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Synergistic enhancement of immunogenicity and protection in mice against *Schistosoma japonicum* with codon optimization and electroporation delivery of SjTPI DNA vaccines

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ABSTRACT

Schistosomiasis is an endemic, zoonotic parasitic disease caused by *Schistosoma japonicum* that remains a public health concern and an effective vaccine is needed. Triose-phosphate isomerase from *S. japonicum* is a promising schistosome vaccine antigen shown to be immunogenic when delivered as a DNA vaccine, however, the previous *S. japonicum* triose-phosphate isomerase (SjTPI) DNA vaccine needs to be further optimized to achieve higher protection. In the current study, codon optimization of SjTPI DNA insert, combined with electroporation but not with the addition of a tPA leader or heat-shock protein in-frame with the SjTPI gene insert, enhanced Th1-type antibody and cytokine production and most significantly, achieved great than 50% reduction of infection against challenge with *S. japonicum* cercariae, a major milestone in *S. japonicum* vaccine development. Our results suggest that the combination of a codon optimized vaccine design and an efficient vaccine delivery system can greatly improve the potential of a SjTPI DNA vaccine as a viable schistosome vaccine candidate.

1. Introduction

Schistosomiasis, a disease caused by trematode blood flukes from *Schistosoma* species of the genus *Schistosoma*, was first reported in 1852. This tropical parasitic disease is endemic and zoonotic, and has been recognized as one of the most important human helminth infection in terms of morbidity and mortality in many developing countries. Approximately more than 200 million people are infected with schistosomes and 779 million people are at risk of infection in over 70 countries [1–3]. Five schistosome species infect humans: *Schistosoma (S.) japonicum*, *S. mansoni*, *S. mekongi*, *S. intercalatum*, and *S. haematobium* [2,4]. Infection by *S. japonicum* remains one of the most prevalent parasitic diseases in China [5–7]. Despite tremendous efforts to control this disease, *S. japonica* remains endemic in Southern China including Hunan, Hubei, Jiangxi, Anhui, and Jiangsu provinces, the lowland marsh areas or the lakes regions, and Sichuan and Yunnan provinces, in the mountain areas; a combined area that covers over 90 counties with more than a half of million of actively infected individuals [5,7–10].

As a zoonotic disease, there are more than 40 animal reservoir hosts in addition to intermediate hosts (snails, *Oncomelania hupensis*) for *S. japonicum* [11,12]. Despite remarkable progress in chemotherapeutic approaches with the development of highly effective drugs, the spread of schistosomiasis into new areas continues [5]. Strategies with annual mass chemotherapy, such as praziquantel (PZQ) treatment, and reductions in the numbers of intermediate host snails have not effectively blocked the transmission of the disease or reinfection. Within a period of 6–8 months following chemotherapy, schistosomiasis prevalence returns to baseline levels among exposed populations in most endemic areas [2]. In addition, the possibility of drug-resistance may reduce the effectiveness of chemotherapy in schistosomiasis infection [13]. Therefore, there is a need to develop an effective vaccine to control and prevent schistosomiasis [2,6,12,14–17]. With the discovery of potential protective antigens and improved understanding of immune mechanisms that control schistosomiasis infection, the development of subunit-based vaccines may become possible.

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Based on the schistosome life cycle, after the cercariae have entered into the mammalian hosts, they develop and grow into paired worms that produce eggs; the eggs are then passed in the feces to begin a new life cycle [3,18]. Both the worms and eggs may cause schistosome pathogenesis in the host although it has never been demonstrated that the worms themselves cause pathogenesis. However, the schistosome does not reproduce within the mammalian host [2]. Reductions in worm and egg numbers have been considered the gold standard for schistosome vaccine development to prevent the infection and reduce the fecundity [2,19].

Recently, several potential protective antigens from the adult worms and all stages of the *S. japonicum* life cycle have been identified. These antigens included glutathione S-transferase (Sj26GST) [20,21], triose-phosphate isomerase (SjTPI) [22–24] and paramyosin (Sj97) [25,26], fatty acid binding protein (FABP, Sj14) [27], and 23 kDa membrane protein (Sj23) [21,23,27,28]. They have been proven to produce partial protection in the mouse model when used as subunit-based vaccines, including peptide vaccines, recombinant protein vaccines, and DNA vaccines [2,29]. These antigens were recommended by WHO/TDR as candidates for vaccine development. However, most of these antigens only produced worm reduction rates below 40% in mouse models [25,26,28,30]. Although partial protection may reduce the pathogenesis, morbidity, transmission rates [17], and improve the control of schistosomiasis when combined with praziquantel treatment in humans and live stock [31,32], it is also critical to improve the protection efficacy for an independent prophylactic vaccine.

SjTPI, a glycolytic pathway enzyme, is found in each stage of the life cycle for *S. japonicum*. Our previous studies have shown that a SjTPI DNA vaccine could produce a 27.9–30.2% worm reduction in mice [23,30], in addition to transmission-blocking potential in larger animals, such as pigs [22]. In the current study, we tested whether a codon optimized version of this SjTPI DNA vaccine, with or without delivery via electroporation, can further improve immunogenicity and possibly protection efficacy of this vaccine.

2. Materials and methods

2.1. Parasites

A Chinese strain of *S. japonicum*-infected *Oncomelania hupensis* was obtained from Jiangsu Institute of Parasitic Diseases. Cercariae of *S. japonicum* were collected from infected snails.

