Safety and immunogenicity of a recombinant vaccine against *Trypanosoma cruzi* in Rhesus macaques

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Abstract

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi* is one of the most important neglected parasitic diseases in the Americas. Vaccines represent an attractive complementary or even an alternative for the control of *T. cruzi* infection and pre-clinical studies in mice demonstrated that trypomastigote surface antigen (TSA-1) and the flagellar calcium-binding (Tc24) parasite antigens are promising candidates for vaccine development. We performed here the first evaluation of the safety and immunogenicity of two recombinant vaccine antigens (named TSA1-C4 and Tc24-C4) in naïve non-human primates. Three rhesus macaques received 3 doses of each recombinant protein, formulated with E6020 (Eisai Co., Ltd.), a novel Toll-like receptor-4 agonist, in a stable emulsion. All parameters from blood chemistry and blood cell counts were stable over the course of the study and unaffected by the vaccine. A specific IgG response against both antigens was detectable after the first vaccine dose, and increased with the second dose. After three vaccine doses, stimulation of PBMCs with a peptide pool derived from TSA1-C4 resulted in

the induction of TSA1-C4-specific TNFα-, IL-2-and IFNγ-producing CD4$^+$ in one or two animals while stimulation with a peptide pool derived from Tc24-C4 only activated IFNγ-producing CD4$^+$T cells in one animal. In two animals there was also activation of TSA1-C4-specific IL2-producing CD8$^+$ T cells. This is the first report of the immunogenicity of *T. cruzi*-derived recombinant antigens formulated as an emulsion with a TLR4 agonist in a non-human primate model. Our results strongly support the need for further evaluation of the preventive efficacy of this type of vaccine in non-human primates and explore the effect of the vaccine in a therapeutic model of naturally-infected Chagasic non-human primates, which would strengthen the rationale for the clinical development as a human vaccine against Chagas disease.

1. Introduction

Chagas disease, also known as American trypanosomiasis, is a vector-borne zoonotic disease caused by the protozoan parasite *Trypanosoma cruzi*. It is one of the most important parasitic diseases in the Americas, causing 0.55 million disability-adjusted life years lost, and 10,600 annual deaths [1–3], and an estimated $7.2 billion in annual economic losses [3].

The disease begins by an acute phase of a few weeks duration with non-specific signs and symptoms, followed by a chronic phase initially asymptomatic but 20–30% of patients will develop cardiac alterations leading to chagasic cardiomyopathy, associated with arrhythmias, which are sometimes fatal, aneurysms, or a dilated cardiomyopathy and eventually cardiac failure [4]. Some patients may also present a digestive form of the disease, with megaesophagous or megacolon. Disease progression is associated with increased mortality [5–7]. Current drug treatments with benznidazole or nifurtimox are effective during the acute phase, but treatment outcomes can be very variable during the chronic phase [8, 9], and do not halt or slow the progression of fibrotic heart disease in patients showing signs and symptoms of Chagasic cardiomyopathy [10]. In addition, these drugs are associated with adverse effects that can be severe and often lead to treatment interruption. Therefore, there have been some efforts at exploring complementary or alternative treatment regimens and combination therapies to reduce treatment duration and drug doses, with the objective of maintaining therapeutic efficacy while reducing adverse effects [9].

Therapeutic vaccines may represent an attractive complementary or alternative for the treatment or control of *T. cruzi* infection [11]. Indeed, extensive proof-of-principle studies have shown that different vaccine formulations can control a *T. cruzi* infection in mice (reviewed in [12]), and reduce fibrosis [13]. They are also effective in regimens of vaccine-linked chemotherapy [14]. These studies have shown that while antibodies may help clear circulating blood parasites, the induction of a cellular immune response, and particularly the activation of cytotoxic CD8$^+$ T cells, is also critical for the control of *T. cruzi* infection, as the parasite is mostly intracellular in its mammalian hosts [15, 16]. Additional economic modeling demonstrates that a Chagas disease vaccine would contribute to lowering health care costs associated with the disease [17–19].

Trypomastigote surface antigen (TSA-1) and the flagellar calcium-binding Tc24 parasite antigens have emerged as promising candidates for further vaccine development of a therapeutic vaccine against *T. cruzi* [20]. Process development for the large-scale production

of both recombinant antigens has been initiated and both antigens have been found able to at least partially control *T. cruzi* infection in mice when formulated with a variety of Th1 adjuvants, including the Toll-like receptor-4 agonist, E6020 (Eisai Co., Ltd.) [21–24]. Specific mutations have been made in both proteins, to facilitate production and increase stability, while maintaining immunogenicity in mice [25–27]. The native antigens are also able to recall an antigen-specific immune response in Chagasic patients [28], indicating that they are adequately processed by the human immune system during natural infection. As a key step prior to clinical trials, we performed here the first evaluation of the safety and immunogenicity of recombinant TSA1-C4 and Tc24-C4 antigens formulated in a stable emulsion that also contained E6020 adjuvant in naïve non-human primates. Macaques are indeed a very valuable model that has repeatedly found to be a much better predictor of immune responses in humans than mouse models [29–32]. In particular, macaques naturally infected with *T. cruzi* also present an immune response that mirrors that observed in human Chagasic patients [33], as well as comparable cardiac alterations [34].

