Oral Multicomponent DNA Vaccine Delivered by Attenuated *Salmonella* Elicited Immunoprotection Against American Trypanosomiasis

Silvia I. Cazorla,1,2,a Marina N. Matos,1,2,a Natacha Cerny,1,2 Carolina Ramirez,1,2 Andrés Sanchez Alberti,1,2 Augusto E. Bivona,1,2 Celina Morales,3 Carlos A. Guzmán,4 and Emilio L. Malchiodi1,2

1Cátedra de Inmunología and Instituto de Estudios de la Inmunidad Humoral Dr. R. A. Margni, Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad de Buenos Aires (CONICET-UBA), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, 2Instituto de Microbiología y Parasitología Médica (IMPaM), UBA-CONICET and Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, UBA, and 3Departamento de Patología, Facultad de Medicina UBA, Instituto de Fisiopatología Cardiovascular, Buenos Aires; Argentina; and 4Department of Vaccinology and Applied Microbiology, Helmholtz Centre for Infection Research, Braunschweig, Germany

We have reported that attenuated *Salmonella* (*S*) carrying plasmids encoding the cysteine protease cruzipain (*Cz*) protects against *Trypanosoma cruzi* infection. Here, we determined whether immunoprotection could be improved by the oral coadministration of 3 *Salmonella* carrying the plasmids that encode the antigens *Cz*, *Tc*52, and *Tc*24. *SCz*+*STc*52+*STc*24–immunized mice presented an increased antibody response against each antigen compared with those in the single antigen–immunized groups, as well as higher trypomastigotes antibody-mediated lyses and cell invasion inhibition compared with controls. *SCz*+*STc*52+*STc*24–immunized and –challenged mice rendered lower parasitemia. Weight loss after infection was detected in all mice except those in the *SCz*+*STc*52+*STc*24 group. Moreover, cardiomyopathy-associated enzyme activity was significantly lower in *SCz*+*STc*24+*STc*52–immunized mice compared with controls. Few or no abnormalities were found in muscle tissues of *SCz*+*STc*24+*STc*52–immunized mice, whereas controls presented with inflammatory foci, necrosis, and amastigote nests. We conclude that a multicomponent approach that targets several invasion and metabolic mechanisms improves protection compared with single-component vaccines.

Keywords. Chagas disease; American trypanosomiasis; *Trypanosoma cruzi*; multicomponent vaccine; DNA delivery system; *Salmonella enterica*; oral vaccine.

*Trypanosoma cruzi*, the causative agent of the neglected tropical disease American trypanosomiasis, or Chagas disease, is an obligatory intracellular parasite that affects 10–12 million people in Latin America. Although vectorborne transmission has been reduced to 70% in Southern Cone countries, mother-to-child transmission, mother-to-child transmission, transfusion- and organ transplantation-associated infections, and ingestion of contaminated foods have emerged as important transmission routes [1–5].

In addition, due to regional and global migration caused by economic hardship, the geographic region of Chagas disease has expanded from rural to urban areas and to nonendemic countries in Europe and North America, a phenomenon referred to as the globalization of Chagas disease [6–8]. The treatment of every affected individual is an ethical imperative. Nevertheless, major antiparasitic drugs are toxic, sometimes unavailable, and ineffective during the chronic stage. Current prevention strategies are insufficient for controlling disease, thus a prophylactic vaccine against *T. cruzi* is urgently needed.

*Trypanosoma cruzi* contains a major cysteine proteinase called cruzipain (*Cz*). Protease-deficient
parasites rapidly activate host macrophages and cannot survive [9]. We have reported the efficacy of attenuated Salmonella enterica (S) carrying a plasmid encoding Cz (SCz) in protecting against T. cruzi infection [10]. Development of multicomponent vaccines constitutes an attractive strategy for triggering immune response against several target structures, thereby affecting different key processes (eg, invasion, survival, metabolism). In this regard, we selected antigens that play a key role in the parasite life cycle and determined whether immunoprotection elicited by SCz could be improved by coadministration of Salmonella strains carrying plasmids encoding a thiol-transferase (Tc52) and a 24-kDa flagellar calcium-binding protein (FCaBP or Tc24).

Tc52 is a T. cruzi thiol-disulfide oxidoreductase release protein described as the missing link between the reduced glutathione- and trypanothione-based metabolisms [11]. Tc52 exerts regulatory effects on host inflammatory and immune responses [12], and it is considered a virulence factor crucial for parasite survival [13, 14]. Tc24 is a 24-kDa highly immunogenic protein that is present in all developmental stages of the parasite and that plays a key role in flagellar motility, leading to cell invasion by trypanomastigotes [15, 16].