2.2. Codon optimization of the SjTPI gene

The codon usage of the SjTPI gene was analyzed with the MacVector software 7.2 against codon preference of *Homo sapiens*. The less optimal codons in the SjTPI gene were changed to the preferred codons in mammalian systems to promote higher expression of the TPI protein. The codon optimization strategy was not limited to changing of codons for mammalian usage. Sequence optimization was also performed to make the mRNA more stable and the gene more favorable for transcriptional and translational processes. During the sequence optimization, following cis-acting sequence motifs were avoided: internal TATA-boxes, chi-sites and ribosomal entry sites; AT-rich or GC-rich sequence stretches; ARE, INS, CRS sequence elements; cryptic splice donor and acceptor sites; and branch points. Despite such DNA sequence changes, the final codon optimized SjTPI gene sequence should still produce the same SjTPI amino acid sequences as in the original parasite. The codon optimized SjTPI gene was chemically synthesized by Geneart (Regensburg, Germany) with added restriction enzyme sites of HindIII and BamHI for subcloning purpose immediately upstream of the start codon and downstream of the stop codon, respectively.

2.3. Construction of DNA vaccines

The DNA vaccine, pcDNA3.1-SjTPI containing the triose-phosphate isomerase (TPI) gene of *Schistosoma japonicum* has been constructed in our lab as previous described [23,30]. pVAX-mHSP, which contains the gene coding the heat-shock protein 70 of mouse (mHSP70), was kindly provided by Prof. Harn (previously at Harvard School of Public Health).

The optimized SjTPI gene (Geneart, Germany) was initially cloned into DNA vaccine vector pJW4303 [33] at HindIII–BamHI cloning sites to produce the DNA vaccine construct pJW4303/TPI-opt. In a separate cloning process, the same codon optimized TPI gene was cloned into pJW4303 at NheI–BamHI cloning sites under and in-frame with a tPA leader sequence to produce the pJW4303/TPI-opt-tPA DNA vaccine construct.

In order to have a fair comparison among different DNA vaccines, all DNA vaccine constructs in this study were made in the same DNA vaccine vector (pcDNA3.1), which was provided by Professor Harn. The TPI-opt or tPA-TPI-opt genes were cleaved from the relevant DNA vaccine constructs, pJW4303/TPI-opt or pJW4303/tPA-TPI-opt, respectively, and cloned into pcDNA3.1 at HindIII/BamHI cloning sites. Consequently, two new codon optimized TPI DNA vaccine constructs were made: pcDNA3.1-TPI.opt and pcDNA3.1-tPA-TPI.opt.

To make the DNA vaccine express TPI-opt fused with mHSP70, the mHSP70 and TPI-opt genes were PCR amplified and constructed into a single pcDNA3.1 vector. The complete mHSP70 coding sequence was amplified from pVAX-mHSP70 with Taq DNA polymerase (Promega) using sense (5′-CGCGGATCCATGGCCAAGAACACGGCGATC-3′) and antisense (5′-CGCGAATTCCTAATCCACCTCCTCGATGGT-3′) primers containing BamHI and EcoRI restriction sites, respectively. The mHSP PCR product was cloned into BamHI/EcoRI-cleaved pcDNA3.1 and constructed recombinant plasmid pcDNA3.1-mHSP70. Then, the optimized TPI gene, with the removal of stop codon, was amplified with a pair of primers: sense (5′-CGCAAGCTTATGAGCAGCAGCCGGAAGTTC-3′) and antisense (5′-CGCGGATCCCTGCCGGGCCTTGCAGATGTC-3′) containing HindIII and BamHI sites, respectively. This TPI-opt gene was then cloned into the HindIII/BamHI sites of pcDNA3.1-mHSP, which became the DNA vaccine construct pcDNA3.1-TPI.opt-mHSP expressing a TPI-mHSP fusion protein.

All of the above DNA vaccine plasmids, pcDNA3.1-TPI, pcDNA3.1-TPI.opt, pcDNA3.1-TPI.opt-mHSP70, pcDNA3.1-tpA-TPI.opt, pcDNA3.1-tpA-TPI.opt-mHSP70, were verified by restriction enzyme analysis and DNA sequencing (Shanghai Sangon Biological Engineering Co). The DNA vaccine plasmids were prepared using Qiagen Plasmid Mega Kit according to the manufacturer's instructions. The final plasmid DNAs were in 0.01 M phosphate buffered solution (PBS) and verified by restriction enzyme digestion for transfection and mouse immunizations.

2.4. DNA immunization

Female BALB/c mice, 4–5 weeks age, were purchased from Shanghai Slac Laboratory Animal Co, Ltd, and used for DNA vaccination studies. The mice were randomly divided into different vaccination groups (14 mice/group). Each mouse received 100 μg of a designated DNA vaccine by intramuscular injection or electoporation at Week 0, 3, and 6. For electroporation, a pair of electrode needles, 5 mm apart, were inserted into the muscle to cover the DNA injection sites and electric pulses were delivered using an electric pulse generator (Programmed Control Stimulator YC-2), as previously described [34]. Four pulses of 100 V each were delivered to each injection site at the rate of one pulse per second, each pulse lasting for 40 ms. Then four pulses of the opposite polarity

were applied as described above. Serum samples were collected 2 days prior to the initial DNA vaccination and at 4 weeks after the 3rd immunization before challenge for detection of antibody responses.

2.5. TPI-specific antibody detection

ELISA was performed to measure TPI-specific antibody responses. The recombinant protein, rSjCTPI, was diluted with carbonate buffer (pH 9.6) to 10 μg/ml and used as the antigen source for ELISA. HRP-conjugated goat-anti-mouse IgG, IgG1, and IgG2a (SouthernBiotech) were used. In brief, ELISA plates (Bio Basic Inc.) were coated with rSjCTPI (100 μl) in 50 mM carbonate buffer, and stored overnight at 4 °C. Each plate was washed three times with PBS (pH 7.6) containing 0.05% Tween-20 (PBST), and blocked with 1% (w/v) bovine serum albumin (BSA) in PBS for 1 h at 37 °C. The plates were further washed three times with PBST, and then incubated with the sera diluted with PBS (diluted 1:100) for the detection of IgG, IgG1, and IgG2a antibodies at 37 °C for 1 h. Pre-vaccination sera were used as a negative control and sera from infected mice were used as a positive control. The plates were washed five times with PBST, followed by incubation with HRP-conjugated goat-anti-mouse IgG, IgG1, and IgG2a (diluted 1:4000) for 1 h at 37 °C. The plates were washed five times with PBST and were developed with tetramethylbenzidine (TMB) substrate (Jingmei Biotech) for 5–10 min. The reaction was stopped by adding sulphoric acid (2 M). The optical density (OD) was read at 450 nm using a Microplate Reader (Antobio).

