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(54) **Title**: VACCINE FOR CONTROL OF TRYPANOSOMA CRUZI INFECTION AND CHAGAS DISEASE

(57) **Abstract**: Provided herein are vaccine compositions for control of Trypanosoma cruzi infection and Chagas disease. The compositions comprise plasmids encoding o GPI- anchored genes ASP-2, TcG-1, TcG2 and TcG4 from Trypanosoma cruzi; plasmids encoding cytokines IL12 and GM-CSF; and plasmids encoding a gene expression system. Certain vaccine compositions comprise recombinant proteins, selected from TcG-1, TcG2 and TcG4 from Trypanosoma cruzi. In another vaccination strategy, the recombinant proteins are replaced by lysates comprising Trypanosoma rangeli cells.

VACCINE FOR CONTROL OF *TRYPANOSOMA CRUZI* INFECTION AND CHAGAS DISEASE

Cross-Reference to Related Applications

This international application claims benefit of priority under 35 U.S.C. §119(e) of provisional application U.S. Serial No. 61/276,274, filed September 10, 2009, now abandoned, the entirety of which is hereby incorporated by reference.

Federal Funding

The invention was supported, in whole or in part, by Grant No. R03AI072538 from the National Institutes of Health. Consequently, the Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to the field of human and animal health and in particular to vaccination approaches for control of *Trypanosoma cruzi* infection and Chagas disease.

Description of the Related Art

American trypanosomiasis or Chagas disease caused by *Trypanosoma cruzi* infection is the prime cause of death in young adults in endemic areas of the American continent and results in over 50,000 deaths, 1 million new cases, and loss of 2.74 million disability-adjusted years per year.

The prevalence rate of T. cruzi infection in dogs may reach up to 84%, determined by serological procedures and xenodiagnosis, in endemic areas (e.g. rural Argentina, Chiapas state of Mexico) [1, 2]. Dogs are the most frequent blood meal source for the domestic triatomines (T. barberi and T. pallidipennis in Mexico [3], T. infestans in Argentina [2]). Likewise, a high prevalence of seropositive dogs [4-6] and infected triatomines is routinely noted in rural and urban developments in southern US states [4, 7, 8] and suggested to maintain T. cruzi transmission in the human habitat. Triatomines are several times more likely to take their blood meal from dogs than from humans. The ratio of

dog blood meals to human blood meals in the engorged guts of triatomines is estimated to be 2.3-2.6 times the ratio of the number of dogs to the number of humans in a household [9]. Thus, the probability of infecting an insect in one blood meal from dogs is estimated to be 200 times higher compared to the probability from adult humans [2]. These studies conclude: a) dogs are important host blood sources for domiciliary triatomines, b) the risk of T. cruzi infection in humans is increased by the presence of infected dogs, and c) strategies that can limit T. cruzi infection in the reservoir host would be effective in interrupting the parasite transmission to the vector, and consequently, to the human host.

The mathematical models based on epidemiological data suggest that vector control would be the most effective strategy against T. cruzi transmission [10]. However, sustained vector control, followed by constant surveillance, requires large-scale insecticide spraying every year that is not cost-effective and affordable for developing countries. Concerns also remain that insecticide use in the long-term may not be efficacious in blocking T. cruzi transmission, owing to the development of drug resistance by triatomines and reinfestation of homes by secondary sylvatic vectors, e.g., Triatoma sordida, in Brazil and other South American countries [11]. The same epidemiological models indicate that dog vaccination would be the second most efficient approach.

The efforts towards vaccine development are numerous. Based upon numerous studies in animal models, a successful vaccine that can provide protection from T. cruzi infection is envisioned by the research community to be composed of defined antigens capable of inducing strong neutralizing and lytic antibody response and type 1-biased T cell responses. Yet currently no vaccine is available for control of T. cruzi infection and disease development in humans and dogs.

Thus, there is a recognized need in the art for a vaccine for control of T. cruzi infection and disease development in humans and dogs. The present invention fulfills this long-standing need and desire in the art.

SUMMARY OF THE INVENTION

The present invention is directed to a DNA vaccine comprising at least one plasmid encoding one or more GPI-anchored genes from *Trypanosoma cruzi*; at least one plasmid encoding a cytokine; and a pharmaceutically acceptable carrier.

The present invention is further directed to a DNA-protein vaccine comprising at least one plasmid encoding one or more GPI-anchored genes from *Trypanosoma cruzi*; at least one plasmid encoding a cytokine; one or more recombinant GPI-anchored proteins from *Trypanosoma cruzi*; and a pharmaceutically acceptable adjuvant.

The present invention is directed further to a vaccine comprising at least one plasmid encoding one or more GPI-anchored genes from *Trypanosoma cruzi*; at least one plasmid encoding a cytokine; one or more lysates comprising cells from *Trypanosoma rangeli* or other protozoa that are non-infective to humans; and a pharmaceutically acceptable adjuvant.

Other and further objects, features, and advantages will be apparent from the following description of the presently preferred embodiments of the invention, which are given for the purpose of disclosure.

BRIEF DESCRIPTIONS OF THE DRAWINGS

So that the matter in which the above-recited features, advantages and objects of the invention, as well as others which will become clear, are attained and can be understood in detail, more particular descriptions of the invention briefly summarized above may be had by reference to certain embodiments thereof which are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate preferred embodiments of the invention and therefore are not to be considered limiting in their scope.

Figure 1 depicts the computational screening strategy used to screen the *T. cruzi* sequence database for the identification of potential vaccine candidates.

Figures 2A-2F depict expression plasmids used in the DNA vaccines. **Figure 2A** depicts CMVI.UBF3/2 encoding ASP-2. The cDNA fragment of ASP-2 (GenBank accession no. U77951) encoding amino acid residues 61 to 705 (SEQ ID NO: 1) was cloned at *BglII* and *SmaI* restriction sites of multiple cloning sequence (MCS) in eukaryotic expression plasmid pCMV.UBF3/2CMVI.UBF3/2 mammalian expression plasmid containing the cytomegalovirus (CMV) immediate-early gene promoter, a Synthetic 5' intron, and a modified 3' untranslated region from the human growth hormone. The cloned genes were fused to a ubiquitin-encoding gene at the 5' end to promote targeting of the expressed protein to the proteosome and entry into the MHC class I pathway of antigen presentation. **Figure 2B** shows pCDNA3 encoding TcG1 – TcG8 cDNAs. The cDNA fragment of *TcG1, TcG2, TcG3, TcG4, TcG5, TcG6, TcG7,* and *TcG8* were amplified using the oligonucleotides depicted in **Figure 2C**, and cloned at indicated restriction sites in the eukaryotic expression plasmid pCDNA3.1. **Figure 2C** depicts oligonucleotides used to amplify TcG1-TcG8 for cloning in pCDNA3. **Figure 2D** depicts eukaryotic expression plasmids encoding murine granulocyte-macrophage colony-stimulating factor (GM-CSF; pCMVI.GM-CSF). **Figure 2E**

depicts eukaryotic expression plasmids encoding murine cytokine interleukin-12 (IL-12; pcDNA3.msp35 and pcDNA3.msp40). **Figure 2F** depicts pCDNA3 encoding dog IL-12.

Figures 3A-3D depicts C57BL/6 mice immunized with pCDNA3 encoding TcG1, TcG2, and TcG4 plus IL-12 and GMCSF-expression plasmids (25 μg each plasmid/mouse, i.m., 3-week intervals) and then with recombinant protein cocktail (rTcG1, rTcG2, and rTcG4) with saponin adjuvant (25 μg each protein/mouse, i.d., 3-week intervals). Mice were challenged with *T. cruzi* 2-weeks after last immunization. **Figure 3A** shows ab response after immunization and during acute (25 dpi) and chronic (>120 dpi) stages. **Figures 3B-3C** show Cytokine profile of splenocytes, in vitro activated with Tclysate. **Figure 3D** shows H&E staining, Controls+ were immunized with empty plasmid and saponin only (8 mice/group).

Figure 4 depicts serological detection of anti-*T. cruzi* antibodies in vaccinated dogs. 96-well plates were coated with *T. cruzi* antigen (5 x 10^5 parasite equivalents/well) or recombinant antigen (10 μg/well) and sequentially incubated with sera samples (1:20 dilution, 100 μl/well) added in triplicate; 100 μl/well HRP-labeled goat anti-dog IgG+M (1:5000 dilution) (KOMA); and 100 μl/well of Sure Blue TMB substrate (K&P). Absorbance was measured at 650 nm. Background signal (without serum) was subtracted before the data were calculated.