Incorporation of any of these 3 antigens in a multicomponent prophylactic or therapeutic vaccine resulted in reduced parasitemia [14, 17–20]. Here, we demonstrate that a multicomponent oral vaccine based on Salmonella as a DNA delivery system for Cz, Tc24, and Tc52 elicited strong humoral and cellular immune responses, conferring improved protection against T. cruzi infection with respect to monovalent formulations.

**MATERIALS AND METHODS**

Recombinant Proteins and Eukaryotic Expression Plasmids Encoding Cz, Tc52, and Tc24

Recombinant Cz purification as well as the construction of eukaryotic plasmid with 1404 bp encoding the full length Cz was previously described [19]. Tc24 was cloned and expressed similarly. The cloning and expression of Tc52 in prokaryotic and eukaryotic plasmids is described by Matos et al [21].

Immunization and Challenge

Experiments that used animals were approved by the Review Board of Ethics of the Instituto de Estudios de la Inmunidad Humoral and conducted in accordance with the guidelines established by the Consejo Nacional de Investigaciones Científicas y Técnicas [22]. Five or 6 female C3H/HeN 6- to 8-week-old mice per group were immunized on days 0, 10, 20, and 30 by oral route with 10⁸ CFU of S. enterica serovar Typhimurium aroA strain SL7207 carrying pCDNA 3.1(+) (GI; control) or with pCDNA 3.1(+) coding for Cz (GII; SCz), Tc-52 (GIII; STc52), Tc24 (GIV; STc24), and Cz, Tc52, and Tc24 (GV; SCz+ STc52+STc24). Fifteen days after the last immunization, mice were killed by cervical dislocation. Blood and intestinal lavage samples were taken, as well as spleen cells, in order to analyze the immune response. Other sets of immunized mice were challenged with 1000 bloodstream trypomastigotes (RA strain) by intraperitoneal route.

Antibody Determination

Protein-specific antibody titers were determined using enzyme-linked immunosorbent assay (ELISA) in plates sensitized with 0.2 µg of Cz, Tc52, or Tc24 per well. Peroxidase-conjugated goat immunoglobulins to mouse immunoglobulin (Ig) G or biotinylated rat monoclonal antibodies to mouse IgG1 or IgG2a subclasses were used as the secondary antibody, followed by streptavidin-peroxidase when appropriate. Plates were developed by adding o-phenylenediamine (OPD)/H₂O₂. Antigen-specific intestinal secretory IgA (sIgA) was detected by ELISA using plates coated with anti-IgA antibodies and purified sIgA as the standard.

Delayed-type Hypersensitivity Reaction

The delayed-type hypersensitivity (DTH) test was performed 13 days after the last immunization by intradermal challenge with 5 µg of the corresponding protein into the footpad. Results are expressed as the difference in thickness of footpads before and 48 hours after the inoculation [10].

Cell Invasion Inhibition

Vero cells (5 × 10⁵ cells/well) were infected with transfected blood trypomastigotes expressing β-galactosidase [23] at a parasite-to-cell ratio of 10:1 for 24 hours at 37°C. Trypomastigotes were previously incubated in triplicate with diluted (1:50) serum from mice belonging to each immunization group. Controls included uninfected Vero cells pretreated with Rosewell Park Memorial Institute 1640 medium alone (0% infection), and cell monolayers were infected with untreated trypomastigotes (100% infection) [24]. After 24 hours of coculture, plates were washed with phosphate-buffered saline and fresh complete medium was added. The assay was developed 5 days later as described [25, 26].

Complement Mediated Killing of Parasites

Trypomastigotes were incubated in triplicate with diluted (1:10) decomplemented sera from mice belonging to each immunization group. Incubation for 30 minutes at 37°C with 5% CO₂ was performed in the presence of normal donor fresh serum [24]. Then, the number of living parasites was determined by counting in a Neubauer chamber.

Measurement of Muscle Damage

Muscle injury was evaluated through the determination of a panel of myopathy-linked enzyme markers at 100 days post-infection (dpi). The assays were performed as described [10]. The histological features of heart and skeletal (quadriceps)
muscles from vaccinated and infected mice (100 dpi) were also investigated. A blind histological test was performed as previously described [27].