2.6. Cytokine measurement

Two days before challenge, two mice randomly selected from each group were sacrificed for measurement of T cell immune responses. Single-cell suspensions were prepared by pooling splenocytes of the two mice from each group. Red blood cells were lysed using lysis buffer and the cells were cultured in RPMI 1640 media supplemented with 10% fetal calf serum. 6×10^5 cells per well were cultured in triplicate wells for each group for 72 h at 37 °C with 5% CO_2 in 96-well plates (Corning) in the presence of rSjCTPI (10 μg/ml), ConA (10 μg/ml), or media alone. Cytokine levels (specified in Section 3) in supernatant were measured by flow cytometry analysis on BD FACSCalibur Flow Cytometer using Cytometric Bead Array Mouse Th1/Th2 Cytokine Kit (BD), according to the manufacturer's instructions.

2.7. Anti-schistosome protection efficacy

At 4 weeks after the last DNA immunization, each mouse was challenged with 40 ± 1 *S. japonicum* cercariae by abdominal skin penetration. Forty-two days post-challenge, all mice were sacrificed and perfused to determine worm and liver egg burdens. Mouse livers were removed, weighed, and digested overnight with 5% KOH (5 ml) at 37 °C. The worm and egg reduction rates in the liver were calculated. The worm reduction rate is equal to (the average worm burden in control group − the average worm burden in test group)/the average worm burden in control group × 100%. The egg reduction rate is equal to (the number of eggs per gram in control group − the number of eggs per gram in test group)/the number of eggs per gram in control group × 100%.

2.8. Statistics

One-way analysis of variance (ANOVA) was used to compare the protection efficacy including the percent reduction in worm and egg burdens. Student's *t*-test was used to analyze the antibody responses. A *p*-value of less than 0.05 was considered statistically significant.

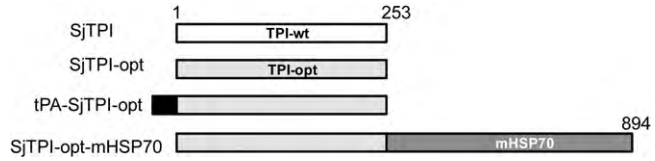

Fig. 1. Schematic diagrams for SjTPI DNA vaccine inserts. The numbers indicate the amino acid positions. The wild type (-wt) and codon optimized (-opt) SjTPI and mHPS70 sequences are indicated. The black square at the N-terminus of tPA-TPI-opt indicates the tissue plasminogen (tPA) leader sequence.

3. Results

3.1. Design of SjTPI DNA vaccines

In this study, four DNA vaccines expressing various forms of the SjTPI gene insert were constructed (Fig. 1). The first SjTPI DNA vaccine was developed, as previously described, using the wild type SjTPI gene sequence. The second DNA vaccine used the codon optimized SjTPI gene insert. The third one was the same codon optimized SjTPI gene insert with an addition tPA leader sequence at the N-terminus. The last DNA vaccine insert was produced by fusing the SjTPI gene with a downstream mHSP70 protein gene. Each of these four SjTPI DNA vaccine inserts was individually cloned into the DNA vaccine vector, pcDNA3.1, and the resulting vaccines were verified by restriction enzyme digestion and sequencing (data not shown).

3.2. Codon optimized SjTPI DNA vaccine improved Th1-type antibody and cytokine responses

The relative immunogenicities of the wild type (TPI) and codon optimized (TPI-opt) SjTPI DNA vaccines were evaluated in mice. At 4 weeks after the 3rd DNA immunization by intramuscular needle injection, TPI-specific antibody responses in mouse sera were measured against the recombinant TPI antigen by ELISA. The TPI-opt DNA vaccine was able to elicit higher level anti-TPI IgG responses than the TPI DNA vaccine (Fig. 2A). Further analysis demonstrated that increased antibody responses by the codon optimized SjTPI DNA vaccine were mainly due to increased IgG2a ($p < 0.001$) but not IgG1 (Fig. 2B). Therefore, our data suggest that the codon optimization of the SjTPI DNA vaccine was more effective in eliciting Th1-type antibody responses. Mice that received empty DNA vaccine vector immunization kept low background without showing significant antibody responses against TPI.

This finding was further confirmed by the measurement of cytokines in immunized animals. At 4 weeks after the 3rd DNA immunization by intramuscular needle injection, splenocytes from immunized mice in each vaccine group were harvested. The cells were then stimulated with recombinant SjCTPI protein (10 μg/ml) or media alone (negative control). Concentrations of Th1 cytokines (IL-2, IFN-γ, and TNF-α), in 72-h culture supernatant, were measured. The results indicated that splenocytes from animals that received the codon optimized TPI DNA vaccine produced higher levels of Th1 cytokines when compared to the cells harvested from the wild type TPI vaccine group (Fig. 3). The cells stimulated with mock medium or the cells from mice immunized with the empty vector immunized did not show significant levels of cytokine responses. The Th2 cytokines, IL-4 and IL-5, were also measured, but these Th2 cytokines were below detectable level in supernatant fluids collected 72 h after culture.

