of *T. cruzi*. The attempts to enhance the protective efficacy by codelivery of TS family members as a multiantigen vaccine with or without cytokine adjuvants (13) or by a DNA prime-protein boost approach (34) have not been successful. The limited protective efficacy of vaccine cocktails was attributed to the fact that TS family members with shared epitopes represent only a minor proportion of all of the possible target molecules in *T. cruzi*, and
additional candidate antigens will be required to elicit an immune response of sufficient magnitude to efficiently control *T. cruzi* infection.

We have previously conducted an in silico analysis of a *T. cruzi* sequence database to identify putative vaccine candidates. The selection strategy was designed to disregard TS family members and select candidate antigens that exhibit the characteristics of GPI-anchored or secreted proteins (2). Of the 19 selected sequences, 8 (*T. cruzi* G1 [TeG1] to TeG8) were phylogenetically conserved in diverse *T. cruzi* strains and expressed in the infective and intracellular mammalian stages of *T. cruzi* (2), TeG1-, TeG2-, and TeG4-encoded antigens were expressed on the plasma membrane of the mammalian stages of *T. cruzi* (trypanostagote/amastigote) and elicited significant levels of antiparasite lytic antibody responses in mice (2), thus forming the basis for testing of their vaccine efficacy in this study. Our data show that the three antigens (TeG1, TeG2, and TeG4), delivered as a DNA vaccine, elicited effective immunity that provided resistance to acute *T. cruzi* infection in a murine model. Sterile immunity was not achieved; however, vaccinated mice exhibited moderate to no cardiac immunopathology and tissue damage. These results validate the applicability of a rational computational approach in the identification of novel vaccine candidates and demonstrate that vaccines capable of controlling the tissue parasite burden below a threshold level will be effective in preventing the chronic pathology of the heart in Chagas’ disease.

**MATERIALS AND METHODS**

**Parasites and mice.** Trypomastigotes of *T. cruzi* (Stylvio X 10/4 strain) were maintained and propagated by continuous in vitro passage in C2C12 cells. C57BL/6 female mice (6 to 8 week old) were obtained from Harlan Labs (Indianapolis, IN). Animal experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the University of Texas Medical Branch Animal Care and Use Committee.

**T. cruzi genes and plasmid construction.** TeG1-encoded protein (accession no. AY727914) is 76% identical to the *Leishmania donovani* 23-kDa cell surface protein (accession no. X86551) (5) and also showed significant homology to the 23-kDa cell surface protein (accession no. XM_816508) suggested to encode a hypothetical protein (291 amino acids) (10). The *T. cruzi* genes and plasmid construction. TeG1-encoded protein (accession no. AY727914) is 76% identical to the *Leishmania donovani* 23-kDa cell surface protein (accession no. X86551) (5) and also showed significant homology to the 23-kDa cell surface protein (accession no. XM_816508) suggested to encode a hypothetical protein (291 amino acids) (10).

The cdDNAs for TeG1, TeG2, and TeG4 were cloned into eukaryotic expression plasmid pCDNA 3.1 (2) for vaccination purposes. The eukaryotic expression plasmid encoding the murine cytokine interleukin-12 (IL-12) (pcDNA3/mpsp5 and pCDNA3/mpsp4) and murine granulocyte-macrophage colony-stimulating factor (GM-CSF) (pCMV1.GM-CSF) were previously described (13). Recombinant plasmids were transformed into Escherichia coli DH5α competent cells grown in L broth containing 100 μg/ml ampicillin and purified by anion-exchange chromatography with the Qiagen maxi prep kit (Qiagen, Chatsworth, CA) according to the manufacturer’s specifications.

**DNA immunization and challenge infection.** C57BL/6 mice were injected in the quadriceps muscles thrice at 2-week intervals with antigen-encoding plasmids (pCDNA3.TcG1, pCDNA3.TcG2, and pCDNA3.TcG4, individually or in combination; 25 μg per DNA/mouse) and cytokine-encoding plasmids (pCDNA3.mp35, pCDNA3.mp80 [IL-12], and pCMV1.GM-CSF; 25 μg per plasmid DNA/mouse). Two weeks after the last immunization, mice were challenged with culture-derived *T. cruzi* trypanostagotes (2.5 × 10⁶/mouse, intraperitoneally) and sacrificed at days 30, 75, and 120 postinfection (p.i.), corresponding to the acute phase of peak parasitemia, the intermediate phase of immune control of parasites, and the chronic phase of disease development, respectively.

