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A multivalent chimeric vaccine composed of *Schistosoma mansoni* SmTSP-2 and Sm29 was able to induce protection against infection in mice

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Summary

Schistosoma mansoni is a blood fluke parasite responsible for schistosomiasis. The best long-term strategy to control schistosomiasis is through immunization combined with drug treatment. In this study, we cloned, expressed and purified SmTSP-2 fused to the N- and C-terminal halves of Sm29 and tested these chimeras as vaccine candidates using an adjuvant approved to be used in humans. The results demonstrated that vaccination with SmTSP-2 fused to N- or C- terminus of Sm29 induced reduction in worm burden and liver pathology when compared to control animals. Additionally, we detected high levels of mouse specific IgG, IgG1 and IgG2a against both chimeras and significant amounts of IFN-γ and TNF-α and no IL-4. Finally, studies with sera from patients resistant to infection and living in schistosomiasis endemic areas revealed high levels of specific IgG to both chimeras when compare to healthy individuals. In conclusion, SmTSP-2/Sm29 chimeras tested here induced partial protection against infection and might be a potential vaccine candidate.

Key words: *Schistosoma mansoni*, vaccine, Sm29, TSP-2

1. Introduction

Schistosomiasis is one of the most important chronic parasitic diseases (1). Currently, it is estimated 207 million people are infected with schistosomes distributed throughout 76 countries (2-3). The major intervention used to control the disease is treatment with the anthelmintic drug praziquantel, accompanied by the provision of safe water, adequate sanitation and, where possible, control of the

snail intermediate host (1-2, 4-5). Nevertheless, the current schistosomiasis control programs have particular limitations, particularly the fact that mass treatment does not prevent reinfection. The development of drug resistance by the parasite is also a concern that has to be considered (6). Likewise long-term effective disease control will benefit from the combination of vaccination and chemotherapy, plus sanitation and public health control measures (6).

Vaccination against schistosomes can be targeted towards the prevention of infection and/or the reduction of parasite fecundity. A reduction in worm numbers is the "gold standard" for anti-schistosome vaccine development (7). Therefore, using this "gold standard" methodology some of the most promising *S. mansoni* vaccine antigens discovered in the last years are the tegument proteins SmTSP-2 (8-9) and Sm29 (10) . Vaccination of mice with the recombinant protein SmTSP-2 formulated in Freund's adjuvant followed by challenge infection with *S. mansoni* resulted in reductions of 57%, 64% and 65-69% for adult worm burdens, liver egg burdens and fecal egg counts, respectively (9). Additionally, vaccination with the recombinant Sm29 also formulated with Freund's adjuvant provided 51%, 60% and 50% reduction in adult worm burdens, intestinal eggs and in liver granuloma numbers, respectively (10). Therefore, we combined these two molecules in a chimeric recombinant form to increase Sm29 solubility due to TSP-2 properties and to enhance protection efficacy induced by single antigen vaccination.

An interesting strategy to induce higher protection is the use of multivalent vaccines. Such a strategy is normally used in DNA vaccine studies, and induces higher protective efficacy than when the antigen is tested alone (11-13). In an attempt of test a multivalent vaccine against schistosomiasis formulated with CpG- alum as adjuvant, we evaluated the protective effect of a SmTSP-2/Sm29 recombinant protein chimera in mice. Two recombinant proteins termed chimera A which present two predicted epitopes of Sm29 (SmTSP-2 plus the N-terminus of Sm29) and chimera B which present tree predicted epitopes of Sm29 (SmTSP-2 plus the C-terminus of Sm29) were produced and tested as potential candidate vaccines against *S. mansoni* infection. The epitopes were predicted using the computer program BCPREDS (14). Mice immunized with both chimeras plus CpG-Alum adjuvant had reduced worm burden and liver pathology after challenge infection with *S. mansoni*. Furthermore, sera from patients living in schistosomiasis endemic areas strongly recognized both recombinant chimeras A and B.

2. Materials and methods

2.1.Mice and parasites

Female C57BL/6 mice aged 6–8 weeks were purchased from the Federal University of Minas Gerais (UFMG) animal facility. Cercariae of *S. mansoni* (LE strain) were maintained routinely in *Biomphalaria glabrata* snails at CPqRR (Centro de Pesquisa René Rachou-Fiocruz) and prepared by exposing infected snails to light for 1 h to induce shedding. Cercarial numbers and viability were determined using a light microscope prior to infection. All protocols were approved by the Committee for Ethics in Animal Experimentation (CETEA) at Universidade Federal de Minas Gerais UFMG under permit 179/2010.