Figures 5A-5C depicts dogs (n=6) immunized with TcVac3^R and two weeks later, challenged with *T. cruzi* (2300 parasites/kg). Control+ Dogs given empty vector/saponin only followed by challenge infection. **Figure 5A** shows Parasitemia determined at 2-day intervals, beginning day 7 pi. **Figure 5B** shows heart pathology, displaying ventricle fibrosis and atrial dilation in control$^+$ group. **Figure 5C** shows that at 60 d post infection lab-reared triatomines were fed on vaccinated and control dogs (6 bugs/dog) Gut-parasitemia in triatomines was determined by light microscopic examination of diluted feces at 2 weeks-post feeding

DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Remington's Pharmaceutical Sciences, 18th Edition (Easton, Pa.: Mack Publishing Company, 1990); Methods In Enzymology (S. Colowick and N. Kaplan, eds., Academic Press, Inc.); and Handbook of Experimental Immunology, Vols. I-IV (D. M. Weir and C. C. Blackwell, eds., 1986, Blackwell Scientific Publications); Sambrook, et al.,

Molecular Cloning: A Laboratory Manual (2nd Edition, 1989); Handbook of Surface and Colloidal Chemistry (Birdi, K. S. ed., CRC Press, 1997); Short Protocols in Molecular Biology, 4th ed. (Ausubel et al. eds., 1999, John Wiley & Sons); Molecular Biology Techniques: An Intensive Laboratory Course (Ream et al., eds., 1998, Academic Press); PCR (Introduction to Biotechniques Series), 2nd ed. (Newton & Graham eds., 1997, Springer Verlag); Peters and Dalrymple, Fields Virology, 2nd ed., Fields et al. (eds.) (B.N. Raven Press, New York, N.Y.).

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

As used herein the specification, “a” or “an” may mean one or more. As used herein in the claim(s), when used in conjunction with the word “comprising”, the words “a” or “an” may mean one or more than one. As used herein "another" or “other” may mean at least a second or more of the same or different claim element or components thereof. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. "Comprise" means "include." It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description. Although methods and materials similar or equivalents to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. Furthermore, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

The term “antigen” as used herein is defined as a compound, composition, or substance that can stimulate the production of antibodies or a T cell response in an animal, including compositions that are injected or absorbed into an animal. An antigen reacts with the products of specific humoral or cellular immunity, including those induced by heterologous immunogens. The term "antigen" includes all related antigenic epitopes. "Epitope" or "antigenic determinant" refers to a site on an antigen to which B and/or T cells respond. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance.

An "antigen" can be a tissue-specific antigen, or a disease-specific antigen. These terms are not exclusive, as a tissue-specific antigen can also be a disease specific antigen. A tissue-specific antigen is expressed in a limited number of tissues, such as a single tissue. Specific, non-limiting examples of a tissue specific antigen are a prostate specific antigen. A disease-specific antigen is expressed coincidentally with a disease process. Specific non-limiting examples of a disease-specific antigen are an antigen whose expression correlates with, or is predictive of, tumor formation, such as prostate cancer. A disease specific antigen may be an antigen recognized by T cells or B cells.

The term "amplification" of a nucleic acid molecule (e.g., a DNA or RNA molecule) refers to use of a technique that increases the number of copies of a nucleic acid molecule in a specimen. An example of amplification is the polymerase chain reaction, in which a biological sample collected from a subject is contacted with a pair of oligonucleotide primers, under conditions that allow for the hybridization of the primers to a nucleic acid template in the sample. The primers are extended under suitable conditions, dissociated from the template, and then re-annealed, extended, and dissociated to amplify the number of copies of the nucleic acid. The product of amplification may be characterized by electrophoresis, restriction endonuclease cleavage patterns, oligonucleotide hybridization or ligation, and/or nucleic acid sequencing using standard techniques. Other examples of amplification include strand displacement amplification, as disclosed in U.S. Pat. No. 5,744,311; transcription-free isothermal amplification, as disclosed in U.S. Pat. No. 6,033,881; repair chain reaction amplification, as disclosed in WO 90/01069; ligase chain reaction amplification, as disclosed in EP-A-320 308; gap filling ligase chain reaction amplification, as disclosed in U.S. Pat. No. 5,427,930; and NASBA.TM. RNA transcription-free amplification, as disclosed in U.S. Pat. No. 6,025,134.

The term "antibody" as used herein includes Immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. A naturally occurring antibody (e.g., IgG, IgM, IgD) includes four polypeptide chains, two heavy (H) chains and two light (L) chains interconnected by disulfide bonds. However, it has been shown that the antigen-binding function of an antibody can be performed by fragments of a naturally occurring antibody. Thus, these antigen-binding fragments are also intended to be designated by the term "antibody." Specific, non-limiting examples of binding fragments encompassed within the term antibody include (i) a Fab fragment consisting of the V_L, V_H, C_L and C_{H1} domains; (ii) an F_d fragment consisting of the V_H and C_{H1} domains; (iii) an Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (iv) a dAb fragment (Ward et al., Nature 341:544-546, 1989) which consists of a V_H domain; (v) an

isolated complimentarily determining region (CDR); and (vi) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region.

Immunoglobulins and certain variants thereof are known and many have been prepared in recombinant cell culture (e.g., see U.S. Pat. No. 4,745,055; U.S. Pat. No. 4,444,487; WO 88/03565; EP 256,654; EP 120,694; EP 125,023; Faoulkner et al., Nature 298:286, 1982; Morrison, J. Immunol. 123:793, 1979; Morrison et al., Ann Rev. Immunol 2:239, 1984).

The term "animal" as used herein refers to living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term mammal includes both human and non-human mammals. Similarly, the term "subject" includes both human and veterinary subjects.

The term conservative variation includes the use of a substituted amino acid in place of an unsubstituted parent amino acid, provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide. Non-conservative substitutions are those that reduce an activity or antigenicity.

The term "cDNA" (complementary DNA) refers to a piece of DNA lacking internal, non-coding segments (introns) and regulatory sequences that determine transcription. cDNA is synthesized in the laboratory by reverse transcription from messenger RNA extracted from cells.

The term "diagnostic" refers to identifying the presence or nature of a pathologic condition, such as, but not limited to, prostate cancer. Diagnostic methods differ in their sensitivity and specificity. The "sensitivity" of a diagnostic assay is the percentage of diseased individuals who test positive (percent of true positives). The "specificity" of a diagnostic assay is 1 minus the false positive rate, where the false positive rate is defined as the proportion of those without the disease who test positive. While a particular diagnostic method may not provide a definitive diagnosis of a condition, it suffices if the method provides a positive indication that aids in diagnosis. "Prognostic" is the probability of development (e.g., severity) of a pathologic condition, such as prostate cancer, or metastasis.

An "epitope" as used herein, is an antigenic determinant. These are particular chemical groups or peptide sequences on a molecule that are antigenic, i.e. that elicit a specific immune response. An antibody specifically binds a particular antigenic epitope on a polypeptide. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope

typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66, Glenn E. Morris, Ed (1996).

The term "expression control sequence" refers to Nucleic acid sequences that regulate the expression of a heterologous nucleic acid sequence to which it is operatively linked. Expression control sequences are operatively linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus expression control sequences can include appropriate promoters, enhancers, transcription terminators, a start codon (i.e. ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The term "control sequences" is intended to include, at a minimum, components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Expression control sequences can include a promoter.

The term "promoter" refers to a minimal sequence sufficient to direct transcription. Also included are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell-type specific, tissue-specific, or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the gene. Both constitutive and inducible promoters are included (see e.g., Bitter et al., Methods in Enzymology 153:516-544, 1987). For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage lambda, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used. In one embodiment, when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) can be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the nucleic acid sequences.

As defined herein, the term "host cell" refers to cells in which a vector can be propagated and its DNA expressed. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used.

The term "immune response" refers to a response of a cell of the immune

system, such as a B cell, T cell, or monocyte, to a stimulus. In one embodiment, the response is specific for a particular antigen (an "antigen-specific response"). In one embodiment, an immune response is a T cell response, such as a CD4+ response or a CD8+ response. In another embodiment, the response is a B cell response, and results in the production of specific antibodies.

A "chimeric molecule" is a targeting moiety, such as a ligand or an antibody, conjugated (coupled) to an effector molecule. The term "conjugated" or "linked" refers to making two polypeptides into one contiguous polypeptide molecule. In one embodiment, an antibody is joined to an effector molecule (EM). In another embodiment, an antibody joined to an effector molecule is further joined to a lipid or other molecule to a protein or peptide to increase its half-life in the body. The linkage can be either by chemical or recombinant means. In one embodiment, the linkage is chemical, wherein a reaction between the antibody moiety and the effector molecule has produced a covalent bond formed between the two molecules to form one molecule. A peptide linker (short peptide sequence) can optionally be included between the antibody and the effector molecule.

An "isolated" biological component (such as a nucleic acid or protein or organelle) as defined herein, has been substantially separated or purified away from other biological components in the cell of the organism in which the component naturally occurs, i.e., other chromosomal and extra-chromosomal DNA and RNA, proteins and organelles. Nucleic acids and proteins that have been "isolated" include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

A “label” as defined herein, is a detectable compound or composition that is conjugated directly or indirectly to another molecule to facilitate detection of that molecule. Specific, non-limiting examples of labels include fluorescent tags, enzymatic linkages, and radioactive isotopes.

Lymphocytes as defined herein are a type of white blood cell that is involved in the immune defenses of the body. There are two main types of lymphocytes: B cells and T cells.

The term “mammal” as used herein includes both human and non-human mammals. Similarly, the term "subject" includes both human and veterinary subjects.

The term “oligonucleotide” refers to a linear polynucleotide sequence of up to about 100 nucleotide bases in length.

Open reading frame (ORF) is defined as a series of nucleotide triplets (codons) coding for amino acids without any internal termination codons. These sequences

are usually translatable into a peptide.

The term "operably linked" refers to a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

As used herein, the term "vector" refers to a nucleic acid molecule as introduced into a host cell, thereby producing a transformed host cell. A vector may include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector may also include one or more selectable marker genes and other genetic elements known in the art.

