ISSN 1672-9145 **Acta Biochimica et Biophysica Sinica** 2005, **37**(11): 751–758 **CN** 31-1940/Q

Identification of Immunodominant Th1-type T cell Epitopes from *Schistosoma japonicum* 28 kDa Glutathione-S-transferase, a Vaccine Candidate

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Abstract Th1-type cytokines produced by the stimulation of Th1-type epitopes derived from defined schistosome-associated antigens are correlated with the development of resistance to the parasite infection. *Schistosoma mansoni* 28 kDa glutathione-S-transferase (Sm28GST), a major detoxification enzyme, has been recognized as a vaccine candidate and a phase II clinical trial has been carried out. Sheep immunized with recombinant *Schistosoma japonicum* 28GST (Sj28GST) have shown immune protection against the parasite infection. In the present study, six candidate peptides (P1, P2, P3, P4, P7 and P8) from Sj28GST were predicted, using software, to be T cell epitopes, and peptides P5 and P6 were designed by extending five amino acids at the N-terminal and C-terminal of P1, respectively. The peptide 190–211 aa in Sj28GST corresponding to the Th1-type epitope (190–211 aa) identified from Sm28GST was selected and named P9. The nine candidate peptides were synthesized or produced as the fusion protein with thioredoxin in the pET32c(+)/BL21(DE3) system. Their capacity to induce a Th1-type response *in vitro* was measured using lymphocyte proliferation, cytokine detection experiments and flow cytometry. The results showed that P6 (73–86 aa) generated the strongest stimulation effect on T cells among the nine candidate peptides, and drove the highest level of IFN-γ and IL-2. Therefore, P6 is a functional Th1-type T cell epitope that is different from that in Sm28GST, and will be useful for the development of effective vaccines which can trigger acquired immunity against *S. japonicum*. Moreover, our strategy of identifying the Th1-type epitope by a combination of software prediction and experimental confirmation provides a convenient and cost-saving alternative approach to previous methods.

Key words *Schistosoma japonicum*; epitope; Sj28GST; Th1 epitope; IFN-γ

Schistosomiasis is a serious parasitic disease that infects over 200 million people and kills about one million people annually [1]. Although praziquantel is an effective drug for the treatment of schistosomiasis, reinfection and the drug resistance of the parasite have become a problem [2]. Therefore, the development of an effective vaccine against schistosomiasis is important to control this disease [3,4]. In the past few years, many vaccine strategies have focused on defense against invasion of cercariae, to reduce worm burden by inducing humoral immunity with schistosome vaccine candidates, but the high-level antigen induced-specific antibodies could not adequately protect the host from infection [5,6]. Immunoepidemiological studies have established a correlation between specific immune response and resistance (acquired immunity) or susceptibility to schistosome infection [7–9]. Some researchers found that Th1-type cytokines, such as IFN-γ and IL-2, are correlated with the development of resistance to reinfection with *Schistosoma mansoni* or *Schistosoma japonicum* [10]. Thus, the identification of antigen molecules and their epitope types (Th1 or Th2) possess important significance to the discovery of immunity mechanisms and the development of effective vaccines

Received: May 24, 2005 Accepted: August 25, 2005

This work was supported by a grant from the National Natural Science Foundation of China (No. 30271166)

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DOI: 10.1111/j.1745-7270.2005.00111.x

for schistosomiasis [3].

The host's immunity against schistosome infection is mainly mediated by $CD4^+$ helper T cells that can be activated by the specific Th1 or Th2 epitopes on the antigen, which bind to major histocompatibility complex class II molecules and are presented by antigen-presenting cells [11]. Identification of these T cell epitopes may help us study the effect of Th1- or Th2-type responses on anti-schistosome infection, and it is also a pivotal step for the study of pathogenesis and immunity, especially for the effective development of multiple Th1 or Th2 epitope vaccines against schistosomiasis. The 28 kDa antigen in *S. mansoni* was identified as a glutathione-S-transferase (28GST) [12]. *S. mansoni* 28GST (Sm28GST) has been recognized as an effective vaccine candidate [13,14], and its phase II clinical trial has been carried out [15]. Its specific T cell epitope has been studied [1]. Recombinant *S. japonicum* 28GST (Sj28GST) prepared from *Escherichia coli* was used to immunize sheep and cattle and could induce worm reduction by 50.4%–68.5% [17]. However, the effects of protection from the Th1 or Th2 polarization induced by Sj28GST, which led to cellular and humoral immunity, are still not clear. Nor is there a report on the identification of T cell epitopes from Sj28GST antigen.