Statistics
Statistical analyses were carried out with the Prism 3.0 Software (GraphPad, San Diego, California) using 1-way analysis of variance. The log-rank test was used for survival curves. P values < .05 were considered significant.

RESULTS

Multicomponent Vaccine Elicits Systemic and Local Humoral Immune Responses
To determine if the efficacy of vaccination with an attenuated Salmonella carrying a eukaryotic expression vector encoding Cz (SCz) could be improved, we coadministered SCz with Salmonella carrying plasmids encoding Tc52 and Tc24 (STc52 and STc24). Fifteen days after the last immunization rCz-, rTc52- and rTc24-specific IgG antibody responses in every group
those obtained using sera from mice in the control group at *P < .05, **P < .01, and ***P < .001. The results are representative of 3 independent experiments. The star at the top of a bar indicates that values were statistically different with respect to controls. Mice immunized with the multicomponent vaccine presented an increased humoral response against every antigen compared with control mice, as well as with respect to the SCz and STc24 groups (Figure 1A). These results indicate that there are no problems of immune dominance impairing responses to individual target antigens from the multicomponent formulation and suggest that improved immune protection could be achieved with the multicomponent vaccine. When IgG isotypes were measured as an indirect mode to determine the induced Th bias, we found that all immunized mice display a Th1-biased immune response, with 5–12 times more IgG2a than IgG1 (Figure 1B).

Considering that T. cruzi is able to penetrate intact mucosae, the ability of a vaccine candidate to efficiently stimulate both systemic and local immune responses would represent a real asset. Accordingly, we determined whether the vaccination protocols were able to stimulate sIgA in intestinal lavage fluids. Antigen-specific sIgA was detected in all vaccinated animals (Figure 1C). Taken together, these results show that for vaccination using Salmonella as a DNA delivery system for Cz, Tc52, and Tc24 induce antigen-specific antibody responses at mucosal and systemic levels.

**Antigen-Specific Antibodies Kill Parasites and Limit T. Cruzi Infection In Vitro**

To further characterize the antibody immune responses, we determined whether the antibodies stimulated in vaccinated mice were able to induce complement-mediated killing of T. cruzi trypomastigotes. A significant reduction in the number of parasites was observed when trypomastigotes were incubated with serum of SCz+STc52+STc24-immunized mice with respect to control mice (P < .001; Figure 2A). More interesting, these antibodies were also able to inhibit the in vitro invasion of trypomastigotes into mammalian cells (Figure 2B). Surprisingly, antibodies generated after STc24 immunization were able to mediate only weak parasite lysis or inhibition of cell invasion, which was not statistically significant with respect to controls. In contrast, sera from SCz- and STc52-immunized mice also killed parasites and inhibited Vero infection by trypomastigotes, although with lower significance than sera from mice receiving the multicomponent formulation.

**Cellular Immune Response**

We analyzed the cellular response in vivo by performing a DTH test by intradermal injection of the corresponding antigens into the footpad of immunized mice. All mice receiving the monoclonal formulations displayed significant DTH responses (Figure 3A) as compared with controls. Mice vaccinated with the multicomponent formulation (SCz+STc24+STc52) showed strong DTH responses against every antigen. These results demonstrate that the cellular immune response elicited to 1 antigen was not abolished by coadministration of other T. cruzi antigens (Figure 3A).

To assess the cytokine profiles in immunized mice 15 days after the last boost, we examined the levels of interleukin (IL)-12, interferon-gamma (IFN-γ), and IL-10 in supernatants of splenocytes after in vitro restimulation with rCz, rTc52, and/or rTc24 (10 µg/mL). Cells from SCz- and STc52-immunized mice released higher levels of Th-1–associated cytokines than...
controls (Figure 3B). In contrast, the level of cytokines produced by cells from STc24-immunized mice was not significantly different with respect to controls. In accordance with these results, when we analyzed cytokine levels in the group receiving the multicomponent vaccine, we observed a strong release of IFN-γ and IL-12 upon Cz and Tc52 in vitro restimulation, whereas no differences were observed upon Tc24 stimulus with respect to controls. In addition to the Th1 cytokines, the immune modulatory cytokine IL-10 was released by cells from the SCz+STc24+STc52 group upon stimulation with every antigen.