As used herein, the term "transduction" encompasses all techniques by which a nucleic acid molecule might be introduced into such a cell, including transfection with viral vectors, transformation with plasmid vectors, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration. A transduced cell is a cell into which has been introduced a nucleic acid molecule by molecular biology techniques.

As used herein, the term "T Cell" refers to a white blood cell critical to the immune response. T cells include, but are not limited to, $CD4^+$ T cells and $CD8^+$ T cells. A $CD4^+$ T lymphocyte is an immune cell that carries a marker on its surface known as "cluster of differentiation 4" (CD4). These cells, also known as helper T cells, help orchestrate the immune response, including antibody responses as well as killer T cell responses. $CD8^+$ T cells carry the "cluster of differentiation 8" (CD8) marker. In one embodiment, a CD8 T cell is a cytotoxic T lymphocyte. In another embodiment, a CD8 cell is a suppressor T cell.

The term "purified" as used herein, does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified nucleic acid is one in which the nucleic acid is more enriched than the nucleic acid in its natural environment within a cell. Similarly, a purified peptide preparation is one in which the peptide or protein is more enriched than the peptide or protein is in its natural environment within a cell. In one embodiment, a preparation is purified such that the protein or peptide represents at least 50% (such as, but not limited to, 70%, 80%, 90%, 95%, 98% or 99%) of the total peptide or protein content of the preparation.

A "recombinant nucleic acid" is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of

nucleic acids, e.g., by genetic engineering techniques.

As used herein, the term "selectively hybridize" refers to hybridization under moderately or highly stringent conditions that excludes non-related nucleotide sequences. In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity, nucleotide sequence composition (e.g., GC v. AT content), and nucleic acid type (e.g., RNA versus DNA) of the hybridizing regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter. One of skill in the art can readily determine these conditions (e.g., Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989). As mentioned above, optimal conditions will vary, depending on the particular hybridization reaction involved, and can be determined empirically.

The term "polynucleotide" or "nucleic acid sequence" refers to a polymeric form of nucleotide at least 10 bases in length. A recombinant polynucleotide includes a polynucleotide that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA) independent of other sequences. The nucleotides can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single- and double-stranded forms of DNA.

The term peptide, as used herein refers to any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation).

The term "probe" comprises an isolated nucleic acid attached to a detectable label or reporter molecule. The term "primer" includes short nucleic acids, preferably DNA oligonucleotides, 15 nucleotides or more in length. Primers may be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods known in the art. One of skill in the art will appreciate that the specificity of a particular probe or primer increases with its length. Thus, for example, a primer comprising 20 consecutive

nucleotides will anneal to a target with a higher specificity than a corresponding primer of only 15 nucleotides. Thus, in order to obtain greater specificity, probes and primers may be selected that comprise 20, 25, 30, 35, 40, 50 or more consecutive nucleotides.

The term "promoter" as described herein, is an array of nucleic acid control sequences that directs transcription of a nucleic acid. A promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements which can be located as much as several thousand base pairs from the start site of transcription. Both constitutive and inducible promoters are included (see e.g., Bitter et al., Methods in Enzymology 153:516-544, 1987). Specific, non-limiting examples of promoters include promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used. Promoters produced by recombinant DNA or synthetic techniques may also be used. A polynucleotide can be inserted into an expression vector that contains a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific nucleic acid sequences that allow phenotypic selection of the transformed cells.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers of use are conventional. Remington's Pharmaceutical Sciences, by E. W. Martin, Mack Publishing Co., Easton, Pa., 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of the vaccines herein disclosed.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (e.g., powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

As used herein, the term "subject" refers to any target of the treatment. Preferably, the subject is a mammal, more preferably, the subject is a canine or a human.

In some embodiments of the present invention there is provided a DNA vaccine comprising at least one plasmid encoding one or more GPI-anchored genes from

Trypanosoma cruzi; at least one plasmid encoding a cytokine; and a pharmaceutically acceptable carrier.

Further to these embodiments, representative GPI-anchored genes are ASP-2, TcG-1, TcG2, TcG3, TcG4, TcG5, TcG6, TcG7 and TcG8. These GPI-anchored genes encode proteins with sequences shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9. Additionally, in some embodiments, representative cytokines are selected from the group comprising IL12, GM-CSF, CD40L, Flt3L and RANTES. In some embodiments, GPI-anchored genes are selected from ASP-2 encoding a protein with a sequence shown in SEQ ID NO: 1, TcG-1 encoding a protein with a sequence shown in SEQ ID NO: 2, TcG-2 encoding a protein with a sequence shown in SEQ ID NO: 3, and TcG4 encoding a protein with a sequence shown in SEQ ID NO: 5; and the cytokines are IL12 and GM-CSF. In some embodiments, the DNA vaccine comprises 100 μg of each plasmid.

In some embodiments of the present invention, there is provided a DNA-protein vaccine comprising at least one plasmid encoding one or more GPI-anchored genes from *Trypanosoma cruzi*; at least one plasmid encoding a cytokine; one or more recombinant GPI-anchored proteins from *Trypanosoma cruzi*; and a pharmaceutically acceptable adjuvant. In some of these embodiments, representative GPI-anchored genes include but are not limited to ASP-2, TcG-1, TcG2, TcG3, TcG4, TcG5, TcG6, TcG7 and TcG8. These GPI-anchored genes encode proteins with sequences shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9. Further to these embodiments, representative recombinant GPI-anchored proteins may be ASP-2, TcG-1, TcG2, TcG3, TcG4, TcG5, TcG6, TcG7 and TcG8. In some of these embodiments, recombinant GPI-anchored proteins have sequences of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9. In some embodiments, the adjuvant is a saponin adjuvant.

In another embodiment of the present invention there is provided a vaccine comprising at least one plasmid encoding one or more GPI-anchored genes from *Trypanosoma cruzi*; at least one plasmid encoding a cytokine; one or more lysates comprising cells from *Trypanosoma rangeli* or other protozoa that are non-infective to humans; and a pharmaceutically acceptable adjuvant.

Certain embodiments of the invention comprise methods of vaccination comprising one or more of the vaccines described supra. In certain embodiments, these methods are used of vaccinating dogs. In certain embodiments, the vaccines comprise from about 50 μg to about 500 μg of each plasmid. In embodiments, the vaccines may comprise

from about 50 μg to about 500 μg of each recombinant GPI-anchored protein. In certain embodiments, the vaccines comprise from about 50 μg up to about 900 μg of the bacterial cell lysates comprising *Trypanosoma rangeli* cells.

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion.

EXAMPLE 1

Vaccine Development

A transfection approach is employed to express ovalbumin (model antigen) in different cellular compartments of T. cruzi. Using these transfectants, it has been demonstrated that parasite secreted antigens and GPI-proteins (released by default in host cell cytoplasm) would be capable of entering the class I and II pathways of antigen presentation and elicit antibody and T cell responses, and, thus, would be the best choice as vaccine candidates [12].

EXAMPLE 2

Computational screening

An unbiased computational/bioinformatics approach was developed and the *T. cruzi* sequence database was screened for the identification of potential vaccine candidates [18] (Figure 1). This strategy was chosen because testing of ~8,000 genes, estimated to be present in the haploid genome of *T. cruzi* (5-8 x 10^7 bp), as subunit vaccine candidates was not economically feasible. Strategic analysis of the database led to a selection of 71 candidate sequences of which eight (TcG1-TcG8, Table 1: SEQ ID NOs 2-9) were found to be phylogenetically conserved in clinically important strains of *T. cruzi,* and expressed in the infective and intracellular stages of the parasite [18]. When delivered as a DNA vaccine in mice, TcG1, TcG2, TcG4 elicited a significant trypanolytic antibody response and Th1 cytokine (IFN-γ), a property associated with immune control of *T. cruzi* [18] (Table 2). These novel vaccine candidates, thus, increased the pool of protective vaccine candidates against T. cruzi.

EXAMPLE 3

Protective efficacy of TcG1, TcG2, and TcG4 in mice

TcG1, TcG2, and TcG4 were cloned in eukaryotic expression vector pCDNA3.1 (for DNA vaccination) and in E. coli expression vector to generate recombinant proteins (for protein vaccination). One group of mice was immunized with four doses of DNA vaccine (pcDNA3 encoding TcG1, TcG2, and TcG4 + IL-12 and GM-CSF expression

plasmids as illustrated in Figures 2A-2F, intramuscular delivery). Second group of mice was immunized with 2 doses of DNA vaccine and 2 doses of recombinant proteins with saponin adjuvant (intradermal). Both groups of mice immunized with DNA-DNA or DNA-protein vaccine elicited anti-parasite humoral and cellular immune responses (Figures 3A-3D) [19].

The DNA-protein vaccine of the present invention induced significantly higher levels of *T. cruzi*- and antigen-specific humoral responses that were maintained after challenge infection and during chronic disease phase. Likewise, the DNA-protein vaccine elicited a stronger, Th1 biased cellular response (IFN-γ, TNF-α cytokines, $CD8^+$T cells) that was effective in controlling the acute parasitemia and tissue parasite burden during acute phase. Due to controlled acute infection, DNA-protein vaccinated mice exhibited remarkable reduction in immunopathology, a hallmark of chronic Chagas disease. Overall DNA-protein vaccine polarized the B & T cell immune response towards Th1 type that controlled parasites during acute phase and towards Th2 type after acute infection that reduced chronic inflammation during disease phase, respectively. These studies suggested that DNA/protein vaccination would be a better approach in eliciting protective immunity against *T. cruzi* infection and disease.