In the present study, the effective Th1 epitopes from Sj28GST were identified through a combination of epitope prediction software and experimental approach *in vivo* or *in vitro* to provide for the design of an effective vaccine for *S. japonicum*.

Materials and Methods

Medium and reagents

RPMI 1640 medium and fetal calf serum were purchased from Gibco BRL Life Technologies (Paisley, UK). Complete RPMI 1640 medium was supplemented with 10% heat-inactivated fetal calf serum, 100 U/ml ampicillin, 100 μg/ml streptomycin, 2 mM *L*-glutamine, 5×10^{-5} M 2-mercaptoethanol and 25 mM HEPES. Concanavalin A (ConA) was the product of Sigma (St. Louis, USA).

Mice, snails and cercariae

Female C57BL/6(H-2^b) mice aged 6–8 weeks were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences (Shanghai, China), and kept under conventional germ-free conditions. *Oncomelania* snails with mature cercariae of *S. japonicum* were purchased from Jiangsu Provincial Institute of Schistosomiasis Control (Wuxi, China). The cercariae were released from the snails in a routine method then irradiated with ultraviolet rays at 400 $\mu W\cdot cm^{-2}$ per minute for 1 min before infection.

Recombinant Sj28GST preparation

Recombinant plasmid pET32c(+)-Sj28GST was transfected into *E. coli*, and thioredoxin (Trx)-Sj28GST fusion protein expression was induced with isopropyl-β-*D*-thiogalactopyranoside (IPTG). The fusion protein was purified by Ni^{2+} column affinity chromatography as described previously [18] by the recommended purification module (Pharmacia, Kalamazoo, USA), quantified by a combination of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and ultraviolet spectrophotometry, then stored at –70 ºC until use.

Epitope prediction

The amino acid sequence of Sj28GST was input into the prediction software to predict the T cell epitopes [19, 20], and six candidate peptides (P1, P2, P3, P4, P7 and P8) were chosen according to the prediction scores. Candidate peptides P5 and P6 were designed by extending five amino acids at the N-terminal and C-terminal of peptide P1, respectively. The peptide 190–211 aa in Sj28GST, which corresponds to the Th1 epitope (190–211 aa) in Sm28GST, was selected and named peptide P9.

Candidate peptide synthesis

Candidate peptides P1, P2, P3 and P4 were synthesized by Meilian Bio-Technology (Xi'an, China) on a 9050 Pep synthesizer instrument by using solid-phase peptide synthesis and standard 9-fluorenylmethoxy carbonyl technology (PE Applied Biosystems, Foster City, USA), and were purified by high-performance liquid chromatography. The purity of peptides was 85%–95% as determined by analytical high-performance liquid chromatography and mass spectroscopy analysis. The peptides were dissolved in distilled water before use.

Preparation of recombinant fusion proteins of candidate peptides

The positive and negative chain oligonucleotides of candidate peptides P5, P6, P7, P8 and P9, and control peptide P10, which has no similarity with Sj28GST, were designed and synthesized according to their amino acid sequences (**Table 1**; start codon ATG and termination codon TAA in bold, restriction endonuclease *Nco*I and *Xho*I sites in italic). After synthesis, they were dissolved in distilled water at a concentration of 100 μM and stored at –20 ºC until use. Equal amounts of the positive and negative chain oligo-

Table 1 Oligonucleotide sequences of epitope candidates from recombinant *Schistosoma japonicum* 28GST