2.   Materials and methods

2.1.   Study design

Animals were housed at the Tulane National Primate Research Center (TNPRC) under the care of TNPRC veterinarians, in accordance with the standards incorporated in the Guide for the Care and Use of Laboratory Animals and with the approval of Tulane Institutional Animal Care and Use Committee (IACUC). All procedures were performed on sedated animals. The study followed the ARRIVE guidelines for animal research [35]. Following confirmatory negative diagnostic for *T. cruzi* infection based on serology and PCR, three naïve male rhesus macaques (*Macaca mulatta*) 4–5 years old were enrolled in the study. A baseline blood sample was taken, and animals were then vaccinated with three doses of the vaccine, one month apart (Figure 1). A blood sample was collected in EDTA at each vaccination, and one month after the last vaccine dose to evaluate safety and immunogenicity of the vaccine. General physical examination was also performed at each time point.

2.2.   Expression and purification of recombinant antigens

Recombinant Tc24-C4 (lot #Tc24-C4160117CIFP-02) and TSA1-C4 (lot #TSA1C4–280518-AAP-2) proteins were produced in *Escherichia coli* following established processes described in detail before [24, 27]. These correspond to mutated antigens in which cysteine residues have been replaced [25–27]. TSA1-C4 was produced in 10L batch culture, purified by ion-exchange chromatography, followed by a refolding step, and a final resuspension in PBS. Tc24-C4 was also produced in a 10L fermenter, and purified on a GE Healthcare Q Sepharose XL column, followed by a 4 LGE Healthcare Sephacryl S-200 High Resolution column, and a final elution in PBS. Final purity of both antigens was >95% and endotoxin content was <10 EU/mg of protein. Purified proteins were stored in PBS at −20°C until used.

2.3. Vaccine formulation and immunizations

Each vaccine dose consisted of 100 μg of recombinant protein with 25 μg of E6020 (Eisai Co., Ltd.), a novel Toll-like receptor-4 agonist, in a stable emulsion [36]. Each antigen was mixed with the adjuvant just prior to immunization, which was performed subcutaneously in a volume of 500 μl. Tc24-C4 was administered over the right quadriceps, and TSA1-C4 over the left quadriceps in sedated animals, to avoid any potential interactions between the two proteins if mixed together.

2.4. Vaccine safety

General physical examination of the animals, including body weight and temperature, was performed at each time point to assess the general health following vaccination. Blood chemistry and cell counts were also performed at each time points to assess vaccine safety. Blood chemistry included Na, K, Cl, proline, albumin, globulin, blood urea nitrogen, glucose and creatinine. Cell counts included neutrophils, lymphocytes, monocytes, eosinophils and basophils.

2.5. Evaluation of vaccine-specific humoral response

Antigen-specific antibody responses were measured in plasma samples through western blot and ELISA. For western blot, 2 μg of whole *T. cruzi* parasite lysate from strain WB1 as well as 12 ng of Tc24-C4 and 18 ng of TSA1-C4 vaccine antigens were separated on 12% SDS-PAGE. Proteins were transferred onto nitrocellulose membranes using a Bio-Rad mini protein-II wet transfer unit. The transferred membranes were incubated with 5% nonfat dried milk dissolved in PBS-T buffer for 1 hour blocking at room temperature, followed by incubation with rhesus plasma samples (1:500 dilution, in blocking buffer) overnight at 4°C with gentle agitation. Membranes were washed three times with PBS-T buffer and incubated with a 1:10,000 dilution of peroxidase-labeled secondary antibody anti-rhesus IgG (Sigma-Aldrich, St. Louis, MO) for 1 hour and washed four times. Signal detection was performed with an enhanced chemiluminescence kit (Clarity Western ECL Substrate kit, Bio-Rad, Hercules, CA). Images were captured using Image Quant LAS 4000, with exposure times of 2 minutes.

For ELISA, ninety-six well microplates were coated overnight at 4°C with 0.5 μg/well of Tc24-C4 or 0.1 μg/well of TSA1-C4 vaccine antigens in carbonate buffer, washed three times with PBS, and blocked with 1% BSA and 0.05% Tween 20 in PBS for 1h at 37°C. After three additional washes, serial dilutions of rhesus plasma ranging from 1:500 to 1:200,000 were added in duplicate wells and incubated for 1h at 37°C. Wells were then washed 3 times, and incubated with a peroxidase-labeled antibody against rhesus IgG at a 1:10,000 dilution, for 30 min at 37°C. After three final washes, 3,3',5,5'–tetramethylbenzidine substrate in DMSO and phosphate-citrate buffer (pH 5.0) with 30% hydrogen peroxide were added and incubated for 30 min at room temperature in the dark. Reactions were stopped with 2M H_2SO_4, and plates were read at 450 nm in an ELISA plate reader. Positive responses to calculate end-point antibody titers were defined using a threshold corresponding to the mean of negative controls plus two standard deviations.