**Salmonella-based Multicomponent Vaccine Elicited Protection Against T. cruzi Challenge**

We then determined whether immunization with the different vaccine formulations was able to confer protection against *T. cruzi* infection. Mice received a sublethal challenge with 100 bloodstream trypomastigotes. The challenge was conducted intraperitoneally as a heterologous route for a more rigorous test of vaccine efficacy. Vaccination with SCz, STc52, or SCz+ STc24+STc52 resulted in significantly decreased parasitemia levels between day 14 and day 28 post-infection, as compared with controls (Figure 4A). When we analyzed the area under the curve (AUC) of parasitemia, mice vaccinated with SCz and STc52 presented 2.80- and 2.78-fold reductions, respectively, in the number of circulating parasites with respect to the control group. In contrast, mice vaccinated with STc24 were not able to control the infection, showing parasitemia similar to that of controls (AUC, 53.5 and 63.5, respectively). The protection conferred by the multicomponent vaccine SCz+STc24+ STc52 was significantly higher than that by single-component vaccines, presenting a 6-fold reduction in the AUC compared with controls (Figure 4A).

As an indirect parameter of the protection conferred by vaccination, we evaluated mice weight as a function of time during the acute phase of infection. Mice immunized with

---

**Figure 3.** Cellular immune response in immunized mice. A, Delayed type hypersensitivity test. Mice were immunized with *Salmonella* carrying plasmids encoding cruzipain (SCz), Tc24 (STc24), Tc52 (STc52), all 3 antigens (SCz+STc24+STc52), or pCDNA 3.1 (+) as control. Fourteen days after the last immunization, footpad thickness was measured before and 48 hours after the inoculation of 5 µg of (I) rCz, (II) rTc52, and (III) rTc24. Results are expressed as the differences between the values of measurements performed before and after antigen inoculation. Stars at the top of a bar indicate that values were statistically different with respect to those obtained in the control group at *P* < .05, **P** < .01, and ***P** < .001. B, Cytokine production in immunized mice. Spleen cells were harvested 2 weeks after the last immunization and cultured in duplicate in the presence of every recombinant antigen. Supernatants were collected 48 hours later and assayed in duplicate by capture enzyme-linked immunosorbent assay (BD Bioscience, OptEIA), according to manufacturer’s instructions. Abbreviations: IFN-γ, interferon-gamma; IL, interleukin; SEM, standard error of the mean.

---

**Table**

<table>
<thead>
<tr>
<th>Immunization</th>
<th>SCz</th>
<th>STc52</th>
<th>STc24</th>
<th>SCz</th>
<th>STc52</th>
<th>STc24</th>
<th>SCz</th>
<th>STc52</th>
<th>STc24</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>447 ± 62</td>
<td>213 ± 23</td>
<td>100 ± 51</td>
<td>829 ± 15</td>
<td>444 ± 42</td>
<td>112 ± 37</td>
<td>647 ± 74</td>
<td>614 ± 97</td>
<td>96 ± 21</td>
</tr>
<tr>
<td>IL-12 (pg/mL)</td>
<td>73 ± 10</td>
<td>85 ± 0.9</td>
<td>213 ± 20</td>
<td>163 ± 13</td>
<td>219 ± 16</td>
<td>186 ± 42</td>
<td>138 ± 31</td>
<td>149 ± 19</td>
<td>167 ± 17</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>371 ± 128</td>
<td>42 ± 4.2</td>
<td>221 ± 54</td>
<td>1222 ± 260</td>
<td>198 ± 24</td>
<td>396 ± 265</td>
<td>683 ± 81</td>
<td>160 ± 18</td>
<td>390 ± 142</td>
</tr>
</tbody>
</table>

The results represent the mean ± SEM of three independent experiments.
single-component vaccines showed slightly less weight lost than nonvaccinated controls (>30%) following challenge (Figure 4B). In contrast, mice immunized with the multicomponent formulation (SCz+STc24+STc52) maintained their weight until day 30 post-infection (P < .05 with respect to the control group).

We then determined whether the immunization regimes capable of reducing parasitemia in the acute phase of T. cruzi infection were additionally effective in limiting tissue injury at a later stage of trypomastigotes challenge. To this end, we analyzed the serum levels of cardiomyopathy-associated enzymes, as well as the characteristic tissue damage caused by chronic T. cruzi infection in hearts and skeletal muscle 100 dpi. The creatinine kinase (CK), aspartate transaminase (AST), and lactate dehydrogenase (LDH) activities in sera from SCz+STc24+STc52-immunized mice were significantly lower than in controls (Figure 5A). Mice vaccinated with SCz or STc52 also presented with reduced activity in serum of CK, LDH, and/or AST compared with nonimmunized control mice. In contrast, the serum levels of CK and LDH in STc24-immunized mice post-challenge were similar to those in controls. In accordance with these results, few or no abnormalities were detected in cardiac or skeletal muscles of mice vaccinated with the multicomponent vaccine by microscopic examination. In contrast, control mice presented inflammatory foci, necrosis, and amastigote nests (Figure 5B).