Candidate peptide	Oligonucleotide sequence	
P5	Positive	5′-*C**ATGG***CGatgtcagagagtttggctattgcacgatttatagcgcgaaaa**TAA**C-3′
	Negative	5′-*TCGAG*TTAttttcgcgctataaatcgtgcaatagccaaactctctgacatCGC-3′
P6	Positive	5′-*C**ATGg***ctattgcacgatttatagcgcgaaaacacaacatgatgggc**TAA**C-3′
	Negative	5′-*TCGAG*TTAgcccatcatgttgtgttttcgcgctataaatcgtgcaatagcCGC-3′
P7	Positive	5′-*C**ATGG***cgaaacatctattggccacttcaccaaaactggcgaaatacttatca**TAA**C-3′
	Negative	5′-*TCGAG*TTAtgataagtatttcgccagttttggtgaagtggccaatagatgtttCGC-3′
P8	Positive	5′-*C**ATGG***cgccaaaactggcgaaatacttatcagagagacatgcaacggcattt**TAA**C-3′
	Negative	5′-*TCGAG*TTAaaatgccgttgcatgtctctctgataagtatttcgccagttttggCGC-3′
P9	Positive	5′-*C**ATGG***cgaaacatctattggccacttcaccaaaactggcgaaatacttatcagagagacatgcaacggcattt**TAA**C-3′
	Negative	5′-*TCGAG*TTAaaatgccgttgcatgtctctctgataagtatttcgccagttttggtgaagtggccaatagatgtttCGC-3′
P10	Positive	5′-*C**ATGG***CGgctaagcaatataacatatgttgtaaatttaaagaacttctcgat**TAA**C-3′
	Negative	5′-*TCGAG*TTaatcgagaagttctttaaatttacaacatatgttatattgcttagcCGC-3′

Start codon ATG and termination codon TAA are in bold. *Nco*I and *Xho*I sites are in italic. The coding sequences are in lowercase.

nucleotides were mixed, incubated with annealing buffer at 95 ºC for 5 min, then cooled naturally to room temperature to form double-stranded DNAs. The DNA fragments were inserted into the expression vector pET32c(+) cut with restriction endonuclease *Nco*I and *Xho*I, and identified by DNA sequencing. The positive recombinant plasmids were transformed into *E. coli* BL21(DE3), respectively, and the expression was induced with IPTG. The expressed fusion proteins of candidate peptides with Trx were identified by SDS-PAGE and selected as epitope candidates based on their molecular weights. The Trx fusion proteins were isolated by Ni^{2+} column affinity chromatography, mixed with *n*-octyl-β-*D*-glucopyranoside and separated on polymyxin B-bound agarose gel electrophoresis to remove bacterial endotoxin contamination [21]. After dialysis against phosphate-buffered saline (PBS), the purities and amounts of Trx fusion proteins were identified by SDS-PAGE and ultraviolet spectrophotometry. The products were stored at −70 ºC until use.

Immunization of mice

Fifty female mice were infected with 400±2 ultraviolet ray-irradiated cercariae through the abdominal skin, and boosted subcutaneously with 100 μg of recombinant Sj28GST emulsified in incomplete Freud's adjuvant at the base of the tail 40 d after the first infection.

Preparation and cultivation of spleen cells

The immunized mice were killed 7 d later, and the spleens were removed aseptically. The suspension of single spleen cells was prepared after removing erythrocytes by hypotonic lysis and resuspended in RPMI 1640 medium by vigorous pipetting. The single cell suspension was spread and added into the 96-well flat-bottomed tissue culture plates at 200 μl/well, then cultured at 37 ºC in a humidified atmosphere with 5% CO_2.

Splenocyte proliferation assay

Cell suspension (1.5×10^6 cells/ml) in complete RPMI 1640 medium supplemented with 10 μg/ml ConA, 10 μg/ml synthetic candidate peptide or 20 μg/ml candidate peptide fusion protein, was dispensed in triplet into 96-well flat-bottomed tissue culture plates at 200 μl/well, and incubated at 37 ºC with 5% CO_2. After incubation for 64 h, [^{3}H]thymidine ([^{3}H]TdR) was added at 0.5 μCi/well. The cells were cultured continuously for 8 h and harvested. The incorporated [^{3}H]TdR was measured in a liquid scintillation counter (Wallac Guardian 1414; PerkinElmer, Boston, USA) after harvesting the cell cultures onto glass filters. The data were expressed as counts per minute.

In vitro cytokine detection by sandwich enzyme-linked immunosorbent assay (ELISA)

For *in vitro* cytokine detection, cell suspension (3×10^6 cells/ml) was dispensed in triplicate into 24-well flat-bottomed tissue culture plates at 200 μl/well, and incubated at 37 ºC with 5% CO_2 in the presence of 10 μg/ml ConA, 10 μg/ml synthetic peptide or 20 μg/ml candidate peptide fusion protein. After 24 h, the supernatants were harvested for detection of IL-2, or, after 48 h, for IFN-γ. IFN-γ and IL-2 levels in the supernatants were determined by sandwich ELISA using a Quantikine M kit (R&D Systems, Minneapolis, USA). The absorbance of each well was measured at 450 nm on a microplate reader (CliniBio 128C;

Salzburg, Austria). Cytokine levels were calculated using standard curves constructed with recombinant murine IFN-γ and IL-2.