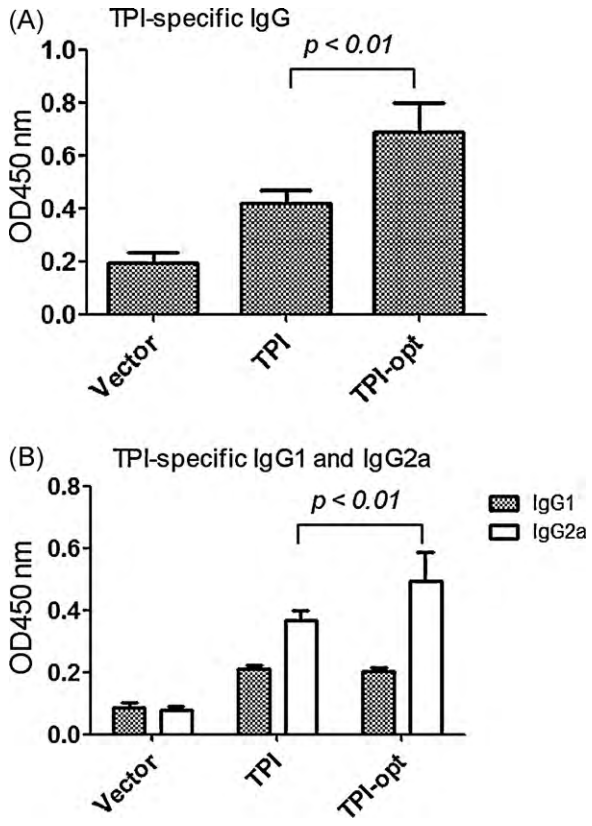

Fig. 2. SjTPI-specific antibody responses induced by wild type and codon optimized TPI DNA vaccines, and empty vector, by intramuscular (i.m.) immunization: (A) IgG responses; (B) IgG1 and IgG2a responses. Data show the average OD values of each group (14 mice) with standard deviations at 1:100 serum dilution. The serum samples were collected at 4 weeks after the 3rd DNA immunization prior to challenge. The statistical differences are indicated by the *p*-values.

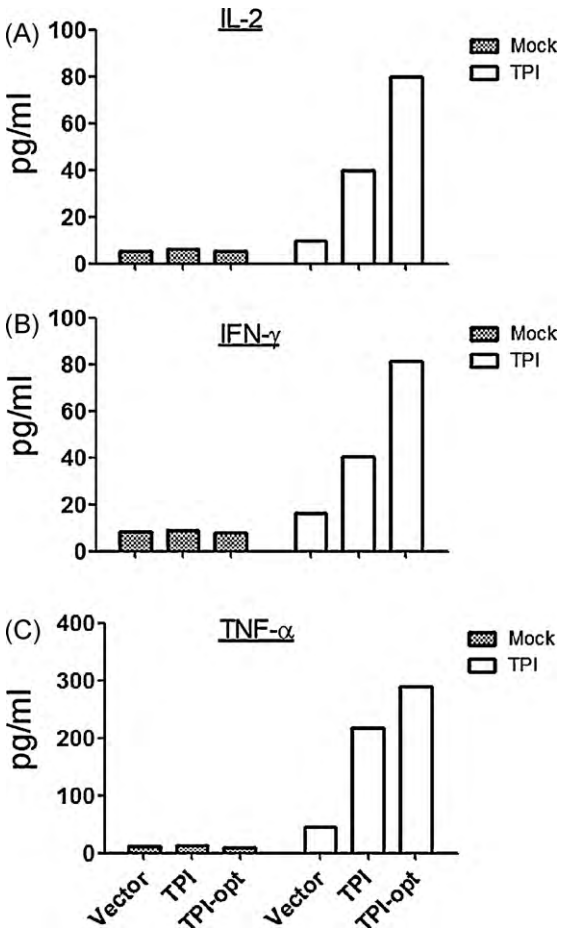

Fig. 3. SjTPI-specific cytokine responses in splenocytes induced by wild type and codon optimized TPI DNA vaccines, and empty vector, using i.m. immunization. Panels A, B and C show IL-2, IFN-γ and TNF-α responses, respectively, with mock or SjTPI stimulation. The splenocytes were collected at 4 weeks after the 3rd DNA immunization prior to challenge.

3.3. Codon optimized SjTPI enhanced the protective immunity against worm and egg challenges

To test the protection efficacy induced by the wild type or codon optimized SjTPI DNA vaccine, immunized mice were challenged with *S. japonicum* cercariae by abdominal skin penetration, at 4 weeks after the 3rd DNA immunization via intramuscular injection. Worm and egg burdens were measured in mouse vena mesenteria and liver tissue at 6 weeks after the infection. Numbers of adult and female worms and eggs were significantly reduced in wild type or codon optimized TPI DNA vaccine groups when compared to the group receiving DNA vector alone (Fig. 4A and B, Table 1). The reduction rates of adult worms, female worm, and eggs were 26%, 30%, and 28%, respectively (Table 1). Furthermore, the group receiving the codon optimized TPI DNA vaccine had higher reduction rates at 36.03%, 40.15%, and 41.17% for adult worms, female worms, and eggs, respectively, indicating that protection was significantly improved when compared to the group that received the wild type TPI vaccine ($p = 0.026$, 0.027, and 0.003, respectively) (Fig. 4C). These results showed that the codon optimized SjTPI DNA vaccine not only elicited higher antibody and cytokine responses but also achieved higher efficacy against *S. japonicum* cercariae infection.

Table 1
Summary of protection efficacies induced by different SjTPI DNA vaccination regimens.