Serum and tissue samples (heart and skeletal muscle) were harvested and stored at −80°C until further use.

**Antibody response.** Serum immunoglobulin G (IgG) antibody response in immunized mice was monitored by use of an enzyme-linked immunosorbent assay (ELISA) (2). Briefly, 96-well U-bottom plates were coated with *T. cruzi* lysate (5 × 10⁶ parasite equivalents/well) and blocked with 1% nonfat dry milk (NFDM). Plates were then sequentially incubated with test sera (1:50 dilution in 0.5% NFDM, 100 μl/well) and horseradish peroxidase-conjugated goat anti-mouse IgG antibody, color was developed with 100 μl/well Sure Blue TMB substrate (KPL, MD), and the ensuing antibody response was monitored at 650 nm with an automated microplate reader ( Molecular Devices). To identify the antibody subtypes, plates were coated and incubated with sera as described above and then incubated with a biotin-conjugated goat anti-mouse IgG1, IgG2a, or IgG2b secondary antibody, followed by horseradish peroxidase-streptavidin. All antibodies were from Southern Biotech and were used at a 1:5,000 dilution in phosphate-buffered saline–0.05% Tween 20-0.5% NFDM.

**Cytokine detection.** *T. cruzi* antigenic lysate was prepared by subjecting parasites (10⁷/ml phosphate-buffered saline; 70% amastigotes, 30% trypomastigotes) to six cycles of frozen thawing and thawing, followed by sonication in an ice-cold water bath for 30 min. Single-cell splenocyte preparation from immunized and control mice was suspended at 2 × 10⁶/ml RPMI-5% fetal bovine serum in 24-well plates. Cells were incubated with 25 μg/ml *T. cruzi* antigenic lysate, and culture supernatants were collected at 48 h for the measurement of IFN-γ and TNF-α with optiELISA ELISA kits (Pharmaning, San Diego, CA) according to the manufacturer’s specifications. Splenocytes incubated with 1 μg/ml concanavalin A were used as positive controls. Serum levels of cytokines were determined by ELISA.

To measure cytokine levels in tissues, we performed real-time reverse transcription (RT)-PCR. Total RNA from tissue samples (50 mg each) was isolated by guanidinium thiocyanate-phenol-chloroform extraction (6), treated with DNase (Ambion) to remove contaminating DNA, and analyzed for quality and quantity with SPECTRAX Plus 384 and for integrity with an Agilent 2100 Bioanalyzer. Total RNA (2.5 μg) was reverse transcribed with 2.5 U of Moloney murine leukemia virus reverse transcriptase (New England Biolabs) and 1 μl poly(dT)₁₈ oligonucleotide, and the first-strand cDNA was used as a template in a real-time PCR on an iCycler thermal cycler (Bio-Rad) with Sybr green Supermix (Bio-Rad) and oligonucleotides for murine cytokines (26). The threshold cycle (*CT*) values for each target gene were normalized to β-actin gene expression, and the relative expression level of each target gene in infected mice was calculated with the formula 2^(-(ΔΔCT)) , where ΔΔCT represents C(T) (infected sample) − C(T) (control). To better visualize the mRNA levels in all groups, bar graphs were generated by plotting the 1/2^CT values on the y axis (16).

**Tissue parasite burden and histopathology.** Tissues (50 mg) were subjected to protease K lysis, and total RNA was isolated by phenol-chloroform extraction and ethanol precipitation. Total DNA (100 ng) was used as the template in a real-time PCR (as described above) with oligonucleotides specific for a sequence encoding the *T. cruzi* 18S rRNA (forward, 5′-TAATGATATGCTTGTTTC-3′; reverse, 5′-GCACACAGATTTACACCGTCT-3′). *CT* values for the *T. cruzi* specific signal were normalized to murine β-actin gene DNA levels. For histological studies, heart and skeletal muscle sections were fixed in 10% buffered formalin for 24 h, dehydrated in absolute ethanol, cleared in xylene, and embedded in paraffin. Five-micrometer tissue sections were stained by hematoxylin and eosin and evaluated by light microscopy. Tissues were scored 0 to 4 in blind studies, according to the extent of inflammation and tissue damage, from normal to total wall involvement (15).