Detection of intracellular cytokines by flow cytometry

After being stimulated with 10 μg/ml synthetic candidate peptide or 20 μg/ml candidate peptide fusion protein, the cells were cultured for 40 h, then treated with a protein transport inhibitor (Brefeldin A) for 8 h. The cells were harvested, fixed, permeabilized (Cytofix/Cytoperm kit; PharMingen, Omaha, USA), and stained with Cy-chrome-labeled anti-CD4 monoclonal antibody (PharMingen). After washing with PBS, the cells were stained with FITC-labeled anti-IFN-γ and anti-IL-4 monoclonal antibodies (PharMingen) for IFN-γ and IL-4 detection. The PE-labeled anti-mouse IgG_2 antibody (PharMingen) staining was used as negative control. After staining, the cells were washed, resuspended in 1% paraformaldehyde in PBS, and then kept protection from light at 4 ºC until analysis on the flow cytometer. Flow cytometric analysis was performed on a FACSCalibur, and 20,000 events were acquired and analyzed with software.

Statistical analysis

For statistical evaluation of data, the two-sided Student's *t*-test was used.

Results

Location of the immunodominant T cell epitopes in Sj28GST antigen

The T cell response to a protein antigen generally focuses on a limited number of potential T cell epitopes [22]. According to the results of software prediction, T cell epitope candidates P1, P2, P3, P4, P7 and P8 in Sj28GST were primarily selected, as shown in **Table 2**. Peptide fragment P1 triggered the strongest proliferation of mice splenocytes and produced the highest level of IFN-γ, but it was composed of only nine amino acids. Thus, five amino acids were extended at the N- and C-terminal of peptide fragment P1 to produce peptide fragments P5 and P6. The epitope candidate P9 was selected based on its location corresponding to the T cell epitope peptide (190−211 aa) in Sm28GST, which overlapped with peptides P7 and P8.

Preparation of the recombinant epitope fusion proteins

The epitope coding fragments inserted in the expression vector pET32c(+) were coincident with those of previous designs, as confirmed by DNA sequencing. The expressed and purified epitope fusion proteins by Ni^{2+} column affinity chromatography were identified by 15% SDS-PAGE. **Fig. 1** shows that the purified Trx-fusion proteins of epitopes were all a single band with a molecular weight of approximately 18 kDa.

Splenocyte proliferation assay

The proliferation responses of irradiated cercariae and rSj28GST-sensitized spleen lymphocytes to each of the synthetic peptides and Trx-fusion proteins are shown in **Fig. 2**. The sensitized spleen cells responded more strongly to candidate peptides P1, P5 and P6 than to control peptide P10.

Th1-type cytokine secretion profile of candidate peptides

To determine the patterns of the Th1-type cytokine

Table 2 Candidate epitopes of recombinant *Schistosoma japonicum* 28GST

Candidate peptide	Amino acid sequence	Location (aa)	Candidate peptide origin
P1	NH_2-**AIARFIARK**-COOH	73–81	Synthetic candidate peptide
P2	NH_2-EPIRMILVAAGVE-COOH	18–30	Synthetic candidate peptide
P3	NH_2-ADVVLIASIDHIT-COOH	159–171	Synthetic candidate peptide
P4	NH_2-DEYYIIEKMIGQVE-COOH	90–103	Synthetic candidate peptide
P5	NH_2-MSESI**AIARFIARK**-COOH	68–81	Trx-fusion protein
P6	NH_2-**AIARFIARK**HNMMG-COOH	73–86	Trx-fusion protein
P7	NH_2-KHLLATS**PKLAKVLS**-COOH	190–204	Trx-fusion protein
P8	NH_2-**PKLAKVLS**ERHATAF-COOH	197–211	Trx-fusion protein
P9	NH_2-KHLLATS**PKLAKVLS**ERHATAF-COOH	190–211	Trx-fusion protein

Peptides P1, P5 and P6, and peptides P7, P8 and P9 have overlapping amino acids as shown in bold respectively. aa, amino acid.