DNA vaccines	No. of mice	Vaccination approach[a]	Adult worms		Female worms		Eggs	
			No. of worms[b]	% reduction	No. of worms[b]	% reduction	No. of eggs[b]	% reduction
Vector	9	IM	24 ± 2		12 ± 1		67625 ± 5289	
SjTPI	10	IM	18 ± 1	26	8 ± 1	30	48450 ± 3523	28
SjTPI-opt	11	IM	15 ± 3	36[c]	7 ± 1	40[c]	39418 ± 7551	42[c]
SjTPI-opt-mHSP70	9	IM	15 ± 2	39[c]	7 ± 1	44[c]	35944 ± 6176	47[c]
tPA-SjTPI-opt	10	IM	15 ± 2	38[c]	7 ± 1	41[c]	37250 ± 7056	45[c]
SjTPI-opt	10	EP	12 ± 3	50[d]	6 ± 1	53[d]	29050 ± 6075	57[d]
SjTPI-opt-mHSP70	11	EP	12 ± 2	52[d]	5 ± 1	55[d]	26090 ± 5782	61[d]

[a] "IM" or "EP" indicates the route of DNA immunization: intramuscular injection or electroporation, respectively.
[b] The numbers are shown as the worm and egg numbers ± standard deviations.
[c] It is statistical significant ($p < 0.05$) when compared to the SjTPI vaccination group.
[d] It is statistical significant ($p < 0.01$) between these two EP groups and their matching IM delivery groups using the same SjTPI-opt or SjTPI-opt-mHSP70 DNA vaccines.

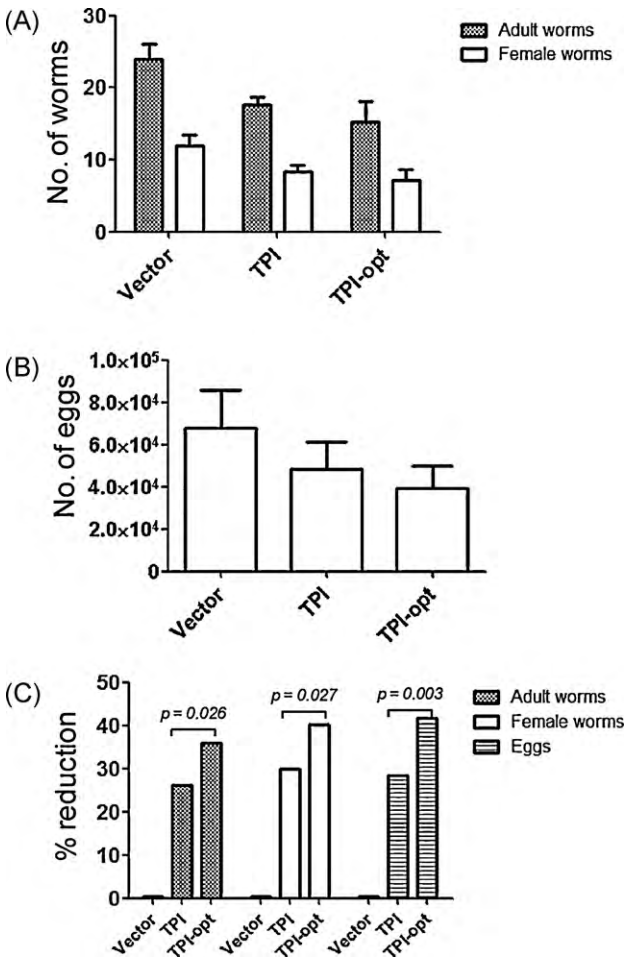

Fig. 4. The protection generated by wild type and codon optimized TPI DNA vaccines, and empty vector, in mice using i.m. immunization. Panels A and B, respectively, show the group average numbers of worms with standard deviations and eggs in liver with standard error. Panel C shows the average reduction rates of adult worms, female worms and eggs in each group. The statistical differences are indicated by the *p*-values.

3.4. Addition of a tPA leader sequence significantly improved antibody responses but has less of an effect on protective immunity

Based on previous reports, the addition of a tPA leader sequence to certain DNA vaccines may enhance antigen secretion in order to increase the immunogenicity of DNA vaccines [33]. Therefore, we tested the effect of adding a tPA leader sequence to a codon optimized SjTPI DNA vaccine. Indeed, the codon optimized TPI DNA vaccine elicited higher IgG1 and IgG2a responses compared to the wild type TPI DNA vaccine (Fig. 5A). However, there was no increase in IL-2, IFN-γ, or TNF-α cytokine production with the addition of tPA leader to the codon optimized TPI DNA vaccine (Fig. 5B). Mice vaccinated with the tPA-TPI-opt had slightly improved reduction of adult and female worm burdens and in egg numbers in liver, but there was no statistical difference when compared with the TPI-opt DNA vaccine (Fig. 5C and Table 1).

3.5. Immune responses induced by DNA vaccine expressing TPI-mHSP fusion antigen

As previously reported, the mouse heat-shock protein 70 (mHSP70) may enhance the immunogenicity of DNA vaccination in mice [35]. In order to test its effects on the codon optimized SjTPI DNA vaccine, mHSP70 was fused to the C-terminus of the codon optimized TPI to express a TPI-mHSP fusion protein (TPI-opt-mHSP70). Regardless of whether the DNA vaccines were delivered via intramuscular needle injection or electroporation, the groups that received the TPI-opt-mHSP70 produced similar levels of antibody (Fig. 6), cytokine responses (Fig. 7), and a similar degree of protection (Fig. 8) to the groups receiving TPI-opt after the 3rd DNA immunization. Therefore, these data indicate that the fusion of mHSP70 to the codon optimized TPI did not further enhance the immunogenicity of TPI DNA vaccines.