2.6. Evaluation of vaccine-specific cellular immune responses

Vaccine-induced cellular immune responses were assessed by flow cytometric analysis of intracellular cytokine secretion in stimulated peripheric blood mononuclear cells (PBMCs). Briefly, PBMCs were purified from blood samples through a Ficoll-hypaque centrifugation, and then cryopreserved. Cryopreserved cells were thawed on the same day as stimulation and re-suspended in complete medium (RPMI-1640, 2 mM/L L-glutamine, 10% heat-inactivated fetal bovine serum, 50 IU/ml penicillin, 50 µg/ml streptomycin, and 10mM HEPES). Prior to stimulation, BD Falcon tubes were coated with GAM (Goat Anti-Mouse IgG (H+L) Kierkegaard and Perry Lab, Ics., Gaithersburg, MD) using 2.5µg GAM/tube in 1.0mL of 50mM TRIS (pH 8.6) and incubation at 37°C for 1 hour. After twice-washing with PBS, anti-CD28 and anti-CD49d antibodies (10ng/mL) were added in 1mL of PBS and incubated at 37°C for 1 hour to facilitate cross-linking. Following this, excess co-stimulatory antibodies were removed, and a minimum of 1.5 million cells per test were added to each BD Falcon tube and were stimulated with pools of peptides predicted to be T cell epitopes from Tc24 and TSA1, respectively (Table 1), and incubated overnight at 37°C with 5% CO_2. Epitope predictions were made for MAMU A*01, A*02, A*11, B*01, and B*17 alleles using NetCTLpan, NetMHCpan, MHC-I Binding Prediction and IEBD analysis algorithms, and the 20 top ranked peptides from each program were compared. Based on this, consensus peptides predicted by all programs were selected. Unstimulated control cells were treated with media only, and positive control cells were stimulated with Staphylococcal enterotoxin B (SEB) (100ng/mL).

After overnight stimulation, cells were washed with PBS and stained with a live/dead amine-reactive dye in PBS, washed, and surface stained with a cocktail of surface antibodies (Table 2) for 20 minutes in the dark at room temperature in 2% FCS-PBS. Samples were resuspended in Fixation/Permeabilization solution (BD Biosciences, San Jose, CA) and incubated for 20 minutes at room temperature, followed by washing with Perm/Wash Buffer (BD Biosciences, San Jose, CA). Intracellular antibodies in cocktail (Table 2) were added to the pellet for 20 minutes at room temperature, and then washed with Perm/Wash and resuspended in Stabilizing Fixative (BD Biosciences, San Jose, CA) until flow cytometry acquisition on a BD LSR Fortessa at the TNPRC Flow Cytometry Core Facility. Flow cytometry analysis was performed using the FlowJo 10.6.1 software (BD Biosciences).

Single cell lymphocytes were selected based on time, single cells, SSC and FSC lymphocyte parameters, and the live/dead discriminator prior to gating CD3+ T cells. Cells were further gated on CD4+ and CD8+ activated T cells producing IFNγ, IL-2 and TNFα. The frequency of antigen-specific cytokine producing CD4+ and CD8+ T cells was calculated by subtracting the background response observed with unstimulated cells (medium alone), and a positive response was defined as a frequency being greater than two-fold of the mean frequency of unstimulated cells.

3. Results

3.1. Vaccine safety

General health of the vaccinated rhesus was unaffected by the vaccine. Their body weights were stable over the 3 months duration of the experiment and body temperature remained normal (Figure 2A). All parameters from blood chemistry (Figure 2B) and blood cell counts (Figure 2C) were also stable over the course of the study and unaffected by each of the vaccine doses, indicating an overall excellent safety profile.

3.2. Vaccine humoral response

Analysis of the humoral response by western blot indicated that naïve animals, that were confirmed to be seronegative and PCR negative for *T. cruzi* infection, had no detectable antibodies against Tc24-C4 or TSA1-C4 antigens at baseline, but antigen-specific IgG were detectable one month after the first vaccine dose (Figure 3A). Importantly, these antibodies also recognized the respective wild type antigens present in a whole *T. cruzi* parasite lysate. Analysis of the time course of antigen-specific antibody titers by ELISA indicated that these strongly increased for both vaccine antigens following the first and second vaccine dose, but then remained high and stable after the third vaccine dose (Figure 3B). Titers above 1:10,000 were measured in most animals for both antigens. Only one animal presented a further increase in IgG titer against TSA1 after the third dose. Also, antibody titers against Tc24-C4 appeared to be somewhat more homogenous than that against TSA1-C4 which were more variable, particularly after the third vaccine dose. Together, these data confirmed the induction of a strong humeral response against both vaccine antigens in naïve rhesus macaques.