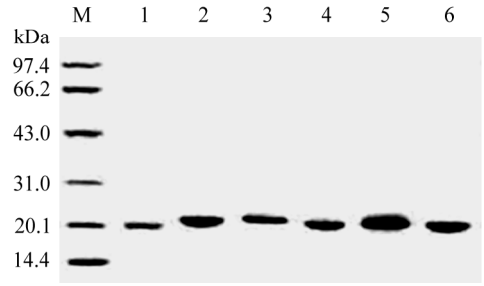

Fig. 1 Identification of recombinant epitope fusion proteins expressed in *Escherichia coli* by sodium dodecyl sulphate-polyacrylamide gel electrophoresis

M, protein molecular weight standard; 1–5, Trx-fusion proteins of peptides P5–P9; 6, Trx-fusion protein of control peptide P10.

profile elicited by the candidate peptides, Th1-type cytokine levels were examined *in vitro* using the splenic cells driven by synthetic peptides or Trx-fusion proteins. As shown in **Fig. 3**, the production of IFN-γ and IL-2 of P1 was high ($P<0.01$) in four synthetic peptides, and P5 and P6 ($P<0.01$) in six Trx-fusion proteins. IFN-γ secretion of synthetic peptides P3, P4 and Trx-fusion protein P10 could not be detected; IL-2 secretion could not be detected in Trx-fusion protein P10.

Production of intracellular cytokines

To detect the production of intracellular cytokines elicited by the candidate peptides, the proportions of $CD4^+$, IFN-γ- and IL-4-producing cells were examined by FACS Calibur using the splenic cells driven by synthetic peptides or Trx-fusion proteins. **Table 3** shows that the proportion of IFN-γ-producing cells was higher than that of IL-4-producing cells in splenic cells stimulated with P1, P5, P6 and P7, but the proportion of IL-4-producing cells was higher than that of IFN-γ-producing cells in the splenic cells stimulated with P2 and P3. Of the splenic cells stimulated with P4, the proportion of the cells secreting IL-4 was obviously higher than that of the cells secreting IFN-γ.

The proportion of $CD4^+$, IFN-γ- and IL-4-producing cells in spleen cells was measured by flow cytometry after the spleen cells from immunized mice were pulsed with different synthetic peptides and recombinant peptide fusion proteins. Therefore, from the results mentioned above, it is evident that candidate peptides P5, P6 and P7 are Th1-type epitopes, and candidate peptide P4 may be a Th2-type epitope.

Discussion

Interest is growing in the concept that mini-gene vaccines, constructed only with multiple epitopes, could be used to promote protection against infectious diseases. The epitope-induced Th1-type response plays an important role in anti-schistosome infection by producing cytokines, such as IFN-γ and IL-2 [23]. It has been shown that, at an early stage of infection, the host's response against the parasite is a Th1-type one [24,25]. Epidemiological surveys of schistosomiasis showed that the individual with a high level of IFN-γ was significantly correlated with resistance to schistosome infection [26]. In

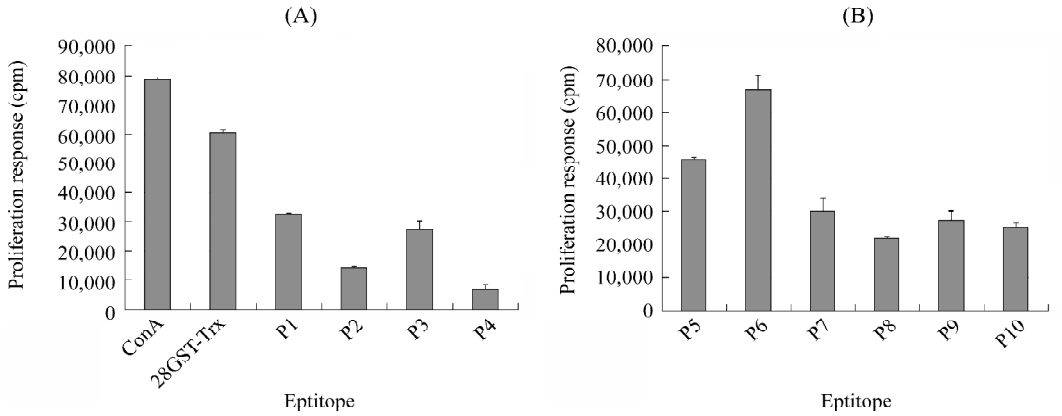

Fig. 2 The proliferation of the splenocytes stimulated with different epitopic peptides

Different synthetic peptides and recombinant epitope fusion proteins of *Schistosoma japonicum* 28GST (Sj28GST) were used to pulse splenocytes from C57BL/6 mice that had been exposed to irradiated cercariae and boosted with recombinant Sj28GST. The synthetic peptide concentration was 10 μg/ml, and the recombinant epitope fusion protein concentration was 20 μg/ml. (A) The stimulation of synthetic peptides. (B) The stimulation of recombinant epitope fusion proteins. ConA, concanavalin A; cpm, counts per minute.