2.2.Cloning of the S. mansoni SmTSP-2/Sm29 cDNA

The chimeras *Sm*TSP-2/*Sm*29A and *Sm*TSP-2/*Sm*29B were cloned into a modified pET41a vector, within the N-terminal GST fusion protein replaced by SmTSP-2 EC-2 into the *Nde*I and *Kpn*I restriction sites. The polymerase chain reaction (PCR) fragments corresponding to Sm29A (N-terminal Val27-Leu87) or Sm29B (C-terminal Cys88-Lys169) were cloned into the *Nco*I and *Xho*I sites of the modified pET41a plasmid generating the chimera A and chimera B, respectively. These constructs were transformed into *Escherichia coli* Rosetta-gami™ electrocompetent cells using the gene Pulser System™ (Bio-Rad). *E. coli* transformants harboring the plasmids were screened on LB agar plates containing kanamycin (50 μg/ml), chloramphenicol (34 μg/ml) and tetracycline (12.5 μg/ml). DNA sequencing was performed to confirm the presence and the correct orientation of the chimeras' open reading frames.

2.3.Expression and purification of the recombinant chimera A and B

Chimeras A and B were expressed in *E. coli* with an in-frame 6x-histidine C-terminal tag using the pET41a expression vector. An *E. coli* Rosetta-gami (DE3) culture containing the recombinant plasmid was grown at 30 ^{o}C to an optical density of approximately 0.5-0.8 at 600nm and gene expression of both chimeras was induced using 1 mM IPTG. After 16 hours of induction, the bacterial cells were harvested by centrifugation at 4,000 *g* for 20 min. The pellet was resuspended in 50 ml of 10 mM

Na_2HPO4, 10 mM NaH_2PO4, 0.5 M NaCl and 10 mM imidazole. Subsequently, the cells were submitted to three cycles of sonication lasting 30 s each and centrifuged at 5400 *g* for 20 min. The chimeras A and B were recovered as inclusion bodies and solubilized in 50 ml of 8 M urea, 10 mM Na_2HPO4, 10 mM NaH_2PO4, 0.5 M NaCl, and 10 mM imidazole. The proteins were purified by affinity chromatography on a Ni-Sepharose column (Hitrap chelating 5 mL) under denaturing conditions using an AKTAprime Plus chromatography system (GE Healthcare, São Paulo, Brazil) according to the manufacturer's protocol. Fractions containing the chimeras A and B were dialyzed against PBS pH 7.0. The dialysis was carried out at 4 ^{o}C using a Spectra/Por2 membrane (MWCO 6 to 8 kDa; Spectrum Medical Industries, Inc., Laguna Hills, CA). These recombinant proteins were quantified using the Bradford's method (Coomassie Protein Assay Kit, Pierce) and used as antigen for vaccination and immunological experiments.

2.4. SDS-PAGE and immunoblotting

SDS-PAGE of purified chimeras A and B were performed (15) and the gel electroblotted onto nitrocellulose membrane (16). The membrane was then blocked with TBST (0·5 M NaCl−0·02 M Tris (pH 7·5), 0·05% Tween 20) containing 5% non-fat dry milk for 16 hrs at room temperature. Subsequently, the membrane was incubated in a 1:2000 dilution of anti-6xHIS antibodies (GE Healthcare) in TBST for 1 hr at room temperature. After three washes using TBST, the membrane was incubated in 1:2000 mouse IgG conjugated with alkaline phosphatase (AP) and treated with AP reaction developing buffer containing nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl-1-phosphate (BCIP). After the reaction was developed, the membrane was washed using distilled water and dried on filter paper.

2.5. Mice Immunization

Six to eight week-old female C57BL/6 mice were divided into four groups of ten mice each. Mice were subcutaneously injected in the nape of the neck with 100 µl containing 25 µg of chimera A, chimera B, Sm29 alone or PBS plus adjuvant as a control group at days 0, 15 and 30 as previously reported by our group (10, 17). The recombinant protein and the control group was formulated with 20 µg of CpG (10104)-Alum (Sigma) as adjuvant.