3.3. Vaccine cellular response

Evaluation of the cellular immune responses was performed by flow cytometry analysis of *in vitro* stimulated PBMCs, which were collected before vaccination and one month after the third vaccine dose. Stimulation with a peptide pool derived from TSA1-C4 resulted in a noticeable induction of CD4$^+$ T cells producing TNFα, IL-2, and IFNγ with positive responses detected in one or two vaccinated animals, depending on the cytokine (Figure 4). Stimulation with a peptide pool derived from Tc24-C4 resulted in a noticeable induction of CD4$^+$ T cells producing IFNγ in two vaccinated animals, but no IL-2 and TNFα producing CD4$^+$ T cells were detected. In addition, an antigen-specific positive CD8$^+$ response with IL-2 production was detected in two vaccinated animals following stimulation with the peptide pool derived from TSA1-C4 but not that from Tc24-C4 (Figure 4). Remarkably, one animal showed induction of all three cytokines from CD4$^+$ T cells in addition to IL-2 expression from CD8+ T cells after stimulation with Tc24-C4 (Figure 5). Together, these data indicate the induction of at least some cellular immune response against the peptide pools derived from TC24-C4 and TSA1-C4 following vaccination.

4. Discussion

There is a critical need to develop novel tools for the control Chagas disease, to overcome the shortcomings of current drug treatments and complement vector control interventions

[11]. While extensive studies in mouse models have provided a strong rationale for the development of a human vaccine against *T. cruzi* based on Tc24 and TSA1 [20], the extrapolation of these encouraging results to humans remains uncertain. Because Rhesus macaques living in outdoor primate colonies are both known natural hosts of *T. cruzi* [37], and have frequently been found to be better predictors of human responses to vaccines than mouse models, we evaluated here for the first time the safety and immunogenicity of two recombinant vaccine candidates in these non-human primates.

Our preliminary evaluation of the safety of the vaccine revealed no evidence of toxicity, as all vaccinated animals remained healthy, and no alterations in liver enzymes or kidney function were detected following three vaccine doses. Blood cell counts were also unaffected, further confirming that the vaccine was very well tolerated, as expected with recombinant proteins [38] and the E6020 adjuvant formulation [36]. To our knowledge this is the first report of safety and immunogenicity for a vaccine formulated with E6020 in a non-human primate model. While further studies are needed for an in-depth evaluation of the safety and toxicity of these two recombinant vaccines, these results are thus encouraging. Future studies should also assess potential local skin reactions at the site of vaccine injection.

In terms of immunogenicity, a strong antigen-specific antibody response was detected against each Tc24-C4 and TSA1-C4 antigens as early as one month after the first dose of the vaccine. Importantly, the response to each antigen was of a comparable magnitude, reaching end-point titers above 1:10,000 in most animals for both antigens after 2–3 vaccine doses. Furthermore, while these antibodies were induced by exposure to the mutated vaccine antigens, in which cysteine residues had been replaced [25–27], the antibodies also recognized the corresponding native antigens from a whole parasite lysate. This is in agreement with previous results in mice indicating that these mutations did not affect the immunogenicity of the recombinant proteins, but improved protein stability and the production process [25–27].

While antibodies may help clear circulating blood parasites, it is well established that a cellular immune response is also needed for the control of *T. cruzi* infection, as the parasite is mostly intracellular in its mammalian hosts [15, 16]. Analysis of T cell responses following vaccination of rhesus macaques confirmed the induction of a limited cellular immune response to the peptide epitope pools derived from both antigens, confirming that they are adequately processed by the immune system of these monkeys. Importantly, there seem to be differences in the profile of T cell activation by each peptide pool, with Tc24-C4 peptides only activating IFNγ-producing CD4$^+$ T cells, while TSA1-C4 peptides also activating IL-2-and TNFα-producing CD4$^+$ T cells, which suggests that the combination of both antigens in a vaccine may broaden the immune response, which may be important for the control of *T. cruzi* parasites. In addition, we detected positive TSA1-specific CD8+ T cell response in two of the vaccinated animals. While we do not know which peptides in our pools contributed to T cell stimulation, and can expect that additional epitopes are present in these proteins, the further identification of individual T cell epitopes from each antigen may allow a finer evaluation of the complete cellular immune response induced by these antigens, and of their possible differences in immunogenicity in non-human primates. Together with

the detection of specific antibodies and a recall cellular immune response to Tc24 and TSA1 in Chagasic patients from southern Mexico [28], these results provide a strong rationale for further evaluation of these antigens in non-human primates including those that are naturally infected with *T. cruzi* and ultimately transition into phase I clinical trials.

