Trans-sialidase delivered as a naked DNA vaccine elicits an immunological response similar to a Trypanosoma cruzi infection

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Abstract

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Trypanosoma cruzi, the protozoan parasite that causes Chagas' disease, does not synthesize sialic acid, but expresses a *trans*-sialidase (TS) that catalyzes the transfer of sialic acid from host glycoconjugates to the parasite surface. Here, we review studies that characterize the immune response to the catalytic domain of the enzyme in humans during Chagas' disease or in mice following immunization with the TS gene. In both cases, there are antibodies that strongly inhibit the enzymatic activity and generation of interferon-γ-producing T cells.

Chagas' disease

Trypanosoma cruzi is a protozoan parasite and the causative agent of Chagas' disease. In spite of considerable efforts to eradicate the triatomine, the disease is still transmitted in several states of Brazil and other South American countries, endangering millions of people. Also, the existence of 10-14 million individuals in the chronic stages of the disease is a major threat for transmission through blood transfusion.

After contact with trypomastigotes of *T. cruzi*, mammalian hosts develop the acute phase of Chagas' disease, characterized by patent parasitemia that lasts for several weeks. The chronic phase initiates when the parasitemia declines significantly, becoming sub-clinical. Most individuals carry this infection for life. Drug treatment is prolonged and

its efficacy is not high. In recently infected children, treatment is 55.8% efficient (1). The efficacy is even lower (19.1%) in adults infected for many years (2). The poor prospect of treatment raises the possibility that immune interventions, such as immunization, could be an additional weapon to increase treatment efficacy in patients who do not respond to conventional chemotherapy.

Structure of the *trans*-sialidase

Several surface antigens of infective forms of *T. cruzi* (trypomastigotes and amastigotes) have been grouped into a super-family defined by the presence of consensus sequences homologous to bacterial sialidases (3-5). These surface antigens are a highly polymorphic family of proteins with molecular masses ranging from 85 to 200 kDa. Many of these

proteins have been implicated in a number of biological processes important for *T. cruzi* interaction with host cells and extra-cellular matrix proteins (6-11). One group of these proteins are enzymes denominated *trans*-sialidase (TS) that catalyze the transfer of sialic acid to ß-galactosyl residues of mucin-like glycoproteins that are present on the surface of *T. cruzi* trypomastigotes and epimastigotes (12,13).

The importance of the acquisition of sialic acid is not completely known. However, there are suggestions that the transfer of sialic acid present on the surface of the host cell to the parasite may help trypomastigote adhesion and penetration into nonphagocytic cells (9,14). Sialic acid also provides a strongly negatively charged cover that protects parasites against human lytic antibodies specific for α-galactosyl residues which are abundant on the trypomastigote surface (Pereira-Chiccola VL, Acosta-Serrano A, Almeida I, Rodrigues MM, Travassos LR and Schenkman S, unpublished results).

In trypomastigote forms found in the vertebrate host, TS is formed by multimeric aggregates with molecular mass ≥400 kDa (15,16), that upon denaturation migrates as multiple bands ranging from 160 to 220 kDa in SDS-PAGE (13,17). Trypomastigote TS has essentially 2 different domains. The N-terminal region contains the catalytic domain of the enzyme (Ref. 18 and Figure 1). The C-terminal, or CTR, is composed of amino acid repetitions. TS is linked to the parasite membrane by a glycosyl-phosphatidylinositol (GPI) anchor and is continuously shed into the supernatant. Details concerning the TS gene and its protein structure have been described by Schenkman et al. (5).

Immunogenic properties of TS during human infection with *T. cruzi*

Serological studies have shown that TS is highly immunogenic during natural human infection with *T. cruzi*. Almost all individuals develop antibodies that recognize an epitope within the CTR of TS (3). Chagasic patients also generate antibodies specific for the N-terminal region of TS that strongly inhibit its enzymatic activity (19,20). Comparatively, chagasic antibodies preferentially recognize the native TS which contains the CTR rather than a recombinant TS which contains only the N-terminal catalytic domain (Figure 2). Nevertheless, there is direct evidence that inhibitory antibodies which recognize the catalytic domain of TS passively protect mice against bloodstream trypomastigote infection (21). These inhibitory antibodies differ from antibodies against the CTR that are unable to block enzymatic activity and were not reported as protective (21,22).