TABLE 1

Genes phylogenetically conserved in clinically important strains of *T. cruzi*.

Gene	SEQ ID NO	SEQUENCE
ASP-2	1	PCEAADAVEGKSGAVQLPKWVDIFVPEKTHVLPKEGSESGVK KAFAAPSLVSAGGVMVAFAEGFSEYNAHENNPFGIRPYEILAG YIKAAESWPSIVAEVNASTWRAHTVIGSRNGNDRLCFLYRPTA VARENKVFLLVGSDTVGYDSDDDMWVKDGWDIQLVEGVATQ STDGKPSKTINWGEPKSLLKHIPKHTQGHLRDVVTAGGSGIV MQNNTLVFPLVVNGKNYPFSSITYSTDNGNNWVFPESISPVG CLDPRITEWETGQILMIVDCGNGQSVYESRDMGTTWTKAVRT LSGVWAISQRGVRSYEIFRVGAIITATIEGRKVMLYTRRGYAS GEKEANALYLWVTDNNRTFHVGPVAMDSAVNETLSNALLYSD GNLHLLQQRANEKGSAISLARLTEELKEIESVLRTWAQLDAFF SKSSTPTAGLVGFLSNTSSGGNTWIDEYRCVNATVTKASKVK NGFKFTGPGPMATWLVNSREDNRQYSFVNHRFTLVATVTIHQ VPKGSTPLLGAGLGDGHGAKIIGLSYSMNKTWETVFYGKKTT SNTTWELGKEYQVTLMLQDGNKGSVYVDGVIVGSPAKIPKVG ALGHEIAHFYFGGGEGDSDSSVTVTNVFLYNRPLSVGELKMV RKSDDKKGNGGDQK

TcG1 2 MVKANYIRAGRLVRIIRGPRQDRVGVVVDIIDGNRVLVENPAD
KKMWRHVQNLKNVEPLKFSVELSRNCSTRTLKNVLAEKKILE
KYAATKSARRIAAKRAFARSTDFERYQLRVAKRSRAFWTRKV
FDENDQKKPVSWHKVALKKLQKNAKKVDSKPAAKKRISN

TcG2 3 MSLSFIESGFVPSDGMRRGVEAADTSAAAELLHLAVPPLMDA
GGKTRVCVAFYEAAQCPFDSRCEHAHHFSELNGYTQNKLLE
TVPVESIPKHFVAPLNSNSSSGNNKNDRTFYATDGNAANYTA
TAAVDGGVAHRSLGGEHGEKEKTSTNRRSKRTARLYDISGSN
TNLCDNSLSSLASSTDTLLLLGSVHDSKDVSPQKGTRRDEGM
EAFRIRLPPLLG

TcG3 4 MLQRTCSGSLYAVLEVARDATPQEIKKAYHRLALRLHPDKTG
GTTTEQFTLIQEAQSILGDPRQRRVYDTFGRMGIESLRQFGD
GMVVMTTAGIRCAFFIIAFWMLLWLLTLVLAIVRFDYNKGWPW
AAVFAPVWVALVPLLLIGGLLVFHGATRREIASTLLGLMCFLVT
FAVAMFVVGLSGALTWTIALAPSAAIYVFQSCFILRYLLPFQFR
NGFAEFIPPGSSVCLSRMYWGFCWKQYLKSCVVSALLVLPCY
RGANRRGRYIKTDLLLDSFYSSYFVLWVHDVCFCRTKIFCGN
SGGAVMSPEPTVPCADGRHRLRQSSFYGMHVGGEVSS

TcG4 5 MSAKAPPKTLHQVRNVAYIFAAWAGLQKGFAEKSANDKMWV
EHQRRLRQENAKRQHAAHALEELKQDEELERSIPTIVPKELHE
LVKALEK

TcG5 6 MGKEKVHMNLVVVGHVDAGKSTATGHLIYKCGGIDKRTIEKF
EKEAAEIGKSSFKYAWVLDKLKAERERGITIDIALWKFESPKS
VFTIIDAPGHRDFIKNMITGTSQADAAVLVIASSQGEFEAGISK
DGQTREHALLAFTLGVKQMVVCCNKMDDKSVNFAQERYDEI
VKEVSAYLKKVGYNVEKVRFIPISGWQGDNMIDKSENMPWY
KGPTLLEALDMLEPPVRPSDKPLRLPLQDVYKIGGIGTVPVG
RVETGTMKPGDVVTFAPANVTTEVKSIEMHHEQLAEATPGD
NVGFNVKNVSVKDIRRGNVCGNSKNDPPKEAADFTAQVIILN
HPGQIGNGYAPVLDCHTCHIACKFAEIESKIDRRSGKELEKN
PKSIKSGDAAMVRMVPQKPMCVEVFNDYAPLGRFAVRDMR

		QTVAVGIIKAVTKKDGGAGKVTKAAAKAAKK
TcG6	7	MQSELSGILSRIPAAVIGTILADESCKTVWFFNPKSREVISMD ALRSLPNPPSNSGADATERHLVYGMMRVRNQGVMFERDHI QRLYENCVLAATSKPLTDEATLPFPVEGVTQSIREYILSEHKE SGDINLKFVTWLPPFSNSLTTAEAWQKFLSDFSYVVYFVKSF FPPKEWYTEGIRISLLYNARRHTPNAKIIQAPLRSRAKSLQDS SGAFEVFFVWDKEAHFLVPEGSRSNYLLVTEDGHLCCSLAV
TcG7	8	MLATHGRGRRVQGAVGAVFSFEEGKRGKTRRAPLTSQNAR KKKTVKSIAASCGADPDILHERNSTALLKEGDGVVYSAVPKY KQSRLGVLLQHPLYSPHVVCCRFVCCVRLRRGWM
TcG8	9	MSDNHQLEYKRGLEDARRHRSRTEDNWLRASVGPLLWFGV PFAVAWLYLRRQAPASAKINPFGGMMEQMMPIKKRQFRVD VKGTKFEDVIGIPEAKQEVQQYVEFLTNPNKFTRLGARLPKG RLLTGEPGTGKTLLAKAVAGEADVPFFSCSGSDFIELMGGS GPKRVRELFEEARSSAPAIVFIDEIDAIGSRAGKIGGSVSSEE NRTINQLLAELDGLNTGTDAIIVIAATNFQDNIDKALLREGRFD RKVNIEMPDKAARVDIFKHYLNRVGTGDPRGRKVDEEGEPL PTNEKVDNLELARELADLTPGVSPATIATIVNEAALQSGIREK RLVDKESILEAVDNTLVGRKHRNRQSVTSLRRTAIHEAGHAL TAWMLPSVKQVLKVSVVPQ

TABLE 2

Screening immunogenic potential of antigens as DNA vaccine in mice.

Immunization with	Mice	Elicitation of immune response by vaccine			Pathological parameters after challenge infection*	
		Antibodies (IgG+M)	CTL activity	Th1 cytokine IFNγ, IL-12	Control of Inflam.	Percent Survival [a-c]
None		-	-	-	-	10 [c]
ASP-1	C3H/HeSnJ, C57BL/6	+/-	+	+	+	40 [b]
TSA-1	Balb/c, C3H/He, C57B/6	+/-	+	+	+	30 [b]
ASP-2	C3H/HeSnJ, C57BL/6	+	++	++	++	62 [b]
ASP-1+ASP-2+TSA-1	C3H/HeSnJ, C57BL/6	+	++	++	++	68 [a]
ASP-2+IL-12+GM-CSF[d]	C3H/HeSnJ, C57BL/6	++	+++	+	+++	80 [a]
TcG1+IL-12+GM-CSF[d]	C57BL/6	++	ND	+	++	90 [b]
TcG2+ IL-12+GMCSF[d]	C57BL/6	++	ND	+	++	92 [b]
TcG4+IL-12+GMCSF[d]	C57BL/6	++	ND	+	+++	100 [b]
ASP-1+ASP-2+ TSA-1+ IL-12+ GM-CSF[d]	C3H/HeSnJ, C57BL/6	+++	+++	++	+++	83 [a]

Mice were intra-muscularly immunized with antigen-encoding plasmids ± cytokine expression constructs (33 μg each DNA/mouse) twice at six-week intervals. Two week after 2nd immunization, mice were either used for measuring immune responses, or challenged with *T. cruzi*. * Immunization protocol provided variable degree of protection in different mouse strains. [a-c] Upon challenge infection, immunized animals exhibited very low (≤10%)[a], moderate (~50%)[b] or similar[c] parasitemia as detected in un-immunized/infected animals (data presented are from the animal model that exhibited best protection). [d] Immunization with these antigens was effective in decreasing the severity of chronic disease, evaluated by histopathological analysis of cardiac tissue biopsies.. A "+" or "-" sign indicates the effectiveness or limitation of the genetic vaccine in eliciting immune responses and protection from *T. cruzi* infection, respectively. ND: not determined.

EXAMPLE 4

Immunogenicity of vaccine candidates in dogs

The protective efficacy of selected vaccine candidates in dogs was determined. The candidate antigens included in dog studies were those that have exhibited maximal protection in murine studies.