3.6. Electroporation further improved the efficacy of SjTPI DNA vaccines

Electroporation, as a novel DNA vaccine delivery approach, has been shown to improve the efficacy of DNA vaccines in our previous study [36]. In the current study, we evaluated whether delivery of the TPI-opt or TPI-opt-mHSP70 DNA vaccine could increase the immunogenicity and protection efficacy against the parasite infection. Mice received the same dose of DNA vaccines at Week 0, 3 and 6 by intramuscular needle injection or electroporation. The groups receiving electroporation immunization with TPI-opt or TPI-opt-mHSP70 produced higher levels of total IgG and IgG2a responses ($p < 0.01$) but similar levels of IgG1 responses compared to the mice receiving the same DNA vaccines by intramuscular inoculation, suggesting an improvement in the Th1-type antibody response. In support of this finding, significantly higher levels of the Th1 cytokines, IL-2, IFN-γ, and TNF-α, in splenocytes were produced in the groups that received the vaccine via electroporation compared to the groups receiving intramuscular inoculation (Fig. 7). The cells stimulated with mock medium or the cells from empty vector immunized mice had no significant production of cytokines. These results also indicated that the electroporation delivery of codon optimized SjTPI DNA vaccines could increase Th1-type antibody and T cell responses. The mice receiving TPI-opt or TPI-opt-mHSP70 DNA vaccine by intramuscular injection or electroporation were then challenged with *S. japonicum* cercariae by abdominal skin penetration at 4 weeks after the 3rd DNA immunization. The groups vaccinated by electroporation had significantly higher reduction rates of adult worms, female worms, and eggs in mouse liver by 50–52%, 53–55%, and 57–61.42%, respectively ($p < 0.01$, compared to intramuscular immunization using the same DNA vaccines) (Fig. 8 and Table 1).

A summary of the protective responses, regarding reductions in worm and egg burdens, induced by different SjTPI DNA vaccines is provided in Table 1. Reductions in worms and eggs in mouse livers were below 30% when the wild type SjTPI DNA vaccine was used. Rates of worm and egg reductions were increased to over 40% by the codon optimized vaccine, and electroporation delivery further improved worm and egg reduction rates to 50–61% (Table 1). These results demonstrate that the codon optimized SjTPI DNA vaccine and the more effective electroporation delivery system could synergistically improve immunogenicity and protection efficacies of the SjTPI DNA vaccine.

4. Discussion

Since the use of DNA vaccines was first reported in the early-1990s, important technical advances have been made to improve the overall immunogenicity of this vaccination approach. In the current study, we incorporated four such improvements in the design of optimized SjTPI DNA vaccines.

First, optimized codon usage has been effective in increasing the overall antigen production and immunogenicity of DNA vac-

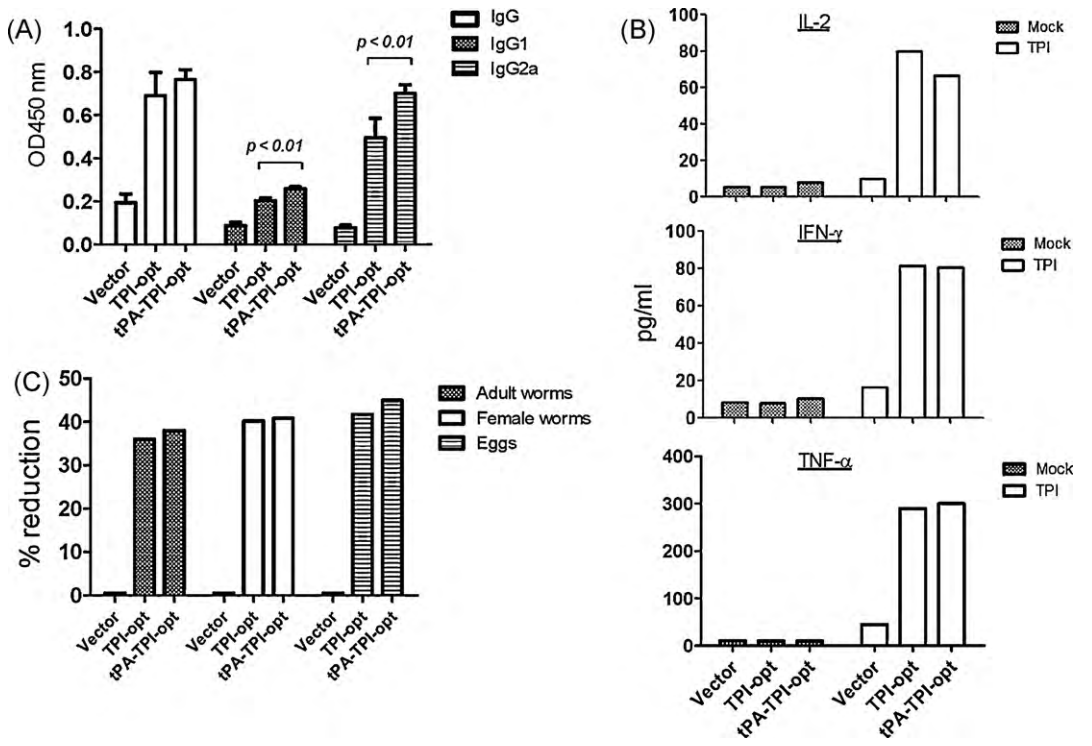

Fig. 5. The immune responses induced by codon optimized SjTPI DNA vaccines with (tPA-SjTPI-opt) or without (SjTPI-opt) a tPA leader sequence in mice using i.m. immunization. (A) TPI-specific IgG, IgG1 and IgG2a responses are shown as the average OD values of each group (14 mice) with standard deviations. The statistical differences are indicated by the *p*-values ($p < 0.01$). (B) TPI-specific IL-2, IFN-γ and TNF-α cytokine responses with mock or SjTPI stimulation. Data show the average values of each group. The serum samples (A) and splenocytes (B) were collected at 4 weeks after the 3rd DNA immunization prior to challenge. (C) The data present the average reduction rates of adult worms, female worms and eggs in each group.