While we immunized each antigen separately and in different sites for this co-administration study, due to the lack of data on potential interactions between the two proteins, it would be key that in the future we could also evaluate a simpler administration of a combined formulation of the antigens, as well as optimize further the vaccine formulation evaluating alternative immunostimulants, doses and route of immunization. In mouse models, immunization with mixtures of TSA1-C4 and Tc24-C4 suggest that these two antigens can indeed be combined (Dumonteil et al., unpublished data), and several Th1 adjuvants such as monophosphoryl lipid A (MPLA) or glucopyranosyl lipid A (GLA, IDRI) can potentiate vaccine efficacy in a similar manner as E6020 [24]. Studies in non-human primates would also allow evaluating vaccine efficacy for the control of *T. cruzi* infection and the development of the chronic Chagasic cardiomyopathy, either as a preventative vaccines or as a therapeutic vaccines.

5. Conclusion

In conclusion, we tested here for the first time the safety and immunogenicity of two co-administered recombinant vaccines against *T. cruzi* in rhesus macaques. The vaccines were safe as did not induce any alterations in blood chemistry and cell counts, indicating normal liver and kidney function. The vaccines also induced high IgG levels against Tc24-C4 and TSA1-C4 proteins, as well some limited antigen-specific cytokine-producing T cells. These preliminary results support the further assessment of the breadth of the cellular immune response induced by the vaccines and the evaluation of their efficacy in non-human primates, to strengthen the rationale for its clinical development as a human vaccine against Chagas disease.

Acknowledgements

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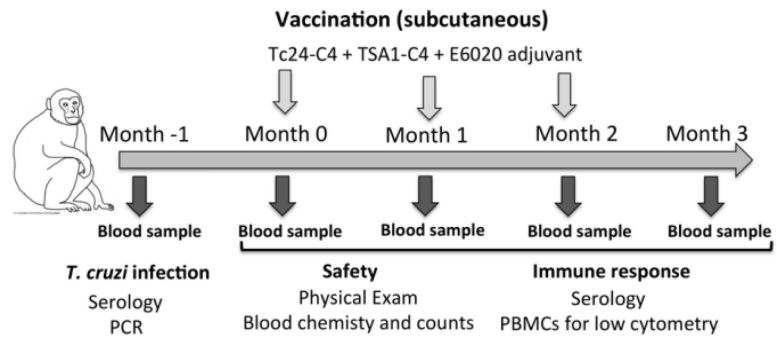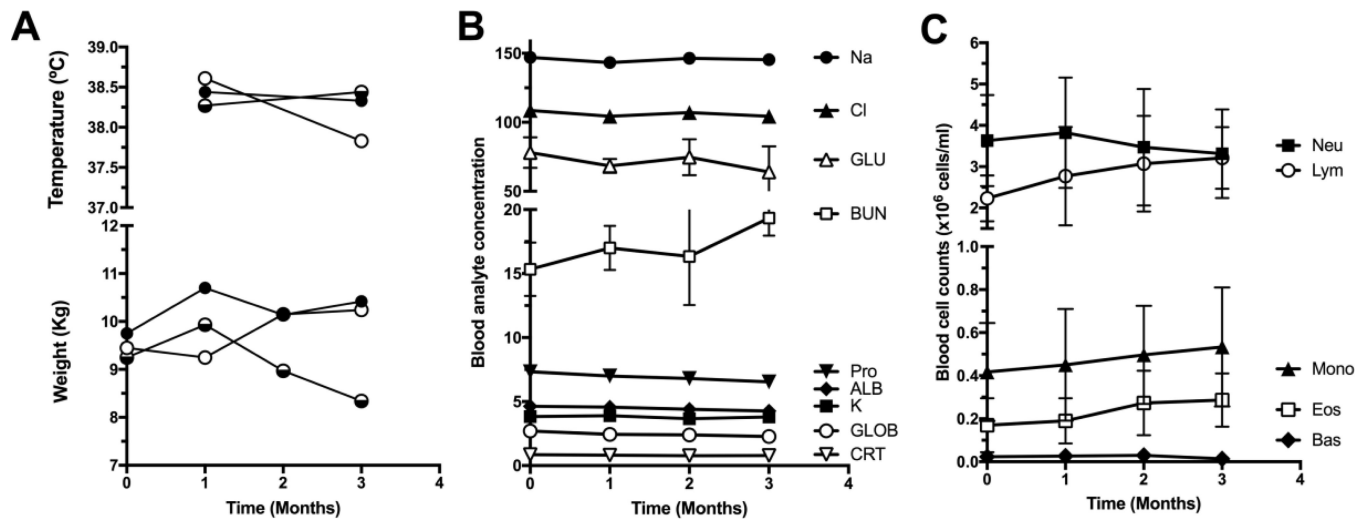
Figure 1. Summary of study design.