TcVac1^R vaccine

pCDNA3 encoding TcG1, TcG2 and TcG4 + IL-12 and GM-CSF expression plasmids. 100 μg each plasmid, total 600 μg DNA. Four doses, intramuscular delivery, 2-week intervals.

TcVac2^R vaccine

Two doses of TcVac1^R followed by two doses of recombinant proteins (TcG1, TcG2, TcG4 with saponin adjuvant (DNA vaccine: im, 600-μg total DNA/dog; protein vaccine: id, 300 μg protein/dog, all doses at 2-week intervals). The recombinant proteins (TcG1, TcG2, and TcG4) were prepared in *E. coli.* No other study has demonstrated the protection afforded by this cocktail of antigens against *T. cruzi* in any model of disease.

EXAMPLE 5

Antibody response

T. cruzi and antigen-specific antibody response were determined in sera obtained from vaccinated dogs before each immunization, and 2 weeks after the last immunization. Negative control: sera from dogs immunized with vector only. Positive control: Sera from *T. cruzi*-infected dogs. It was found that after a second dose of DNA vaccine, antigen-specific antibody responses were elicited and these were enhanced by booster immunization with recombinant protein doses of TcVac2^R. An antibody response to individual candidate genes was elicited in vaccinated dogs, and it was similar to that detected in chronically infected dogs. Shown in Figure 4 are the parasite- and recombinant antigen-specific antibody response in sera collected after the last immunization.

EXAMPLE 6

Trypanolytic activity

Trypanolytic activity correlates with protection from *T. cruzi* infection as antibodies to surface proteins (plus complement) induce damage to infective trypomastigotes [20, 21]. Trypanocidal activity of the antibodies elicited was determined in dogs vaccinated with TcVac1^R using procedures standardized in the lab [18]. The sera from vaccinated dogs, obtained after 4th immunization, provided ≥80% lytic efficiency (1:8 dilution). In comparison, sera from *T. cruzi*-infected dogs exposed to multiple parasite proteins exhibited 90% lytic activity. No parasite lysis was observed with negative control sera from dogs immunized with empty vector, or when heat inactivated immune sera or heat inactivated complement were used. Together, these data show that dogs immunized with

TcVac1^R elicit antigen-specific antibody responses that are trypanolytic in nature, and hence capable of providing protection from *T. cruzi* infection.

TABLE 3

Trypanolytic activity of antisera from vaccinated dogs

Antiserum from dogs immunized with	% lytic activity		
	Sera dilution		
	1:4	1:8	1:16
Vector only	0	0	0
TcVac2^R	90	81	62
T. cruzi-infected	100	92	81

Chronic serum was obtained from lab-infected dogs. Immune sera from vaccinated dogs were obtained after 4th immunization (as for Fig 3). *T. cruzi* trypomastigotes (5 x 10^4 /25 µl were incubated for 4 h at 37°C, 5% CO_2 with 25 µl two-fold dilution of sera samples + 25 µl/well human complement (Sigma). The live, freely moving parasites were counted by light microscopy. Parasites that stained positively with 0.03% trypan blue were considered dead. All samples were analyzed in triplicate. Percent trypanocidal efficiency: (Total parasites-free parasites after incubation/Total parasites) X 100. SD was ≤10%, n=3/gp.

EXAMPLE 7

TcVac3^R vaccine: protective and transmission blocking efficacy

To simplify vaccine composition and reduce the cost of production, TcVac3^R was designed in which recombinant proteins were replaced by equivalent amount of protein lysate of *T. rangeli* (non-pathogenic in mammals and humans).

TcVac3^R vaccine

Two doses of DNA vaccine containing four expression plasmids + cytokine expression plasmids followed by two doses of *T. rangeli* lysates with saponin adjuvant (n=6, DNA vaccine: im, 600-µg DNA/dog; *T. rangeli* lysate: id, 400 µg protein/dog, all doses at 3-week intervals). Vaccinated dogs exhibited up to 8-fold decline in acute parasitemia (Fig. 5A), and a significant decline in myocardial pathology evidenced by decreased fibrosis in left ventricle and decreased dilation of right ventricle (Fig. 5B) when compared to controls that were immunized with empty vector only and infected with *T. cruzi*. Clinical exam (EKG analysis) of TcVac3^R vaccinated dogs detected no alterations while control+ dogs exhibited symptoms of conduction problems, myocarditis and/or pericarditis (Table 4).

TABLE 4

Echocardiographic findings

TcVac1 ^{R}	TcVac3 ^{R}	CONTROL+	CONTROL -
1.- Ventricular dilatation	1.- No alterations	1.-Conduction problems myocarditis	1.- No alterations
2.- Repolarization problems myocarditis	2.- No alterations	2.-Myocarditis	2.- No alterations
3.- No alterations	3.- No alterations	3.- Pericarditis	3.- No alterations

In the above experiments, the infectivity of the dogs to triatomines was determined by feeding the insects on abdomen skin using a membrane-feeding apparatus. Importantly, xenodiagnostic studies (Fig. 5C) showed that >88% of bugs (23/26) fed on control+ dogs were infected while only 50% bugs (15/30) bugs fed on TcVac^{R} vaccinated dogs became infected, thus demonstrating at least 50% reduction in infectivity (Table 5).

Table 5

Transmission blocking efficiency.

Groups	# infected/Total bugs fed on dogs	% Infectivity
TcVac1 ^{R}	10/19	52.63
TcVac3 ^{R}	15/30	50
CONTROL+	23/26	88.46
CONTROL -	0	0

Tratomines (6 per dog), 6 dogs per group were fed on vaccinated or control dogs at day 60 pi. The infectivity of triatomines was determined by light microscopic examination for metacyclic trypomastigotes in diluted feces of bugs.

The following references were cited herein:

1. Gurtler et al. (1990). Ann Trop Med Parasitol 84:313-323.
2. Gurtler et al. (1996). Am J Trop Med Hyg 55:24-31.
3. Guzman-Bracho C. (2001). Trends Parasitol 17:372-376.
4. Barr et al. (1995). Am J Vet Res 56:1037-1044.
5. Bradley et al. (2000). J Am Vet Med Assoc 217:1853-1857.
6. Barnabe et al. (2001) Exp Parasitol 99:73-79.
7. Meurs et al. (1998) J Am Vet Med Assoc 213:497-500.
8. Beard et al. (1988) J Parasitol 74:343-344.

9. Gurtler et al. (1997) J Appl Ecol 34:699-715.
10. Cohen JE and Gurtler RE. (2001) Science 293:694-698.
11. Moncayo A. (2003) Mem Inst Oswaldo Cruz 98:577-591.
12. Garg N et al. (1997). J Immunol 158:3293-3302.
13. Wizel et al. (1997). J Immunol 159:6120-6130.
14. Low et al. (1998) J Immunol 160:1817-1823.
15. Wizel et al. (1998) Infect Immun 66:5073-5081.
16. Garg N and Tarleton RL. (1998) In:Proceedings 10th International Congress of Immunology, New Delhi, India, p 1421-1426
17. Garg N and Tarleton RL. (2002) Infection & Immunity 70:5547-5555.
18. Bhatia et al. (2004) Infect Immun 72:6245-6254.
19. Bhatia V and Garg NJ. (2008) Clin Vaccine Immunol 15:1158-1164.
20. Sepulveda et al. (2000) Infect Immun 68:4986-4991.
21. Umekita LF and Mota I. (2000) Braz J Med Biol Res 33:253-258.

Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. These patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually incorporated by reference.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. It will be apparent to those skilled in the art that various modifications and variations can be made in practicing the present invention without departing from the spirit or scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.

WHAT IS CLAIMED IS:

1. A DNA vaccine comprising:

at least one plasmid encoding one or more GPI-anchored genes from *Trypanosoma cruzi*;

at least one plasmid encoding a cytokine; and

a pharmaceutically acceptable carrier.

2. The DNA vaccine of claim 1, wherein said GPI-anchored genes are selected from the group consisting of ASP-2, TcG-1, TcG2, TcG3, TcG4, TcG5, TcG6, TcG7 and TcG8.

3. The DNA vaccine of claim 2, wherein said GPI-anchored gene encodes a protein having a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9.

4. The vaccine of claim 1, wherein said cytokine is selected from the group comprising IL12, GM-CSF, CD40L, Flt3L and RANTES.

5. The vaccine of claim 1, comprising 100 μg or more of each plasmid.

6. The vaccine of claim 1, wherein said GPI-anchored genes are selected from ASP-2 encoding a protein with a sequence shown in SEQ ID NO: 1, TcG-1 encoding a protein with a sequence shown in SEQ ID NO: 2, TcG-2 encoding a protein with a sequence shown in SEQ ID NO: 3, and TcG4 encoding a protein with a sequence shown in SEQ ID NO: 5; and wherein said cytokines are IL12 and GM-CSF.

7. A DNA-protein vaccine comprising:

at least one plasmid encoding one or more GPI-anchored genes from *Trypanosoma cruzi*;

at least one plasmid encoding a cytokine;

one or more recombinant GPI-anchored proteins from *Trypanosoma cruzi*; and

a pharmaceutically acceptable adjuvant.

8. The vaccine of claim 7, wherein said GPI-anchored genes are ASP-2, TcG-1, TcG2, TcG3, TcG4, TcG5, TcG6, TcG7 and TcG8.

9. The vaccine of claim 7, wherein said GPI-anchored genes encode proteins with sequences shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9.