cines [33,37,38], and, therefore, it was not surprising to see that codon optimization was able to improve the immunogenicity and protection by a SjTPI DNA vaccine, given the difference of codon preference between parasite and mammalian proteins. Second, the use of *in vivo* electroporation was able to further improve the immunogenicity and protection of a codon optimized SjTPI DNA vaccine, a result that is consistent with reports that the electroporation approach was able to markedly enhance the effectiveness of DNA vaccination in eliciting both humoral and cellular immune responses in various animal models against different pathogens [34,36,39–41]. More significantly, the combination use of codon optimization and electroporation was able to achieve a synergistic effect in protection: more than 50% reduction of infection—a high standard for a parasite vaccine. The third approach we tested was the addition of a tPA leader on the immunogenicity of the SjTPI DNA vaccine. While overall antibody responses were increased, possibly due to more secreted TPI antigen and the addition of tPA alone was able to elicit better protection than the wild type SjTPI DNA vaccine, there was no synergistic improvement in protection when combined with the use of codon optimization. Finally, we also tested whether a heat-shock protein can serve as a molecular adjuvant to enhance protective immune response. A SjTPI-HSP fusion insert was more effective than the wild type gene SjTPI DNA vaccine in eliciting better protection, but the combination use of codon opti-

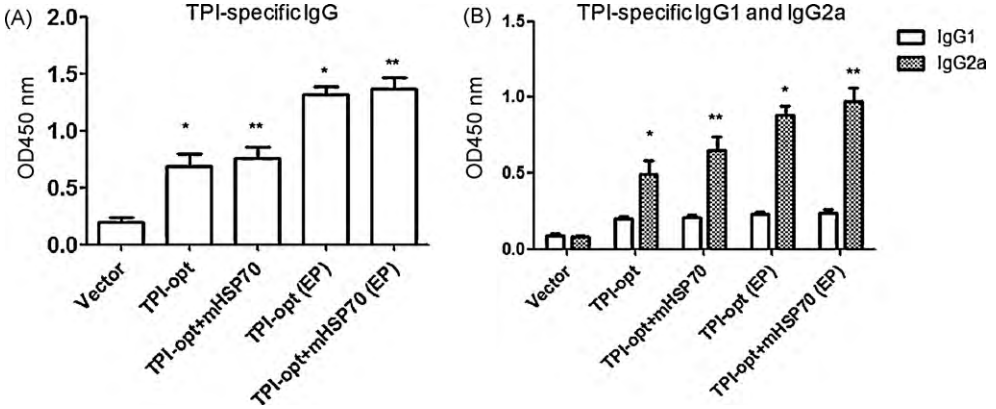

Fig. 6. The SjTPI-specific IgG (A), IgG1 and IgG2a (B) responses induced by TPI-opt or TPI-opt-mHSP70 DNA vaccines via i.m. or electroporation (EP) vaccination as indicated. Data show the average OD values of each group (14 mice) with standard deviations at 1:100 serum dilution. The serum samples were collected at 4 weeks after the 3rd DNA immunization prior to challenge. The statistical differences are indicated by "*" or "**" for matching groups with $p < 0.01$.

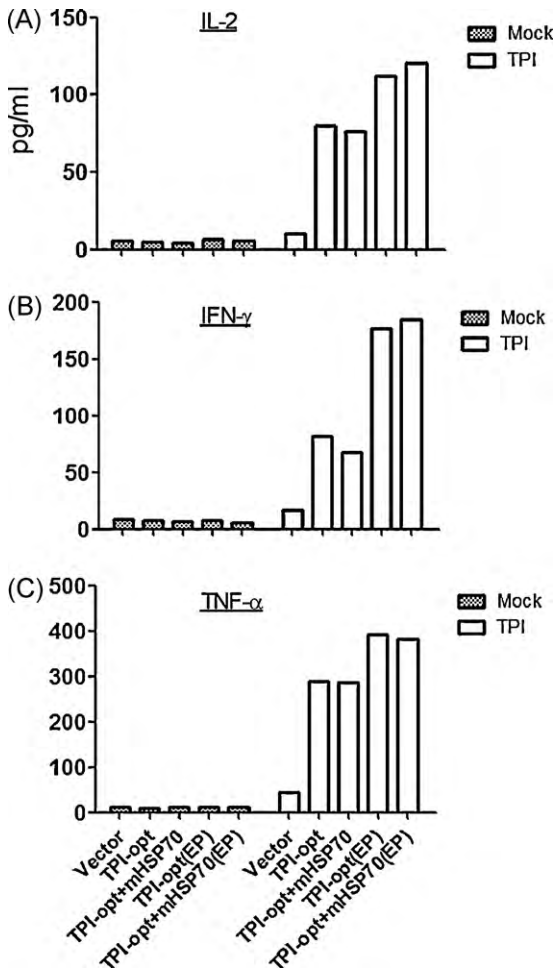

Fig. 7. The SjTPI-specific cytokine responses induced by TPI-opt or TPI-opt-mHSP70 DNA vaccines via i.m. or electroporation (EP) vaccinations as indicated. Panels A, B and C show IL-2, IFN-γ and TNF-α responses, respectively, with mock or SjTPI stimulation. The splenocytes were collected at 4 weeks after the 3rd DNA immunization prior to challenge.