2.6. Challenge infection and worm burden recovery

Fifteen days after the last boost, mice were challenged through percutaneous exposure of abdominal skin for 1 h in water containing 100 cercariae (LE strain). Forty-five days after challenge, adult worms were perfused from the portal veins (18). Two independent experiments were performed to determine protection levels. The protection was calculated by comparing the number of worms recovered from each vaccinated group with its respective control group, using the formula:

PL = WRCG – WREG x 100/ WRCG

Where PL = protection level, WRCG = worms recovered from control group, and WREG = worms recovered from experimental group.

2.7.Measurement of specific antibodies

Following immunization, sera of ten mice from each vaccinated or control group were collected at two-week interval. Measurements of specific antibodies IgG, IgG1 and IgG2a to each immunized group were performed using indirect ELISA. Maxisorp 96-well microtiter plates (Nunc, Denmark) were coated with 5 µg/ml of each protein (chimera A, chimera B and Sm29) in carbonate-bicarbonate buffer, pH 9.6 for 16 hrs at 4 ^{o}C, then blocked for 2 hrs at room temperature with 200 µl/well PBST (phosphate buffer saline, pH 7.2 with 0.05% Tween-20) plus 10% FBS (fetal bovine sera). One hundred microliters of each serum from the chimera A, chimera B and Sm29 immunized group was diluted 1:100 in PBST and added per well in plates previously sensitized with the proteins chimera A, chimera B and Sm29, respectively.

Plate-bound antibody was detected by peroxidase-conjugated anti-mouse IgG, IgG1 and IgG2a (Sigma) diluted in PBST 1:10000, 1:5000 and 1:2000, respectively. Antigen concentration and the dilutions of mouse serum and secondary antibodies were optimized as previously described (17-18) .Color reaction was developed by addition of 100 µl per well of 200 pmol OPD (o-phenylenediamine, Sigma) in citrate buffer, pH 5.0 plus 0.04% H_2O_2 for 10 min and stopped with 50 µl of 5% sulfuric acid per well. The plates were read at 495 nm in an ELISA plate reader (BioRad, Hercules, CA).

2.8. Cytokine analysis

Cytokine experiments were performed using splenocyte cultures from individual mice immunized with chimera A, chimera B, or PBS plus CpG-Alum as control (n=4 for each group). Splenocytes were isolated from macerated spleens of individual mice one week after the third immunization and washed twice with sterile PBS as previously described (17-18) . After washing, the cells were adjusted to $1x10^6$ cells per well for IL-4, IFN-γ and TNF-α assays in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS, 100 U/ml of penicillin G sodium, 100 µg/ml of streptomycin sulfate, 250 ng/ml of amphotericin B. Splenocytes were maintained in culture with medium alone or stimulated with the recombinant proteins chimera A and B (25 µg/ml) or with concanavalin A (ConA) (5 µg/mL) for IL-4 and IFN-γ, and LPS (1 µg/mL) for TNF-α, as positive controls (17-18). In order to neutralize potential effects of LPS presented in the *S. mansoni* recombinant antigens (chimera A- 2.84 EU/ml, chimera B-2.85 EU/ml), Polymyxin B (Calbiochem, Germany; 10 µg/mL) was added to cell cultures every 12 hours during all culture period (19). The 96-well plates (Nunc) were maintained in an incubator at 37 ^{o}C with 5% CO2. The assays for measurement of IL-4, IFN-γ and TNF-α were performed using the Duoset ELISA kit (R&D Diagnostic, Minneapolis, MN) according to the manufacturer's directions.

2.9. Study population

Serum was obtained from individuals living in two different endemic areas for schistosomiasis ("Melquiades" and "Côrrego do Onça", Minas Gerais, Brazil). These individuals were classified in five groups, regarding their infection status and the selection of subjects was performed based only on the criteria for inclusion and exclusion of each group independent of previous knowledge of immune responses for each individual. Non-infected (NI) individuals, natural resistant (NR), infected (INF), resistant (RR) and susceptible (SR) to *S. mansoni* reinfection as described in Table 1. Individuals classified as susceptible to *S. mansoni* reinfection (SR) showed stool-positive examination following treatment (praziquantel, 40 mg/kg). The sera from RR and SR groups were obtained six months after praziquantel treatment and these individuals were examined for *S. mansoni* infection using the Kato–Katz technique before treatment and one, six and 12 months after treatment to check for reinfection rates. The water contact exposure was objectively evaluated by observers and NR, SR and RR groups had at least one contact daily. These individuals were followed for 24 months. Additionally, RR, SR and INF patients were negative for other helminthic infections. These patients or

their legal guardians gave informed consent after explanation of the protocol that had been previously approved by the Ethical Committee of the Federal University of Minas Gerais.