10. The vaccine of claim 7, wherein said recombinant GPI-anchored proteins are ASP-2, TcG-1, TcG2, TcG3, TcG4, TcG5, TcG6, TcG7 and TcG8.

11. The vaccine of claim 7, wherein said GPI-anchored proteins have sequences of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9.

12. The vaccine of claim 7, wherein said adjuvant is a saponin adjuvant.

13. The vaccine of claim 7 comprising

100 μg of each plasmid; and

100 μg of each recombinant GPI-anchored protein.

14. The vaccine of claim 7, wherein said GPI-anchored genes are selected from ASP-2 encoding a protein with a sequence shown in SEQ ID NO: 1, TcG-1 encoding a protein with a sequence shown in SEQ ID NO: 2, TcG-2 encoding a protein with a sequence shown in SEQ ID NO: 3, and TcG4 encoding a protein with a sequence shown in SEQ ID NO: 5; wherein said recombinant GPI-anchored proteins are selected from TcG-1 protein with a sequence shown in SEQ ID NO: 2, TcG-2 protein with a sequence shown in SEQ ID NO: 3, and TcG4 protein with a sequence shown in SEQ ID NO: 5; and wherein said adjuvant is a saponin adjuvant.

15. A vaccine comprising:

at least one plasmid encoding one or more GPI-anchored genes from *Trypanosoma cruzi*;

at least one plasmid encoding a cytokine;

one or more cell lysates comprising of cells from a protozoan that is non-infective to humans; and

a pharmaceutically acceptable adjuvant.

16. The vaccine of claim 15, wherein said GPI-anchored genes are ASP-2, TcG-1, TcG2, TcG3, TcG4, TcG5, TcG6, TcG7 and TcG8.

17. The vaccine of claim 15, wherein said GPI-anchored genes encode proteins with sequences shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9.

18. The vaccine of claim 15, wherein said adjuvant is a saponin adjuvant.

19. The vaccine of claim 15 comprising,
100 μg of each plasmid; and
400 μg of said bacterial cell lysates.

20. The vaccine of claim 15,
wherein said GPI-anchored genes are selected from ASP-2 encoding a protein with a sequence shown in SEQ ID NO: 1, TcG-1 encoding a protein with a sequence shown in SEQ ID NO: 2, TcG-2 encoding a protein with a sequence shown in SEQ ID NO: 3, and TcG4 encoding a protein with a sequence shown in SEQ ID NO: 5;
wherein said protozoan is *Trypanosoma rangeli*; and
wherein said adjuvant is a saponin adjuvant.

21. A method of vaccinating a subject against *Trypanosoma cruzi* infection comprising administering the vaccine of claim 1.

22. The method of claim 18, wherein said subject is a dog.

23. A method of vaccinating an animal against *Trypanosoma cruzi* infection comprising administering the vaccine of claim 6.

24. A method of vaccinating an animal against *Trypanosoma cruzi* infection comprising administering the vaccine of claim 7.

25. A method of vaccinating an animal against *Trypanosoma cruzi* infection comprising administering the vaccine of claim 14.

26. A method of vaccinating an animal against *Trypanosoma cruzi* infection comprising administering the vaccine of claim 15.

27. A method of vaccinating an animal against *Trypanosoma cruzi* infection comprising administering the vaccine of claim 20.

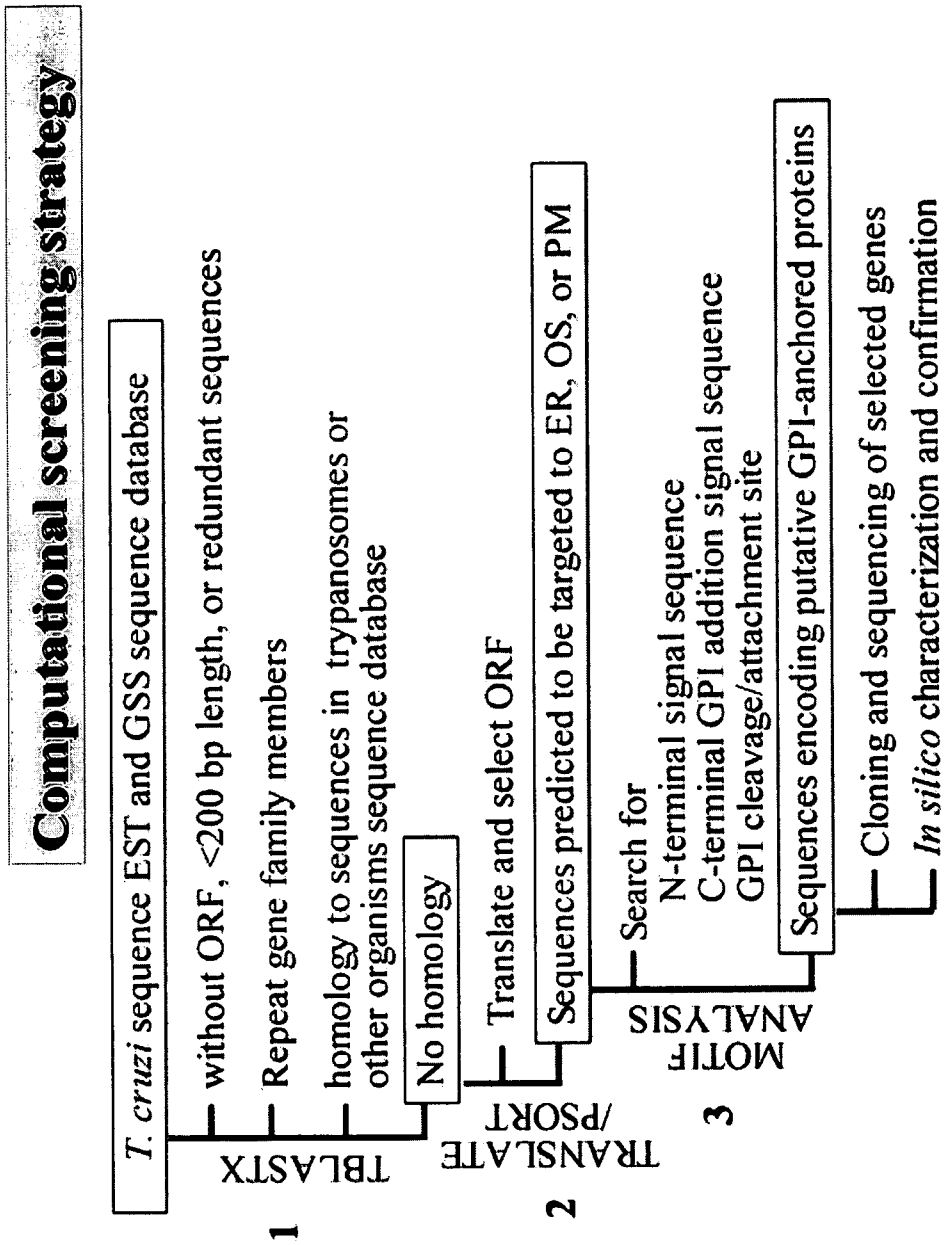
Computational screening strategy
T. cruzi sequence EST and GSS sequence database
1
TBLASTX
without ORF, <200 bp length, or redundant sequences
Repeat gene family members
homology to sequences in trypanosomes or other organisms sequence database
No homology
2
TRANSLATE /PSORT
Translate and select ORF
Sequences predicted to be targeted to ER, OS, or PM
3
MOTIF ANALYSIS
Search for
N-terminal signal sequence
C-terminal GPI addition signal sequence
GPI cleavage/attachment site
Sequences encoding putative GPI-anchored proteins
Cloning and sequencing of selected genes
In silico characterization and confirmation