Figure 2. General health and blood parameters.
(**A**) Body weight and temperature, (**B**) blood chemistry and (**C**) blood cell counts were evaluated during the course of the study. All values are within the normal range. Data are presented as mean ± standard deviation (SD), except in (**A**) where data from individual animals are shown. GLU: glucose; BUN: blood urea nitrogen; Pro: proline; ALB: albumin; GLOB: Globin; CRT: Creatinine; Neu: neutrophils; Lym: lymphocytes; Mono: monocytes; Eos: eosinophils; Bas: basophils.
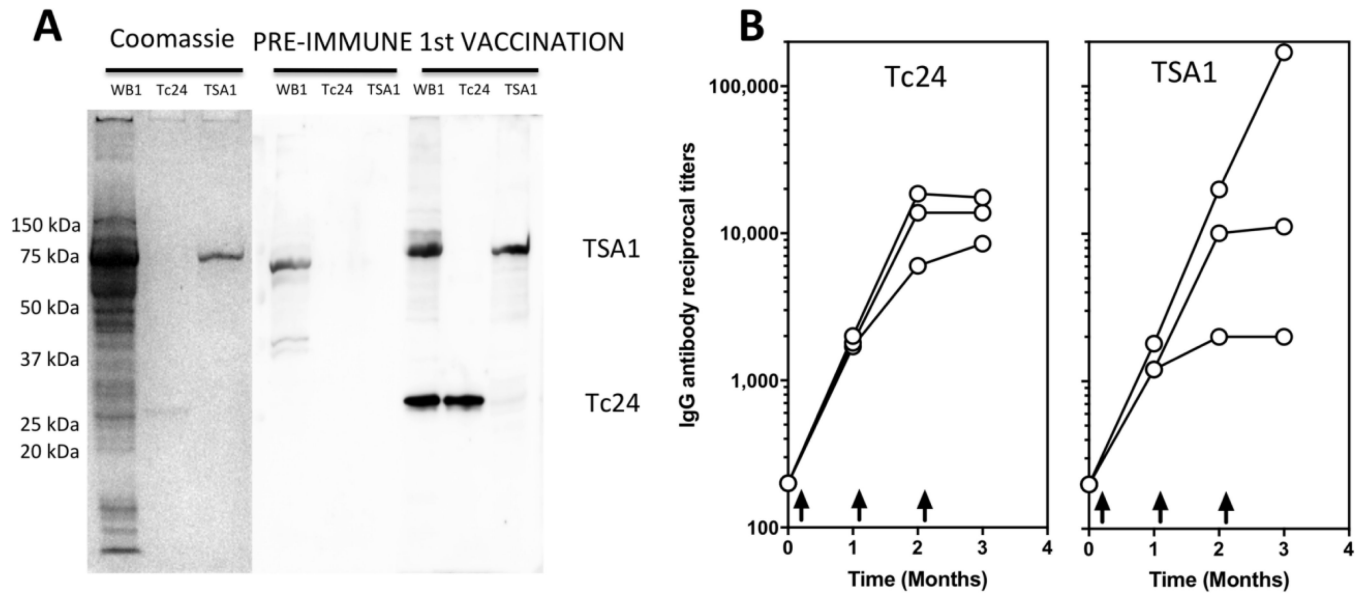
Figure 3. Induction of antigen-specific antibodies in vaccinated rhesus macaques.
(A) Western blot indicating the presence of specific antibodies against Tc24-C4 and TSA1-C4 antigens following the first vaccine dose in Rhesus LB83. These antibodies also recognize the corresponding native antigens in a parasite lysate (WB1 strain). **(B)** Time course of antigen-specific IgG titers in vaccinated rhesus, measured by ELISA. Arrows in the graph indicate the timing of the three vaccine doses. Data points represent individual Rhesus monkeys.