2.10. Measurement of human humoral responses of patients living in endemic areas for schistosomiasis

Sera of schistosomiasis patients living in endemic and non-endemic areas in Brazil were used in an ELISA (20) to measure the levels of total IgG to chimera A or chimera B. The studied population was divided into NI, SR, NR, RR and INF subjects (Table 1). Levels of total IgG to Sm29, chimera A and chimera B antigens were assessed using 96 well flat-bottom microtiter plates (Nunc) that were coated overnight at 4°C with 100 µl of each recombinant protein at a concentration of 5 µg/ml in 0.1 M carbonate bicarbonate buffer (pH 9·6) per well. The plates were then blocked with 10% bovine fetal serum in PBS (pH 7·4) for 2 h at room temperature. Subsequently, the plates were washed three times with PBS plus 0.05% Tween-20 (PBST). For total IgG, serum samples were diluted 1:50 in PBST (100 µl/well added in duplicate) and the plates incubated for 1 h at room temperature. Peroxidase-labeled anti-human IgG (Sigma, St Louis, MO) was added at dilutions of 1:10000 (100 µl/well). After 1 h at 37°C, the plates were washed and orthophenyl-diaminobenzidine plus 0.05% hydrogen peroxide in phosphate citrate buffer (pH 5) was added (100 µl/well). The plates were then incubated for 30 min at room temperature, and the reaction was stopped by addition of 5% H_2SO_4 (50 µl/well). Absorbance was read at 492 nm using a microplate reader (Bio-Rad, Hercules. CA, USA).

2.11. Histopathological analysis

Following vascular perfusion of mice for recovery of the schistosomes, liver sections from mice (8/group) of control and experimental groups were collected to evaluate the effect of immunization on granuloma formation. The livers sections removed from the central part of the left lateral lobe were fixed with 10% buffered formaldehyde in PBS. Histological sections of 5 µm thickness were cut using a microtome and stained with picrosirius. Briefly, tissue slides were deparaffinized, hydrated, stained with 0.1% solution of Sirius Red in saturated aqueous picric acid, washed in 0.01 N HCl and counterstained with Harris' Haematoxylin as previously described (21). The area from each liver section (mm^2) was calculated using the KS300 software connected to a Carl Zeiss image analyzer. For measurements of the total area of granulomas, 20 granulomas with a single well-defined egg from each animal of a group of eight mice were randomly selected and assessed using a microscope with

10× objective lens. Granuloma images were obtained through a JVC TK-1270/RBG microcamera. Using a digital pad in the KS300 software built in a Carl Zeiss image analyzer, the areas were measured and expressed in square micrometers (μm^2).

2.12. Statistical analysis

Statistical analysis was performed with Student's *t*-test using the software package GraphPad Prism (La Jolla, CA, USA). Kruskal-Wallis test was used to compare the levels of IgG induced by the different patient groups.

3. Results

3.1.Production of recombinant chimera A and B

Chimeras A and B were expressed as inclusion bodies and purified under denaturing conditions in 8 M urea by nickel-affinity chromatography. The procedure for protein refolding resulted in a substantial loss of protein via precipitation, but yielded sufficient soluble protein to be used for immunization experiments. The expression and purity of chimeras A and B as 6xHis-tag fusion proteins were assessed by SDS-PAGE and Western blotting analysis, revealing proteins of approximately 18 and 20 kDa for chimeras A and B, respectively (Figure 1), and corresponding to their predicted molecular weights.