Comparison Between a Bicomponent and the Multicomponent Vaccines

Mice immunized with STc24 had parasitemia and weight loss undistinguished from nonvaccinated controls during the acute phase of T. cruzi infection, as well as similar levels of serum enzymes in the chronic phase of the infection. Thus, we determined whether vaccination with a formulation containing just 2 antigens (SCz+STc52) could confer a similar degree of protection as with the multicomponent vaccine. To this end, 3 groups of mice immunized with SCz+STc52, SCz+STc24+STc52, or Salmonella with an empty vector as control were infected with 1000 trypomastigotes by intraperitoneal route, and their parasitemia was analyzed. We did not observe any significant difference in the parasitemia AUC for SCz+STc52- and SCz+STc52+STc24-immunized mice (AUC 11.2 and 10.3 parasites, respectively), although they were significantly different than the control (Figure 6A1). Furthermore, we did not observe any weight loss differences between mice during the acute phase of the infection, although significant weight loss was detected in control mice (Figure 6AII). However, while 83% of the mice receiving the multicomponent vaccine survived until the end of the acute phase of the infection, 50% of the mice receiving SCz+STc52 died between 18 and 37 dpi (Figure 6AIII). We also measured the activity of cardiomyopathy-associated enzymes at 100 dpi in vaccinated mice challenged with a sublethal dose of trypomastigotes (100 parasites). No differences were observed between mice immunized with SCz+STc52 and SCz+STc24+STc52 (Figure 6B), indicating that both immunization regimens were able to protect tissue in the chronic phase of Chagas disease. In contrast, control mice displayed high serum activity of CK, LDH, and AST post-challenge.

**DISCUSSION**

After several years of debate about the pathogenesis of Chagas disease, it has been accepted that preventing infection or controlling the acute parasite load would be effective in decreasing tissue damage. This provides new incentive for the development of prophylactic vaccines against T. cruzi infection. Accordingly, several antigens have been tested in animal models as vaccine
candidates against *T. cruzi* [28]. In parallel, efforts to enhance the protective efficacy of subunit vaccines have included testing of adjuvants [29, 30] or antigen-DNA delivered by bacteria or viruses [31], including heterologous prime-boost protocols [32].

In this context, we previously reported the ability of attenuated *S. enterica* carrying a eukaryotic vector encoding Cz DNA to promote a Th1 protective response against *T. cruzi* challenge [10]. Here, we determined if the degree of protection conferred by this DNA-based vaccine could be further increased by incorporation of additional antigens. To this end, we assessed the efficacy of a multicomponent approach based on the coadministration of *Salmonella* carrying eukaryotic vectors coding for Cz, Tc52, and Tc24. The antigen selection was based on the fact that they are immunogenic in natural infection [33–35], important in different metabolic and/or pathogenesis processes [36], present in the parasite membrane or released [37, 38], phylogenetically conserved in diverse *T. cruzi* strains [39], present in the 3 main developmental stages of the parasite [39], and able to induce protection in prophylactic and/or therapeutic settings [17, 40, 41].

Mice vaccinated by oral route with the multicomponent vaccine (SCz+STc52+STc24) showed no antigenic interference at systemic or mucosal levels, with significant antibody response against all 3 antigens. The antibodies in sera exhibit biological activity, since they were able to both mediate parasite killing in the presence of complement and limit parasite invasion of eukaryotic cells. These results are more significant considering that overall titers were modest, as is expected for a DNA vaccine. This fact strengthens the hypothesis that the quality of antibodies induced by vaccination against *T. cruzi* is far more important than the absolute titers. In this regard, we previously demonstrated that low antibody titers against the N-terminal domains of Cz were able to dramatically reduce *T. cruzi* infection of Vero cells [24]. This has clinical correlations, since it was reported that asymptomatic chronic patients have higher levels of lytic antibodies than those exhibiting clinical disease [42].