**Statistical analysis.** Data are expressed as means ± standard deviations (SD) and were derived from at least triplicate observations per sample (six or more animals per group). Results were analyzed for significant differences by using analysis-of-variance procedures and Student’s t tests. The level of significance was accepted as *P* < 0.05.

**RESULTS**

The development of an antibody response induced by the intramuscular immunization of mice with DNA vaccines was determined by use of an ELISA. *T. cruzi*-specific antibodies became detectable after a second immunization and were enhanced after a third dose (Fig. 1). We detected a substantial level of parasite-specific IgG, IgG1, and IgG2b antibodies in mice immunized with TeG1-, TeG2-, and TeG4-encoded plasmids (individually and in combination, *P* < 0.01). The antigen-
induced antibody response was predominantly of the Th1 type, with IgG2b/IgG1 ratios of >1 (Fig. 1B). Delivery of the three antigen-encoding plasmids in a mixture induced a par level of the IgG and IgG1 responses and an up to 50% higher level of the IgG2 response than was noted in mice immunized with individual antigen-encoding plasmids (P < 0.01). Control mice immunized with plasmid vector alone or cytokine plasmids only exhibited no parasite-specific antibodies (Fig. 1; data not shown).

Upon challenge infection with T. cruzi, vaccinated mice continued to exhibit an approximately two- to threefold higher level of parasite-specific IgG antibodies compared to unvaccinated controls (Fig. 2A, P < 0.01). A maximal antibody response was noted in TcG2-immunized mice (Fig. 2A). After 30 days p.i. (dpi), the overall IgG response appeared to be at par between vaccinated-and-infected and unvaccinated-and-infected mice (data not shown). The IgG2b/IgG1 levels in sera of immunized mice were higher than that noted in unvaccinated-and-infected mice at all stages of infection and disease development (Fig. 2B and C, P < 0.01). Mice immunized with a TcG1-encoding plasmid exhibited an early increase in the IgG2b/IgG1 ratio beginning at 30 dpi, while mice immunized with TcG2- and TcG4-encoding plasmids exhibited a substantial increase in the IgG2b/IgG1 ratio by day 75 p.i. (Fig. 2B and C). T. cruzi-specific IgG2a antibodies were detectable at days 75 and 120 p.i. and were not statistically significantly different among different groups (data not shown). We did not observe an inhibitory or competitive effect on antibody response elicitation in mice immunized with the mixture of antigen-encoding plasmids. Together, the results presented in Fig. 1 and 2 suggest that immunization with DNA vaccines resulted in the elicitation of a strong, parasite-specific, Th1-type antibody response that persisted after challenge T. cruzi infection.

In contrast to the antibody response, splenocyte activation and cytokine production were not enhanced in vaccinated-and-infected mice. Splenocytes were stimulated in vitro with antigenic lysate, and cytokine secretion was assessed via an ELISA. Splenocytes from unvaccinated mice (or mice injected with an empty plasmid vector), harvested at 30 days after a challenge infection with T. cruzi, vaccinated mice contained a strong parasite-specific antibody response after a challenge infection with T. cruzi. Mice were vaccinated as described in the legend to Fig. 1 and infected with T. cruzi (2.5 × 10^7 parasites/mouse). The serum levels of T. cruzi-specific IgG, IgG2b, and IgG1 antibodies were measured during the acute (30 dpi, panel A), intermediate (75 dpi, panel B), and chronic (120 dpi, panel C) phases of infection and disease development. Data (mean ± SD) are representative of three independent experiments. Abbreviations are as in Fig. 1. None, unvaccinated mice challenged with T. cruzi; ND, not determined; *, P < 0.05; **, P < 0.01 (vaccinated-and-infected versus unvaccinated-and-infected mice).
FIG. 3. Splenocyte production of cytokines in vaccinated mice. Mice were vaccinated with antigen-encoding plasmids and challenged with T. cruzi as described in the legend to Fig. 1. Splenocytes from vaccinated-and-infected and unvaccinated-and-infected mice were stimulated in vitro for 48 h with antigenic lysate (gray bars, 30 dpi) or no antigen (open bars), and the levels of IFN-γ (A) and TNF-α (B) expression in cell-free supernatants were measured with an ELISA. Data (mean ± SD) are representative of three independent experiments. Abbreviations are as in Fig. 1 and 2. **, P < 0.01 (vaccinated-and-infected versus unvaccinated-and-infected mice).