3.2.Antibody profile following mice immunization

In order to evaluate the profile of the humoral immune response, total IgG and the subclasses IgG1 and IgG2a to Sm29, chimera A and B, were measured from sera of ten vaccinated and control animals. All vaccinated mice developed significant levels of specific IgG, IgG1 and IgG2a antibodies after the second immunization compared to the non-vaccinated control group and these levels increased significantly after the third immunization for all tested antigens. Mice immunized with chimera B (SmTSP-2 fused to Sm29 C-terminus) showed significantly higher titers of IgG and IgG2a isotypes compared to Sm29 or chimera A antigens (Figure 2).

3.3. Cytokine profile

To evaluate the cytokine profile generated by the immunization of mice with chimera A and chimera B, splenocytes were isolated from vaccinated and control animals after the third immunization. Statistically significant levels of IFN-γ and TNF-α were detected in splenocyte culture supernatants of chimera A and chimera B vaccinated mice compared to the control group (Figure 3). Chimera A immunization induced higher production of IFN-γ (712.7 ± 261.4 pg/ml) and TNF-α (407 ± 94.3 pg/ml) compared to chimera B immunized group (IFN-γ 328.8 ± 113.7 pg/ml; TNF-α 259 ± 38.9 pg/ml). Additionally, no significant amounts of IL-4, a signature of Th2-immune responses, were detected. These results show that immunization with chimeras A and B induces a Th1 type of immune response in mouse T cells, characterized by the production of IFN-γ and TNF-α cytokines. The results of splenocyte stimulation with the positive controls ConA and LPS are not shown but induced high levels of these cytokines as expected.

3.4. Worm burden recovery

Protective immunity induced by vaccination with recombinant Sm29, and chimeras A and B were evaluated 45 days after challenge with 100 *S. mansoni* cercariae. Mice vaccinated with recombinant Sm29 (20.36%), chimera A (27.84%) and B (34.83%) showed statistically significant reduction in worm burden recovery compared to control mice (Table 2).

3.5. Immunization with chimeras A or B induces reduction in liver pathology

Histological analysis by digital morphometry of picrosirius-stained sections obtained from mice immunized with recombinant Sm29, chimera A or chimera B showed reductions of 38%, 48% and 31% on granuloma area, respectively. These findings demonstrated that all antigens tested are important molecules involved in reduction of liver pathology (Figure 4).

3.6. Human IgG responses to chimera A and B

To investigate the presence of specific anti-Sm29, anti-chimera A or anti-chimera B antibodies in sera of individuals living in schistosomiasis endemic area ELISA was performed. Total IgG levels in sera of schistosomiasis patients and non-infected individuals were evaluated. The NR, INF and RR groups

studied had higher levels of IgG anti-chimera A or anti-chimera B compared to the non-infected group (Figure 5). In order to determine whether IgG responses to chimera A or chimera B could differentiate resistant *versus* susceptible individuals, we compared the NR group to INF individuals, and RR patients compared to the SR group. The RR patient group produced elevated levels of IgG to all antigens tested in this study when compared to the SR patient group. In contrast, the NR group did not show statistically significant higher levels of IgG to chimeras A or B when compared with the INF group. However, the level of specific anti-Sm29 IgG was significantly higher in the NR group when compared to INF subjects. Additionally, the RR group showed higher IgG levels against chimeras A and chimera B when compared to Sm29. These results demonstrate that the fusion proteins are better recognized by human total IgG of patients with resistant phenotype compared to Sm29 alone.

4. Discussion

Schistosomiasis is a chronic debilitating parasitic disease that represents a major health problem in endemic areas, such as various parts of South America, Africa, and Southeast Asia (22). Currently, chemotherapy is the major control strategy used, however, extension of endemic areas and constant reinfection of individuals, together with poor sanitary conditions in tropical countries makes drug treatment inefficient (23-24). Therefore, complementary control measures, including vaccines could contribute enormously to disease control (25-27). Tegument proteins are of great importance to schistosome vaccine development since they are a major host–parasite interface (28-29). Recently, many tegument proteins were identified and characterized including the recombinant proteins SmTSP-2 and Sm29. Previously, it was demonstrated that the large extracellular loop of the *S. mansoni* tegument tetraspanin, SmTSP-2, formulated with Freund's adjuvant, is an efficacious vaccine antigen, eliciting high levels of protection in a murine schistosomiasis model of infection (9, 30). SmTSP-2 vaccine antigen is also protective, even when formulated with CpG-alum (30). Sm29 recombinant protein fused to 6xHIS and used to vaccinate C57BL/6 formulated with Freund’s adjuvant showed a reduction of 51% in the number of adult worms, and induced a Th1 immune response in vaccinated mice (6, 10). Furthermore, a preparation containing GPI-anchored proteins, Sm29 plus other molecules engendered a mixed Th1/Th2 type of immune response in mice, which conferred partial protection against cercariae challenge and reduced pathology in the liver (31).