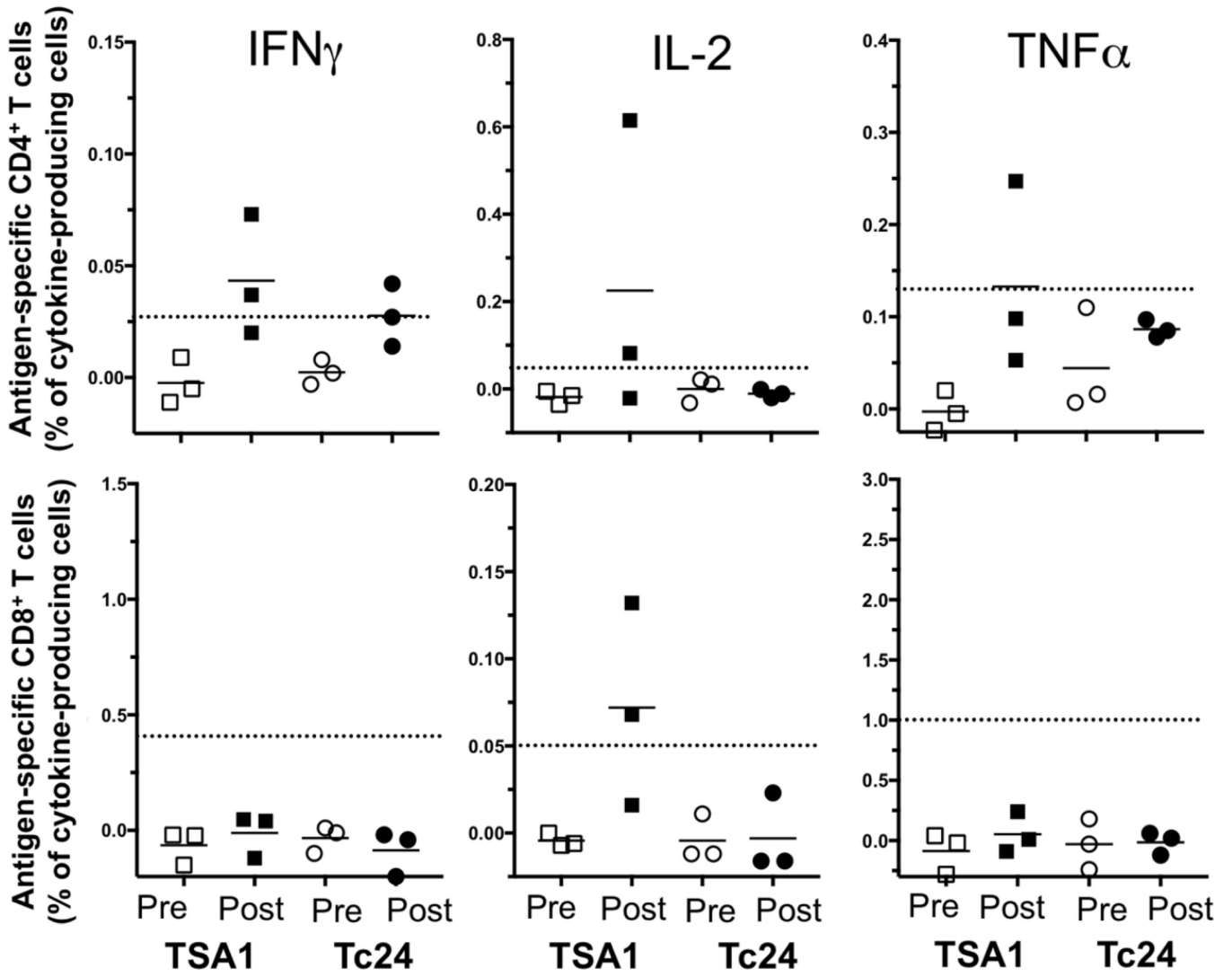
Figure 4. Cytokine-producing T cells in vaccinated rhesus macaques
The cellular immune response was evaluated before vaccination (Pre, open symbols) and one month after the third vaccine doses (Post, closed symbols). The frequency of antigen-specific (Squares: Tc24-C4; Circles: TSA1-C4) cytokine producing CD4+ (Top row of panels) and CD8+ T cells (Bottom row of panels) was calculated by subtracting the background frequency of unstimulated cells to that of peptide-stimulated cells. The dotted horizontal lines indicate the cut-off to consider a positive response, defined as two-fold the frequency of cytokine-producing unstimulated cells. Data points represent individual rhesus macaques.
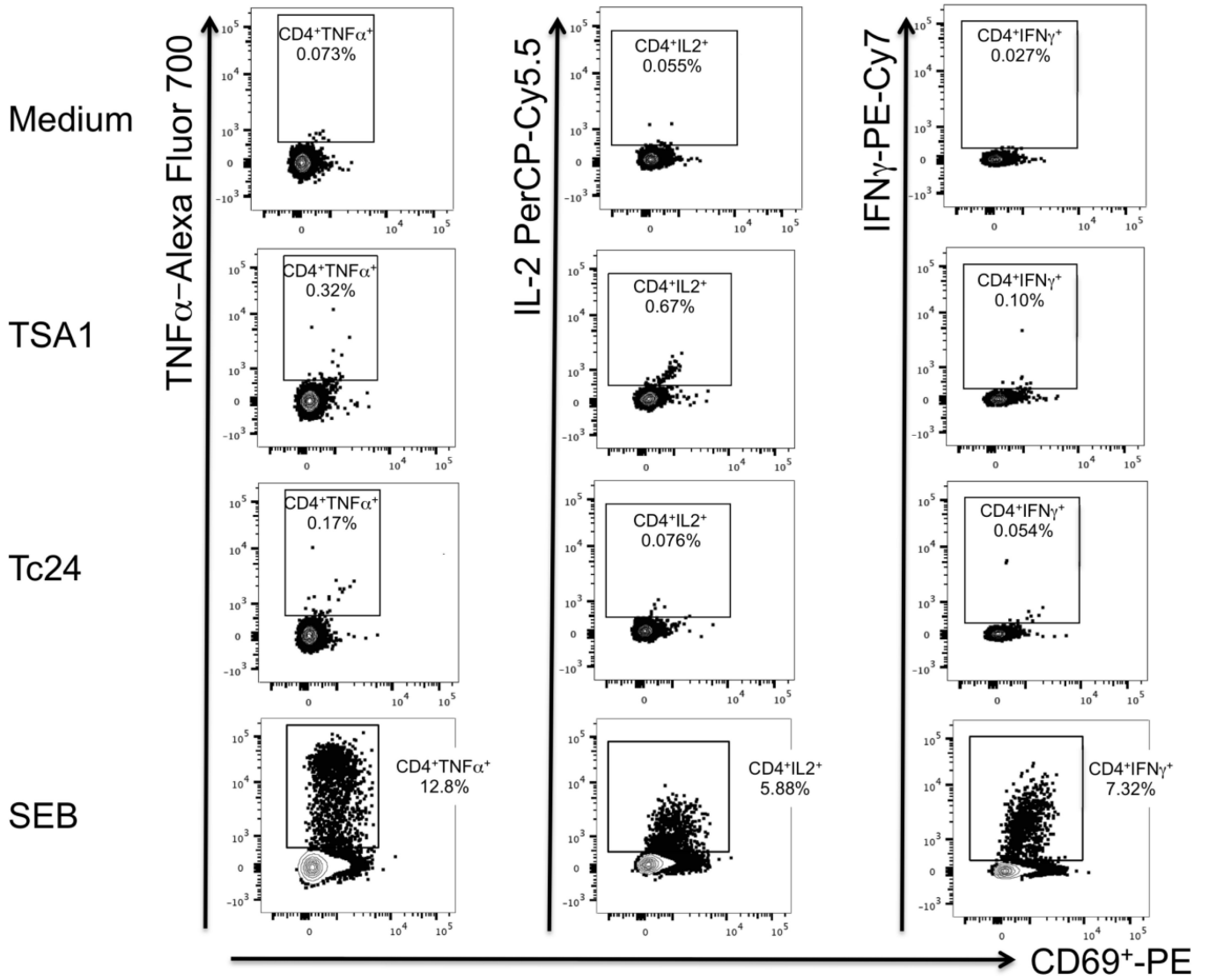
Figure 5. Dot plots of cytokine-producing CD4+ T cells