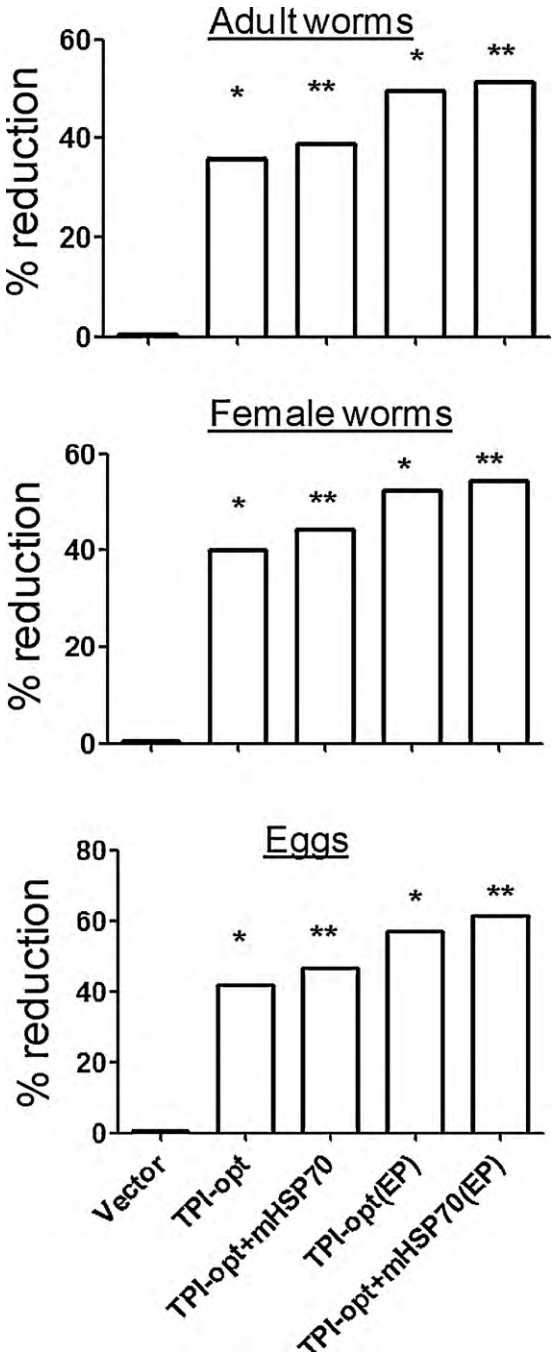

Fig. 8. The protection generated by TPI-opt or TPI-opt-mHSP70 DNA vaccines via i.m. or electroporation (EP) vaccinations as indicated. Panels A, B and C, present the average reduction rates of adult worms, female worms and eggs, respectively, in each group. The statistical differences are indicated by "*" or "**" for matching groups in each graph with $p < 0.01$.

mization and HSP fusion protein was not able to achieve synergistic improvement on protection. The failure of tPA or HSP approaches for synergistic effect with codon optimization suggested that the protection against *S. japonicum* by SjTPI DNA vaccine is elicited via a specific mechanism, such as Th1-type immune responses and not by a conventional antibody response.

In our previous studies, we successfully produced the original SjTPI DNA vaccine based on the wild type SjTPI gene sequence [23,30]. However, the final protection of mice against challenge was moderate, similar to other subunit-based vaccines. In the current study, we modified the SjTPI DNA vaccine via optimization of the coding gene sequence and observed a significant increase in immunogenicity and protection in the same mouse model.

Recently, in a separate study, we demonstrated that electroporation was effective in eliciting improved protective immunity in mice by delivering a polyvalent antigen formulation that included the wild type gene SjTPI and three other DNA vaccines against *S. japonica* [34]. However, a high level of protection was achieved only after these immunized mice received a boost with the recombinant protein antigens. In the current study, a single component codon optimized SjTPI DNA vaccine, delivered by electroporation, was able to achieve a high level of protection without any protein boost. Clearly, the synergistic effect of codon optimization and electroporation with this one component DNA vaccine is beneficial for cost-related reasons and as a practical formulation for clinical applications than would the electroporation delivery of a polyvalent DNA prime plus protein boost formulation.

It was clear that predominantly Th1-type antibody and cytokine responses were observed with either codon optimization or electroporation. Furthermore, the combination of these two approaches did not change this Th1 dominance. This may have contributed to an improved protection efficacy with SjTPI DNA vaccines. By using a viral protein as a model antigen, we have recently shown that both intramuscular injection of DNA vaccines, with

or without electroporation, induced a mainly Th1-type immune response [36]. Results from the current studies demonstrated a similar Th1 dominance for a parasite antigen, suggesting that Th1 dominance may be associated with the method of DNA vaccine delivery rather than the antigen itself. In support of this hypothesis, a gene gun delivery method for DNA vaccines has generally been shown to elicit a Th2-type antibody response [36,42]. In the current report, the measurement of T cell immune responses in vaccinated mice should be considered only as a pilot study given the small sample size. Future studies should include more detailed analysis on polyfunctionality of T cell immune responses when the combination approach of codon optimization and electroporation is used.

In summary, the improvement in protection in a mouse model as measured by reductions in adult worm and liver egg burden by 50–60% with a single antigen-based vaccine is very encouraging. As shown by human schistosomiasis, serious symptoms are generally associated with large numbers of parasites harbored within the body. A vaccine that can effectively reduce the worm burden by 50% or more may have a major impact on disease progression, including decreased morbidity. Furthermore, a recent study in water buffalo showed that a vaccine with 50% efficacy would reduce the transmission of the disease [4]. It was suggested that employment of such a vaccine in water buffalo, combined with praziquantel treatment, could result in significant decreases in transmission of the disease in the lake and marsh regions, based on mathematical models of transmission in these regions of China [43]. Furthermore, use of such vaccines in humans and in bovines, would lead to a dramatic decrease in transmission of schistosomiasis and a reduction in infection. Data presented in the current report support the notion that a codon optimized SjTPI DNA vaccine, when delivered by electroporation, may be one of such vaccines.

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