To complement these serological studies, we used a recombinant protein based on the sequence of the N-terminal catalytic domain to determine whether chagasic patients develop a cell-mediated immune response (CMI) to TS. CMI was measured by *in vitro* T-cell proliferation, and interferon-γ and interleu-

Figure 1 - Schematic view of the trans-sialidase (TS) of T. cruzi trypomastigotes and the recombinant protein based on the N-terminal region.
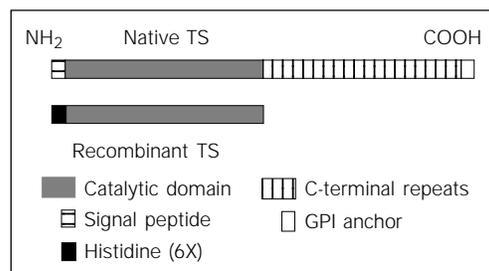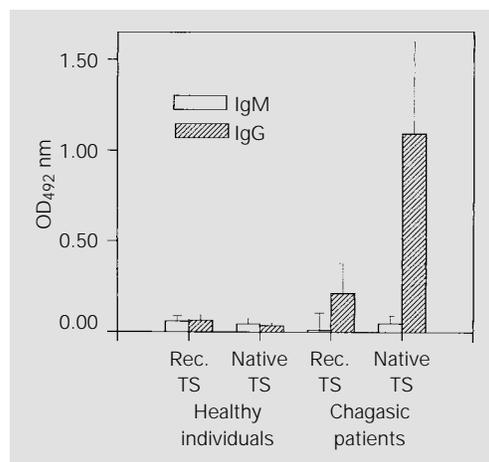

Figure 2 - Human antibody recognition of native and recombinant (Rec.) trans-sialidase (TS) by ELISA.

kin-4 production in response to the recombinant protein. We found that cells from 78% of the chagasic patients proliferated in response to the recombinant TS. Most relevant, cells from 88% of these patients produced interferon-γ upon stimulation with the recombinant protein (Figure 3 and Ribeirão M, Pereira-Chiccola VL, Fragata-Filho A, Renia L, Schenkman S and Rodrigues MM, unpublished results). In contrast, interleukin-4 was not detected in response to TS. CMI was specific, because healthy individuals never exposed to *T. cruzi* infection failed to react with this recombinant protein. From these results, we concluded that the TS catalytic domain is recognized by T cells and antibodies in a large proportion of patients infected with *T. cruzi*.

Immunogenic properties of TS delivered as naked DNA vaccine

Based on the results that support the concept that TS is highly immunogenic during natural infection with *T. cruzi*, we determined whether immunization with plasmids containing the TS gene could elicit immunity against experimental Chagas' disease. Initially we compared the immunogenicity of several plasmid vectors containing a gene encoding the catalytic domain of TS. Additionally, we evaluated the effect of the presence or absence of the signal peptide on the immunization with plasmid DNA. For this purpose, three plasmids were generated containing the coding region for the catalytic domain of TS. The TS gene was inserted into
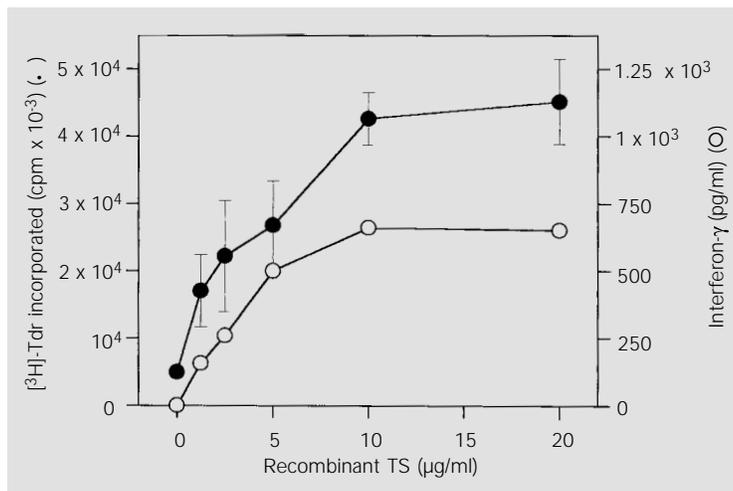

Figure 3 - Proliferative response and interferon-γ production by peripheral blood mononuclear cells of a chagasic patient upon stimulation with recombinant trans-sialidase (TS).