FIG. 1

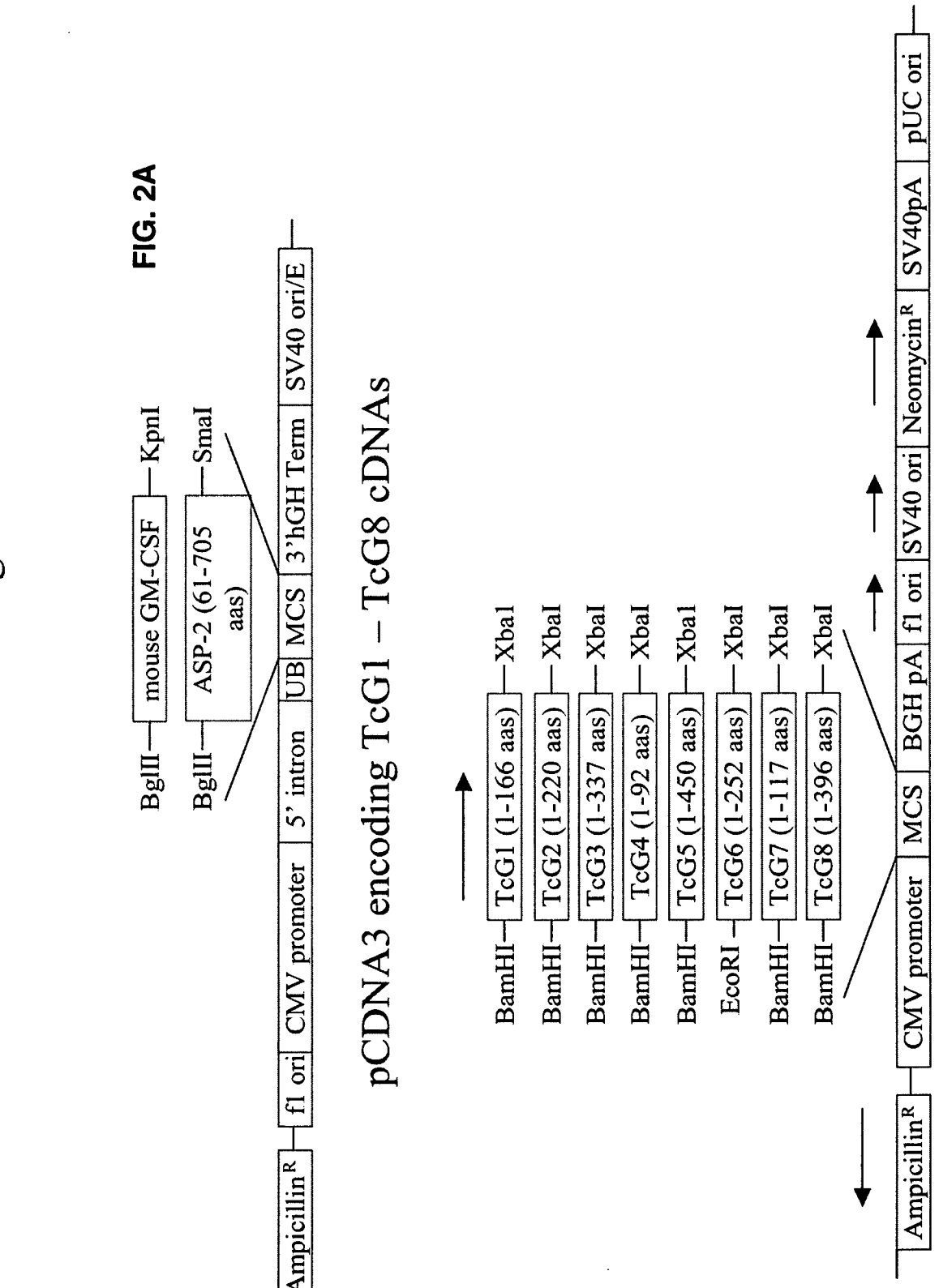
CMVI.UBF3/2 encoding ASP-2
BglII
mouse GM-CSF
KpnI
BglII
ASP-2 (61-705 aas)
SmaI
Ampicillin R
f1 ori
CMV promoter
5' intron
UB
MCS
3'hGH Term
SV40 ori/E

FIG. 2A

pCDNA3 encoding TcG1 – TcG8 cDNAs
BamHI
TcG1 (1-166 aas)
XbaI
BamHI
TcG2 (1-220 aas)
XbaI
BamHI
TcG3 (1-337 aas)
XbaI
BamHI
TcG4 (1-92 aas)
XbaI
BamHI
TcG5 (1-450 aas)
XbaI
EcoRI
TcG6 (1-252 aas)
XbaI
BamHI
TcG7 (1-117 aas)
XbaI
BamHI
TcG8 (1-396 aas)
XbaI
Ampicillin R
CMV promoter
MCS
BGH pA
f1 ori
SV40 ori
Neomycin R
SV40pA
pUC ori

FIG. 2B

TcG1	GGATCCATGGTGAAGGCGAACTATATT	GGGTCTAGATCACGTTCGAGATGCGCTTC	499
TcG2	GGATCCATGTCGCTTTCATTTATCGAGTCAGGG	GGGTCTAGATCACCCAACAGCGGTGGAA	662
TcG3	GGATCCATGCTTCAGCGTACCTGCAGC	GGGTCTAGATCAGCTTGACACTTCGC	1011
TcG4	GGATCCATGTCAGCCAAGGCTCCC	GGGTCTAGATCACTTTTCAAGCGCC	276
TcG5	GGATCCATGGGGAAGGAAAAGGTGC	GGGTCTAGATCACTTCTTAGCGGC	1350
TcG6	AAGGCTATGCTGGCGACAC	GGGTCTAGATCACACAGCAAGGG	756
TcG7	GGATCCATGCTGGCGACACACGG	GGGTCTAGACTACATCCATCCTCGCC	351
TcG8	GGATCCATGTCCGATAACCATCAACTGG	GGGTCTAGA TCACTGTGGTACAACGCTG	1188
TcGPI8	AAGCTTCGAGCATTGTCTATGTGCCTTGAA	CTCGAGCTACAGCAGGTCATATTGTACATC	450

Underlined sequences represent restriction sites.

FIG. 2C

CMVI.UBF3/2 encoding GM-CSF (mouse)

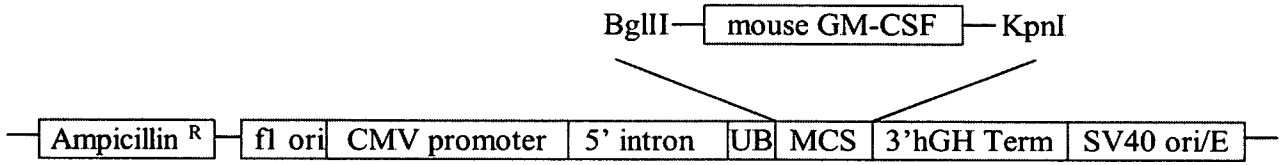

FIG. 2D

PCT/US2010/002465

pCDNA3 encoding mouse IL-12 (msp35, msp40 cDNAs)

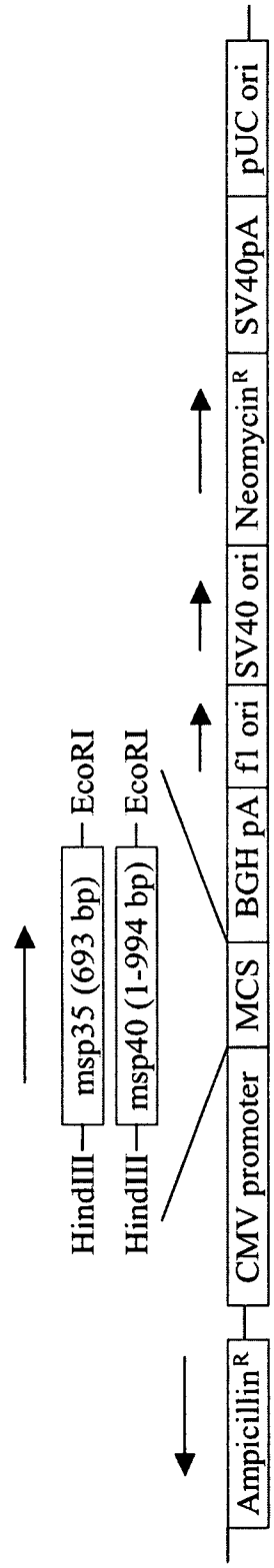

FIG. 2E

pCDNA3 encoding dog IL-12 (msp35 linked to msp40 cDNA)