FIG. 4. Mice vaccinated with antigen-encoding plasmids exhibited substantial control of the tissue parasite burden. C57BL/6 mice were vaccinated with TcG1-, TcG2-, and TcG4-encoding plasmids plus cytokine adjuvants and challenged with T. cruzi. Heart total DNA was used as the template in a real-time PCR for amplification of the T. cruzi 18S rRNA-encoding sequence. The T. cruzi 18S rRNA gene signal was normalized to the murine β-actin gene, and the data represent mean 1/2^−CT values obtained from two independent experiments with heart tissue of at least three mice per experiment. The SD for all of the data points was <10%. *, P < 0.05; **, P < 0.01 (vaccinated-and-infected versus unvaccinated-and-infected mice).

FIG. 5. Inflammatory cytokine response is decreased in vaccinated mice. Mice were vaccinated with antigen-encoding plasmids and challenged with T. cruzi. (A) Serum levels of TNF-α at days 30 (gray bars), 75 (checkered bars), and 120 (dotted bars) p.i. (B and C) Myocardial levels of mRNAs for IFN-γ (B) and TNF-α (C) were determined by real-time RT-PCR as described in Materials and Methods. Shown are the RT-PCR values for the cytokines normalized to β-actin gene expression. The data represent mean 1/2^−CT values obtained from two independent experiments with heart tissue of at least three mice per experiment. The SD for all of the data points in panels B and C was <10%. *, P < 0.05; **, P < 0.01 (vaccinated-and-infected versus unvaccinated-and-infected mice).

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was detected at a very low level in mice vaccinated with TcG1- and TcG2-encoding plasmids (10% of unvaccinated-and-infected controls) (Fig. 4). Infected control mice exhibited increased peracute-level (data not shown). The IFN-γ in the sera of vaccinated-and-infected mice was almost undetectable after the acute phase (data not shown). The IFN-γ (Fig. 5B) and TNF-α (Fig. 5C) mRNA expression in the myocardia of TcG1-, TcG2-, and TcG4-immunized mice was 50 to 80% lower compared to that detected in unvaccinated-and-infected mice (Fig. 5B and C, P < 0.01).

Histopathological analysis of tissue sections from vaccinated-and-infected mice showed an extensive infiltration of inflammatory cells in the heart (Fig. 6) and skeletal muscle (data not presented).
previously identified, by computational analysis of a sequence database, as putative vaccine candidates. The three plasmids along with cytokine expression plasmids. The vaccination or were injected with the empty vector only (Fig. 6). These results demonstrate that the DNA vaccines used in this study were effective in controlling the tissue parasite burden during the acute phase. Mice immunized with TcG4 and TcG1 exhibited better control of T. cruzi infection than did TcG2-immunized mice (Fig. 4), and this protection was correlated with the persistence of a high ratio of serum levels of IgG2b/IgG1 antibodies. At the intermediate and chronic stages, the parasite burden determined by a highly sensitive real-time RT-PCR approach was almost undetectable in immunized-and-infected mice. We surmise that elicitation of a strong IgG2b and IgG1 lytic antibody response by DNA vaccines encoding TcG1, TcG2, and TcG4-specific antibodies are lytic in nature and efficiently killed trypomastigotes in a complement-dependent manner (2). Subsequently, all vaccinated mice exhibited significant control of the tissue parasite burden during the acute phase. Mice immunized with TcG4 and TcG1 exhibited better control of T. cruzi infection than did TcG2-immunized mice (Fig. 4), and this protection was correlated with the persistence of a high ratio of serum levels of IgG2b/IgG1 antibodies. At the intermediate and chronic stages, the parasite burden determined by a highly sensitive real-time RT-PCR approach was almost undetectable in immunized-and-infected mice. We surmise that elicitation of a strong IgG2b and IgG1 lytic antibody response by DNA vaccines encoding TcG1, TcG2, and TcG4 contributed to the effective control of T. cruzi infection.