Importantly, in mice vaccinated with SCz+STc24+STc52, the cellular immune response to every antigen was not impaired by the presence of the other antigens included in the multicomponent vaccine. Furthermore, 2 major cytokines (IFN-γ and
IL-10) were increased in mice receiving SCz+Stc52+Stc24 (Figure 5). Several studies have demonstrated a correlation between production of pro- and anti-inflammatory cytokines by CD4+ T cells with symptomatic and asymptomatic clinical forms of chronic Chagas disease, respectively [43, 44]. IFN-γ–producing CD4+ T cells likely assist phagocytes in parasite killing and provide help for development of CD8+ cytotoxic T lymphocyte effector cells. On the other hand, IL-10+ regulatory T cells are predominantly found in clinically asymptomatic chagasic patients [45], indicating that these cells play an important role by preventing collateral immune damage during T. cruzi infection [46].

Immunization with the multicomponent vaccine promoted efficient protection in mice, which retained their body weight after infection. Animals also showed significantly lower levels of parasitemia than controls or mice immunized with monocomponent formulations (Figure 4). Protection was stable and long lasting, even during the chronic stage of infection. SCz+Stc24+Stc52–immunized mice showed serum levels of the cardiomyopathy-associated enzymes similar to those of noninfected mice (CK, 27.59 ± 9.6; LDH, 1070 ± 124; and AST, 10.8 ± 3.5; Figure 6). Thus, the multicomponent vaccine showed a clear additive effect considering that better scores were systematically obtained for critical protection parameters such as parasitemia, animal weight, and levels of cardiomyopathy-associated enzymes.

Surprisingly, mice vaccinated with Stc24 were not able to restrain T. cruzi infection, showing parasitemia levels similar to those in animals in the control group. The ability of Tc24 to confer immune protection is controversial. An earlier study showed that Tc24 was not a good vaccine candidate, since it induced polyclonal activation of IgM-secreting B cells that facilitate parasite immune evasion [47]. Later, it was reported that immunization or treatment with 2 doses of plasmids encoding Tc24 and Tc24 with alum induced control of the disease, with reduction of parasitemia, mortality, myocarditis, and heart parasite burden in mice and dogs [17, 18, 20, 48].

Therefore, we determined if, under our experimental conditions, immunization with a bicomponent vaccine including SCz+Stc52 would confer similar protection as a trivalent vaccine including Tc24 (SCz+Stc24+Stc52). No differences were observed between both groups in terms of parasitemia and myocardiopathology. Unexpectedly, most of the mice immunized with the trivalent vaccine showed improved survival post-
*T. cruzi* infection during the acute phase compared with those receiving the bivalent vaccine. Considering the immunogenicity profiles and protective efficacy of mono-, bi-, and tri-component vaccines, the underlying mechanisms responsible for the superior efficacy of the tri-component vaccine are unclear. However, the observed improved survival of mice during the acute phase, together with previous evidence on the protective role of the Tc24 antigen in prophylactic or therapeutic vaccines [17, 18, 20, 48, 49], suggest that Tc24 should not be excluded from the multicomponent vaccine. Studies should be conducted to identify parameters that correlate better with parasitemia and myocardial damage in order to elucidate the mechanisms that lead to improved protection by inclusion of Tc24 in the formulation.

These results confirm that *T. cruzi* vaccine efficacy is not only highly dependent on the selected antigen(s) but also on the vaccination strategy [10]. Vaccines based on a single antigen are not likely to be able to confer an adequate level of protection against *T. cruzi* infection. This would be an attainable goal by using a multiantigen approach, which targets several metabolic and pathogenic mechanisms. The experiments described here demonstrate that the *Salmonella*-based trivalent formulation conferred protection superior to that of the mono- or bivalent vaccines. Although complete parasite clearance would probably not have been achieved, mice immunized with the multicomponent vaccine controlled the parasite burden, resulting in limited inflammatory cells in the heart and absence of tissue destruction during the chronic phase. In this regard, prophylactic vaccination should be effective at preventing Chagas disease. Furthermore, a multicomponent vaccine used in mammals may promote effective control of vectorial transmission to humans. Triatomines that feed on vaccinated mammals with low parasitemia will rarely get infected or will have a decline in the number of trypomastigotes eliminated in their feces.

We conclude that immunity improved when additional antigens were added, highlighting the benefits of a multiantigen approach. Thus, vaccines with a single antigen are not likely to be protective, and a multiantigen approach targeting multiple invasion and metabolic mechanisms is likely required to better protect against *T. cruzi* infection and disease.

**References**

21. Matos MN, Cazorla SI, Bivona AE, Morales C, Guzmán CA, Malchiodi EL. Tc52 amino terminal DNA carried by attenuated...