In the present study, we show that it is possible to induce protection in a murine schistosomiasis model of infection using the recombinant proteins SmTSP-2 and Sm29 in chimeric forms when formulated with CpG-alum, a human-approved adjuvant. C57BL/6 mice immunized with chimeras A and B in the presence of CpG-alum showed high levels of specific IgG, IgG1 and IgG2a that appeared after the second immunization. Regarding IgG1 and IgG2a subclasses, specific IgG1 against both chimeras was predominant. However, chimera B induced the highest levels of IgG and IgG2a compared to chimera A or Sm29 alone. Further, we confirmed by cytokine analysis the Th1-type of immune response induced following vaccination with both chimeras, characterized by high levels of IFN-γ and TNF-α and absence of IL-4. Studies using the irradiated cercariae model which induces high levels of protection in mice suggest that protection can be induced with a mixed Th1/Th2 response, a polarized Th1 response or even a polarized Th2 response (32). To determine whether chimera A and B conferred protection against *S. mansoni* infection, immunized mice were challenged with 100 cercariae and worm burden analyzed. Immunization with chimera A and chimera B together with CpG-alum induced 27% and 34% of worm burden reduction, respectively. The involvement of IFN-γ in protective immunity to schistosomiasis is well documented in the murine model (32). In the irradiated cercariae vaccination model treatment with monoclonal anti-IFN-γ antibody totally abrogated the protective immunity achieved (33) showing the importance of high titers of IFN-γ in protective immunity in schitosomiasis. Several *S. mansoni* antigens tested by our group that had a tendency to induce a Th2 type of immune response failed to engender protection in the mouse model (17, 34). In contrast, Th1 antigens induced partial protection against infection (10, 18, 35).

In this study, we show that the chimeric proteins A and B are protective, even when formulated with a human-approved adjuvant combination. Previous studies have demonstrated that vaccination with recombinant Sm29 and SmTSP-2 formulated with Freund's adjuvant provided reduction in worm burden and liver pathology (9-10). Herein, the association of SmTSP-2 fused to Sm29 vaccine, in a chimeric form, not only reduced parasite load but also the pathology and disease associated with schistosomiasis compared to control group that received PBS formulated with CpG-alum. However, when the results of vaccination with the two chimeric forms (A and B) were compared to Sm29 alone we did not detect statistically significant differences in worm burden and liver pathology among these groups.

Studies with sera from patients resident in schistosomiasis endemic areas revealed greater recognition of chimera B in NR, INF and RR groups tested, suggesting that the main B-cell epitopes of Sm29 probably reside predominantly within the C-terminal portion of the protein. IgG1 and IgG3 are associated with protective immune mechanisms including opsonization, cell cytotoxicity and activation of the classical complement pathway (36). Therefore, the recognition of antigens by IgG isotypes in individuals resistant to infection and reinfection is an important step in evaluation of the potential of these antigens as schistosome vaccine candidates.

In conclusion, chimera A and chimera B immunization induced a Th1 type of immune response, worm burden reduction and diminished liver pathology in murine schistosomiasis compared to control group that received PBS with adjuvant. Both chimeras were recognized by sera from natural resistant human subjects, and presented higher IgG levels in subjects resistant to reinfection compared to Sm29 alone, reinforcing earlier studies on the role of these antigens as targets of naturally acquired immunity to schistosomiasis and the potential of these chimeric proteins in the development of an effective anti-schistosomiasis vaccine better than Sm29 formulated alone.

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Figure Legends

Figure 1: Expression and purification of chimeras A and B 6xHis fusion proteins. (A) Coomassie blue stained SDS-12% PAGE profile of the purified chimeras A and B proteins. Lanes: 1, rSm29 control (Accession number AF029222); 2, chimera A; 3, chimera B; molecular weight marker (kDa). (B) Western blot analysis of the purified recombinant protein using anti-6xHIS antibody.