An assessment of studies with either knockout mice or infected animals treated with antibodies to deplete specific immune molecules have shown that, besides the T. cruzi-specific lytic antibody response, efficient control of acute parasitemia also requires concerted activities of macrophages, T helper cells, and cytotoxic T lymphocytes (reviewed in reference 35). The IFN-γ and TNF-α cytokines are believed to be the key activators of macrophage NO production and oxidative burst for parasite clearance in the early stages of T. cruzi infection (17). CD4+ Th1 helper cells stimulate type 1 cytokine production and have been implicated in the induction of protective immunity, while CD8+ T cells are considered important for the killing of infected cells (3, 28). Interestingly, in this study, the analysis of splenocyte proliferation and cytokine production in mice immunized with TcG1-, TcG2-, or TcG4-encoding plasmids showed a substantially reduced proinflammatory response compared to that detected in unvaccinated-and-infected control mice at all stages of infection and disease development (Fig. 3). It was particularly surprising that, even at the acute stage (30 dpi), IFN-γ production by splenocytes...
incubated in vitro with T. cruzi antigenic lysate was significantly lower in immunized mice compared to infected controls. Despite a compromised cytokine profile, all groups of vaccinated mice were better equipped to control the tissue parasite burden throughout the course of infection and disease development. We surmise that the lytic antibody response elicited by immunization with TcG1-, TcG2-, and TcG4-encoding plasmids was sufficiently strong to control the acute phase of infection and the tissue parasite burden in vaccinated mice. This notion is supported by other studies in which mice were immunized with an avirulent CL14 clone of T. cruzi and challenged with a virulent CL strain of T. cruzi. The latter cited elevated IgG1 and IgG2b isotype antibody levels and diminished IFN-γ levels, as well as substantially low parasitemia and a decreased percentage of mortality, in comparison to control infected mice that were not primed with an avirulent strain (21, 25, 32).

A major criticism of vaccine development against T. cruzi has been that elicitation of stronger, potent immune responses by vaccine may exacerbate immune pathology. The persistence of inflammatory responses associated with tissue necrosis and cell death are hallmarks of chronic Chagas’ disease. It has been suggested that CD8+ T cells, the dominant resident immune cells in the heart, have a toxic effect on the host, as many of the CD8+ T cells express granzyme A, which results in nonspecific bystander cell death and tissue necrosis (27). Others have shown that the frequency of IFN-γ-producing T cells specific for T. cruzi correlate with the severity of chronic disease in chagasic humans and experimental animals (7, 33). Circulating levels of IFN-γ and its production by peripheral blood mononuclear cells stimulated in vitro with T. cruzi antigen have been implicated as risk factors in identifying asymptomatic, seropositive patients at risk of developing symptomatic chronic cardiomyopathy (7). In this study, we indeed observed, irrespective of vaccination status, a substantially high infiltration of mononuclear cells in the heart and skeletal muscle in response to a challenge infection with T. cruzi. However, as the chronic phase developed, mice vaccinated with antigen-encoding plasmids exhibited minimal immunopathology. This was evidenced by substantially lower serum and cardiac levels of inflammatory cytokines and minimal to no detection of parasite 18S rRNA gene-specific signal and inflammatory infiltrate in the heart tissue of vaccinated-and-infected mice during the chronic disease phase (Fig. 4 and 6). Similar observations were made with mice immunized with a nonpathogenic strain (CL14 or TCC) challenged with a virulent strain (CL or Tulahuen) of T. cruzi (1, 21). Substantially high levels of inflammatory infiltrate in the heart, skeletal muscle, and intestinal tissue were noted in response to a challenge infection. As the chronic phase developed, the tissue parasite burden and associated inflammation were marginal in mice immunized with an attenuated strain while those directly challenged with a virulent strain maintained severe symptoms of Chagas’ disease (1, 21). Experimental studies with mice suggest a clear correlation of the acute-phase parasite burden, parasite persistence, and the severity of cardiomyopathy symptoms during the chronic phase (37). On the basis of these studies and our data, we conclude that the strong type 1 antibody response elicited in TcG1-, TcG2-, and TcG4-immunized mice (Fig. 1 and 2) prevented parasite replication and propagation in the host (Fig. 4). Though complete parasite clearance was likely not achieved, vaccinated mice controlled the parasite burden below a threshold level, resulting in limited to no activation and infiltration of inflammatory cells in the heart and absence of tissue destruction during the chronic phase (Fig. 6).

In summary, we have demonstrated that vaccination of mice with antigen-encoding plasmids provided resistance to T. cruzi infection and chronic inflammatory pathology of the heart in the order TcG4 < TcG1 < TcG2. The elicitation of a strong IgG2b/IgG1 antibody response in immunized mice contributed to parasite control. Subsequently, splenic production of inflammatory cytokines and cardiac infiltration of inflammatory infiltrate were reduced in vaccinated mice. Our results validate the utility of a bioinformatic-computational approach to the identification of a potent vaccine candidates against T. cruzi.

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