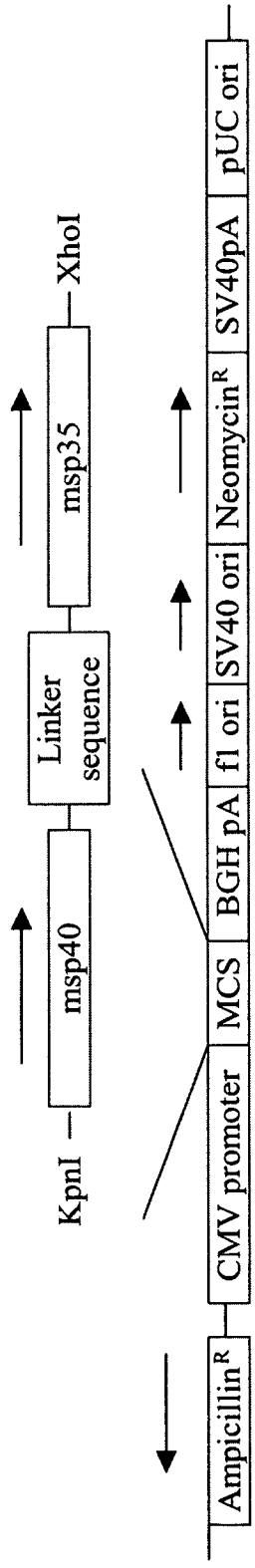

FIG. 2F

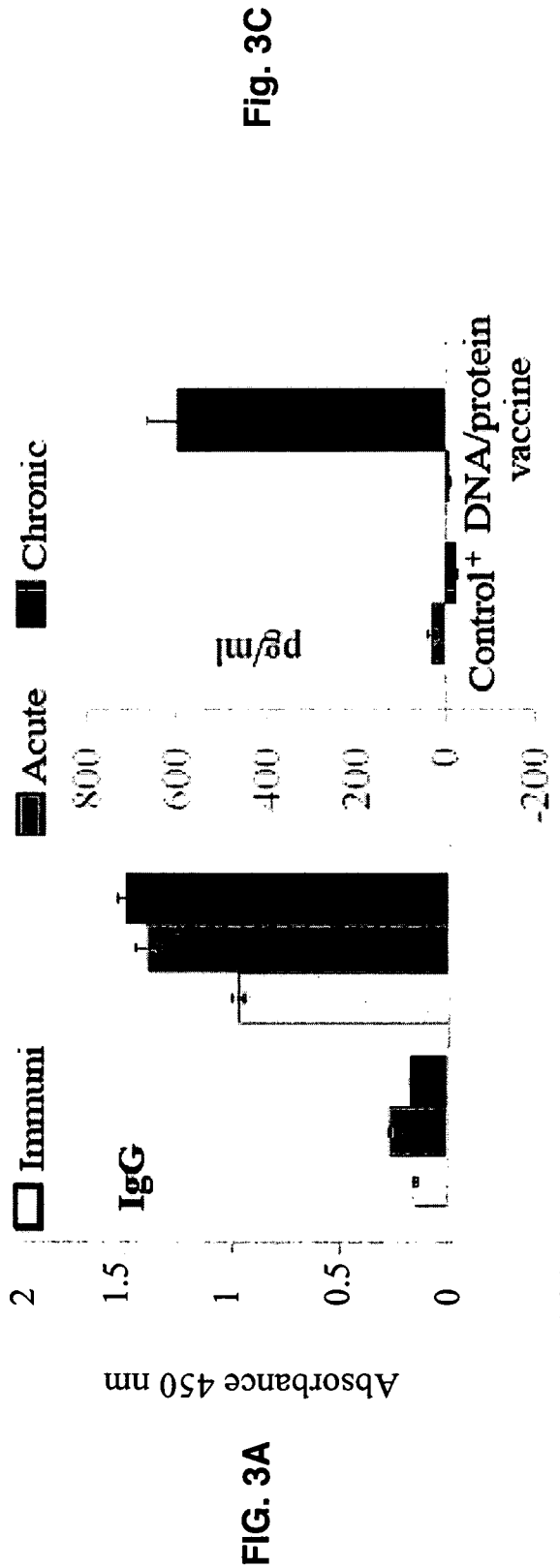

FIG. 3A

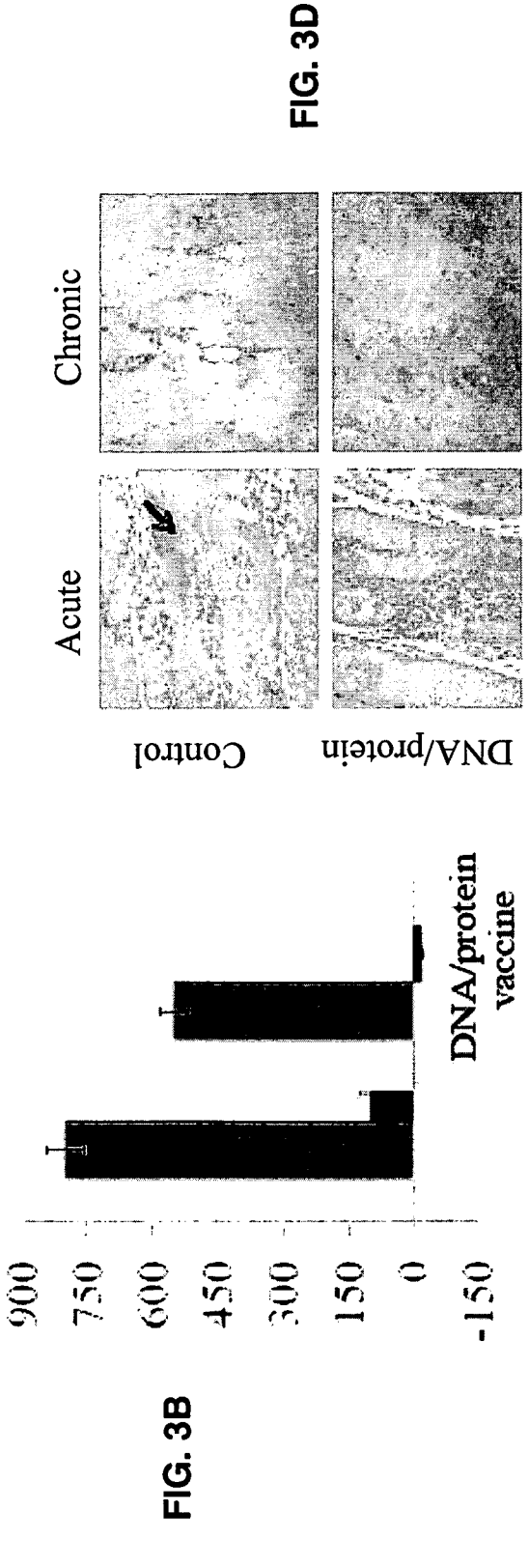

Fig. 3C

FIG. 3D

FIG. 3B

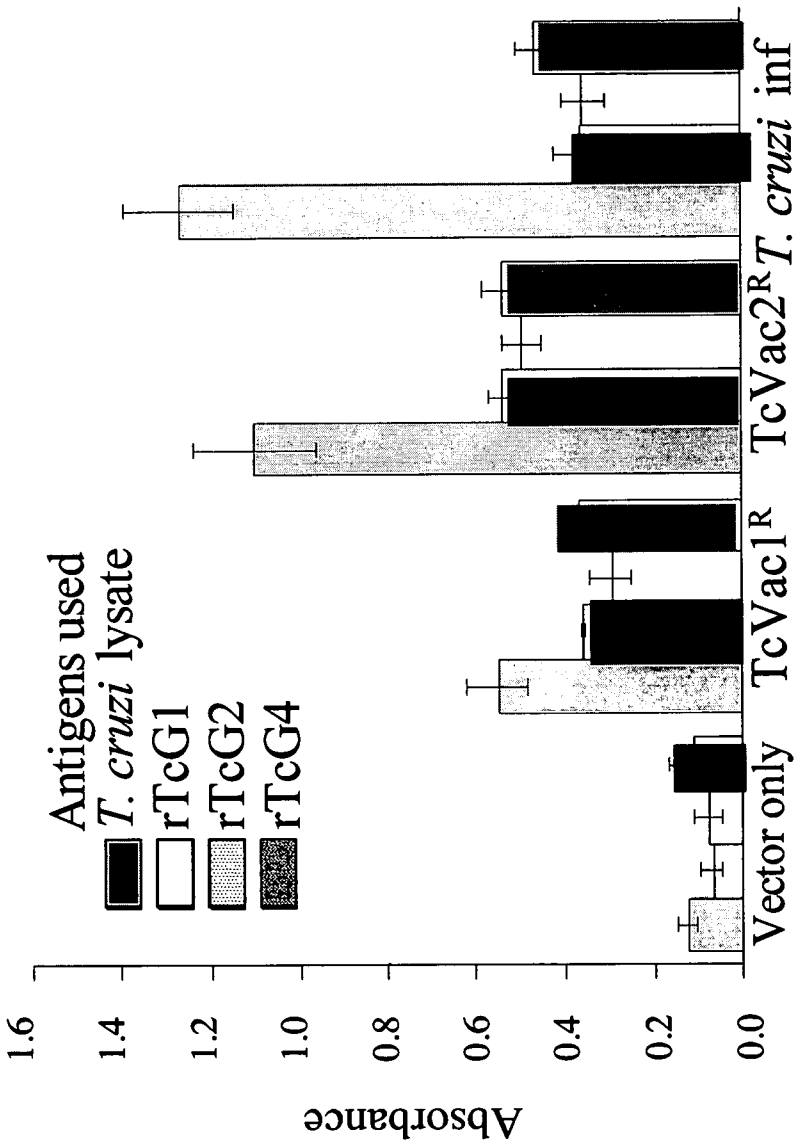
Absorbance
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Antigens used
T. cruzi lysate
rTcG1
rTcG2
rTcG4
Vector only
TcVac1R
TcVac2R
T. cruzi inf

FIG. 4

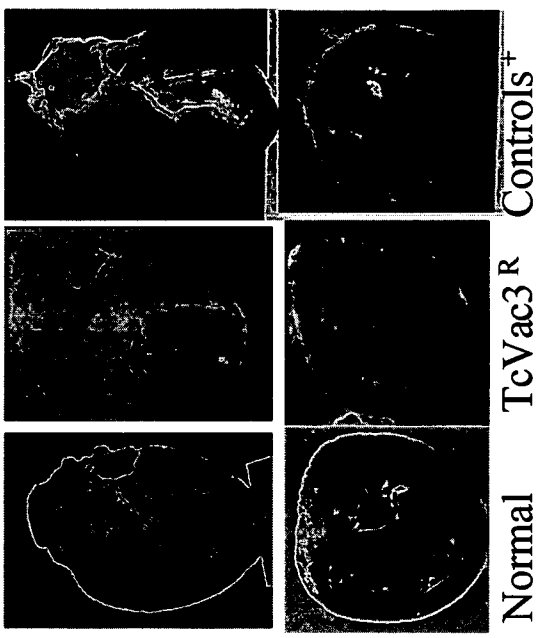

FIG. 5B

FIG. 5C

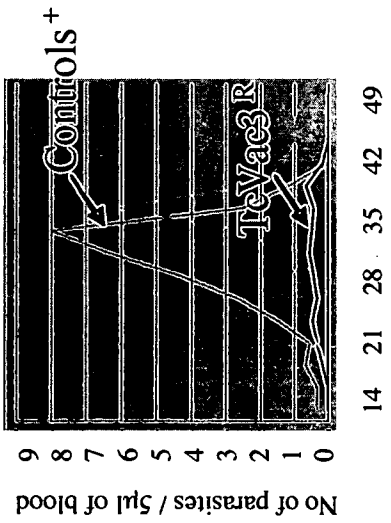

FIG. 5A