Figure 2: Kinetics of specific IgG (A), IgG1 (B) and IgG2a (C) antibody responses to chimera A and B, and Sm29 in mice immunized with each recombinant protein. Sera of ten immunized mice per group were collected at days 15, 30, 45, 60, 75 and 90 after the first immunization and assayed by ELISA. Results are presented as the mean absorbance measured at 492 nm for each group. Results represent the mean of two independent experiments. Statistically significant differences between responses to Sm29, chimera A and chimera B compared to control group are denoted by an asterisk and statistically significant differences of chimera B compared to Sm29 and chimera A are denoted by two asterisks for $p<0.05$.

Figure 3: Cytokine profile of mice immunized with recombinant chimera A and chimera B proteins. One week after the last immunization, splenocytes from four mice were isolated and assayed for (A) IFN-γ and TNF-α and (B) IL-4 production in response to chimera A, chimera B and rSm29. The results are presented as mean ± S.D. for each group. A statistically significant difference of IFN-γ and TNF-α

produced by chimera A, chimera B or rSm29 vaccinated mice compared to negative control group is denoted by one asterisk and the difference between IL-4 produced by ConA stimulated cells compared to the other groups is denoted by two asterisks for $P < 0.05$. Results are representative of two independent biological experiments.

Figure 4: Liver pathology measured in Sm29, chimera A or chimera B vaccinated mice. **(A)** The area of 20 granulomas from each animal of a group of eight mice with a single well-defined egg was measured. The total diameter of granulomas was measured, and the results were expressed as mean square micrometers (μm^2). Statistically significant differences compared to control group are denoted by an asterisk ($p< 0.05$). **(B)** Histological analysis of hepatic tissue from vaccinated mice**.** Animals were sacrificed and their livers were washed in PBS and stored in formaldehyde until sectioning and staining with Picrosirius. (i) PBS control group, (ii) mice vaccinated with Sm29, (iii) mice vaccinated with Chimera A, (iv) mice vaccinated with Chimera B. Images were captured using a 40x objective lens and are representative of two independent biological experiments. Bars, 50 µm.

Figure 5: IgG antibody responses of schistosomiasis patients to Sm29, chimera A or chimera B. Analysis of human IgG in sera of Natural Resistant (NR); Infected (INF); Resistant to Reinfection (RR); Susceptible to Reinfection (SR) and Non-Infected individuals (NI) were performed by ELISA. Results are expressed as mean ± S.D. Statistically significant differences compared to the NI, INF, SR are denoted by a #,*,**, respectively, for $p<0.05$. Statistically significant differences compared to Sm29 in the same group are denoted by & for $p<0.05$.

Table 1. Study population.

Group	Infection status	Description	Age (mean ±SD)	Sex (M/F)
Infected (I) n=8	Infected with *S. mansoni*	Individuals living in an endemic area for schistomiasis with stool positive examinations	17.1 ± 14.9	5/3
Susceptible to reinfection (SR) n=8	Infected with *S. mansoni*	Individuals living in a endemic area for schistosomiasis that present stool positive examination after praziquantel treatment	22.8 ± 12.1	3/5
Resistant to reinfection (RR) n=8	Not infected	Individuals living in a endemic area for schistosomiasis that although water contact, present stool negative examination after praziquantel treatment	18.8 ± 9.3	4/4
Natural resistant (NR) n=8	Not infected	Non-infected individuals exposed to water contaminated with cercariae	34 ± 11.2	3/5
Non-infected (NI) n=8	Not infected	Individuals living out of endemic area for schistosomiasis that never present this disease in their lives	28.3 ± 4.5	2/6

Table 2: Protection level induced in C57BL⁄6 mice by immunization with recombinant Sm29, chimera A or chimera B

Groups	Worm burden (mean±SD)	Protection
PBS + CpG-Alum	46.25 ± 3.11	-
Sm29 + CpG-Alum	36.83 ± 5.81	20.36 % *
Chimera A + CpG-Alum	33.37 ± 5.63	27.84 % *
Chimera B + CpG-Alum	30.14 ± 6.57	34.83 % *

Statistical analysis were performed using Student's *t*-test. *statistically significant ($p < 0.05$) compared to control group. Data are representative of two independent vaccine trials.