Cells were analyzed by flow cytometry, and CD4+ T cells were gated as described in the methods section. Responses from rhesus KL72 are shown for unstimulated control cells (Medium, top row of panels), TSA1 peptide pool (upper middle row of panels), Tc24 peptide pool (lower middle row of panels), and SEB (bottom row of panels) stimulation. Percentages of cytokine-producing CD4+ T cells are indicated in each panel for TNFα (Left column panels), IL-2 (middle column panels) and IFNγ (Right column panels).

Table 1.

Peptides derived from TSA1 and Tc24 antigens

Source antigen	Peptide ID	Sequence
TSA1	TSAP97	LSSSDIVAGY
TSA1	TSAP122	KEYWQAHTV
TSA1	TSAP167	REIDDYIWKA
TSA1	TSAP176	AEAWNIKVI
TSA1	TSAP265	WEIPGGVSSV
TSA1	TSAP377	RTFHLGPFSV
TSA1	TSAP334	RVDALITATI
TSA1	TSAP392	KTFANTLLY
TSA1	TSAP400	YSDDALHLL
TSA1	TSAP497	RATWPVNSRW
TSA1	TSAP514	VDYNFTIVAM
TSA1	TSAP551	GLSYGAGGKW
TSA1	TSAP547	RTKLIGLSY
TSA1	TSAP534	STPLLGASL
TSA1	TSAP580	REYQVALMLQ
TSA1	TSAP616	WTEFSHFYF
Tc24	Tc24P81	DEFTPRVRDIT
Tc24	Tc24P92	KRAFDKARAL
Tc24	Tc24P110	SEDFVEFLEFR
Tc24	Tc24P114	VEFLEFRLML
Tc24	Tc24P117	LEFRLMLCYI
Tc24	Tc24P127	YDFFELTVMFDE
Tc24	Tc24P153	KRAVPKLEAWG
Tc24	Tc24P164	AKVEDPAALFK
Tc24	Tc24P183	SVTFDEFAAW
Tc24	Tc24P189	FAAWASAVKL

Table 2.

Antibodies used for flow cytometric analysis of T cells.

Marker	Fluorochrome	Clone	Manufacturer	Catalog #	Staining
CD3	Alexa Fluor 488	SP34–2	Becton Dickinson	557705	Surface
IL-2	PerCP-Cy5.5	MQ1-17H12	Becton Dickinson	560708	Intracellular
TNFα	Alexa Fluor 700	MAB11	Becton Dickinson	557996	Intracellular
Live-Dead	Aqua Dead Cell Stain Kit		ThermoFisher	L34965	Live-dead
CD8	Brilliant Violet 605	SK1	Becton Dickinson	564115	Surface
CD4	Brilliant Violet 650	L200	Becton Dickinson	563737	Surface
CD69	PE	FN50	Becton Dickinson	560968	Intracellular
IFNγ	PE-Cy7	B27	Becton Dickinson	557643	Intracellular