Table 1 - Antibody induction by immunization with TS plasmids.

[1]Based on the sequence of gene TS 154 cloned from T. cruzi Y strain (17). Length is reported in terms of number of amino acid residues (aa).

Plasmid	Vector	Translated amino acids[1]	Length (aa)	Antibody titers	Inhibition of TS activity
B43	pcDNA3	33-678	645	1.63×10^4	72 ± 21
154/13	pcDNA3	1-678	678	1.69×10^4	86 ± 13
CI-44	VR1012	1-678	678	1.68×10^4	64 ± 27
pcDNA3	pcDNA3	-	-	<1:100	11 ± 7
VR1012	VR1012	-	-	<1:100	1.3 ± 3.3

Table 2 - Survival of BALB/c and A/Sn mice after challenge with T. cruzi trypomastigotes.

Mice were immunized with 4 doses of the indicated plasmid. Each dose consisted of 100 µg of DNA per animal. BALB/c and A/Sn mice were challenged with 6,500 and 250 T. cruzi trypomastigotes, respectively.

Mouse strain	Plasmid	Number of experiments	Number of mice (protected/challenged)	Protection (%)
BALB/c	PCDNA3	3	2/20	10%
BALB/c	154/13	3	20/23	87%
A/Sn	PCDNA3	3	0/15	0%
A/Sn	154/13	3	0/15	0%
A/Sn	VR1012	2	0/10	0%
A/Sn	CI-44	2	0/8	0%

the pcDNA3 vector with or without the coding region for the TS signal peptide. These plasmids were found to be equally immunogenic by inducing antibodies to TS as estimated by ELISA or inhibition of TS enzymatic activity (Table 1 and Ref. 23).

Subsequently, we used one of these plasmids to determine whether the TS gene could elicit T cell-mediated and protective immunity against *T. cruzi* infection in mice. BALB/c mice immunized with plasmid 154/13 containing the gene encoding the catalytic domain of *trans*-sialidase generated specific immune responses, as measured by antibody production and T cell activation (24). Although the major immunoglobulin G produced in response to the TS gene was IgG1, we noticed that T cells preferentially produced interferon-γ. To understand this apparent contradiction, we derived T cell clones from DNA-immunized mice. We found that CD4^{+} T cell clones produced high levels of interferon-γ but failed to secrete IL-4 or IL-10 (TH1 cells). In addition, we isolated CD4^{+} T cell clones that secreted IL-4 and IL-10 but failed to produce interferon-γ (TH2 cells, Rodrigues MM, Ribeirão M, Pereira-Chiccola VL, Renia L, Schenkman S and Costa F, unpublished results). Our results demonstrate that TH1 and TH2 CD4^{+} cells coexist in mice immunized with the TS gene.

In addition to interferon-γ-producing CD4^{+} T cells, we demonstrated that 35% of the interferon-γ secreted came from CD8^{+} T cells. To confirm the existence of this T cell subtype, we also generated T cell clones using as target cells A20J cells transfected with the TS gene. These clones produce large amounts of interferon-γ upon stimulation with TS-transfected syngeneic cells. Considering that interferon-γ has been implicated in resistance against *T. cruzi* infection (25), it is quite possible that both cell types mediated protective immunity in our model. In support of this hypothesis, we observed that upon challenge with blood stages of *T. cruzi*, BALB/c mice showed a significant reduction in the peak of parasitemia (24). Most relevant, 87% of these animals survived the acute infection induced by blood stages of *T. cruzi*. In contrast, there was only 10% survival in control mice immunized with the vector alone (Table 2). The protective immunity observed in BALB/c mice was strain specific since A/Sn mice immunized with this same plasmid failed to survive the infection.

Conclusions

The molecular characterization of *T. cruzi* TS opened the possibility of designing experiments to study naturally acquired human immunity and to compare it with immunity elicited by vaccination in the experimental models. Our data strongly suggest that immunity to TS is deleterious to the parasite, protecting the host. In the experimental model, a similar immunity pattern can be obtained by immunization with a naked DNA vaccine, inducing the production of inhibitory antibodies, as well as the activation of interferon-γ-producing T cells. Although this is clearly a protective response, protective immunity depends on the genetic background of the host. Future studies may reveal whether immunity to TS also contributes to the pathology of chronic Chagas' disease in humans.

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