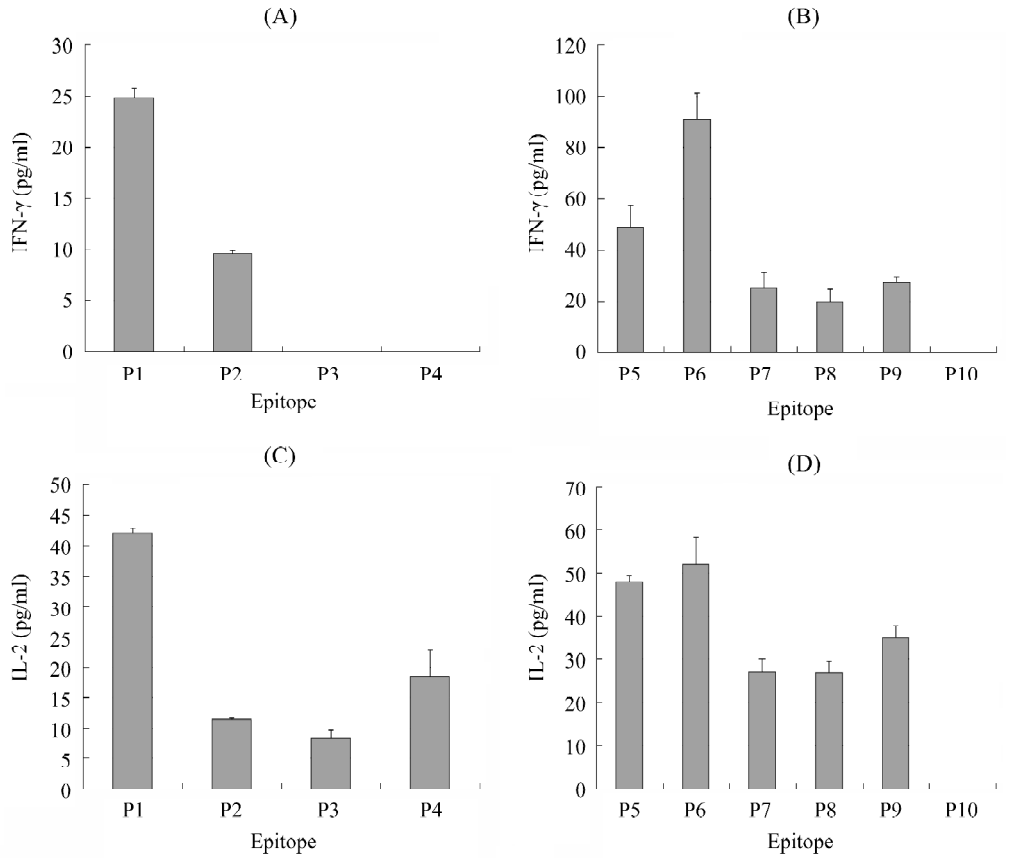

Fig. 3 Different synthetic peptide- and recombinant peptide fusion protein-induced IFN-γ and IL-2 production in primed spleen cells

Spleen cells from immunized mice were assayed for cytokine production after being pulsed with synthetic peptides and Thx-fusion proteins. The responses are given in pictograms per milliliter of IL-2 at 24 h and IFN-γ at 48 h. All assays were performed in triplicate. The mean results of a series of three independent experiments are shown. (A,B) The IFN-γ level induced by synthetic peptides and Thx-fusion proteins. (C,D) The IL-2 level induced by synthetic peptides and Thx-fusion proteins.

Table 3 Results of flow cytometric analysis of the proportion of $CD4^+$, IFN-γ- and IL-4-producing cells in spleen cells

Candidate peptide	Cell proportion			IFN-γ- vs. IL-4-secreting cells
	$CD4^+$	IFN-γ-secreting	IL-4-secreting	
P1	18.30%	19.41%	16.44%	1.18:1.00
P2	22.46%	16.89%	23.11%	1.00:1.37
P3	8.89%	9.56%	13.99%	1.00:1.47
P4	61.05%	7.47%	19.50%	1.00:2.60
P5	29.97%	23.46%	17.20%	1.36:1.00
P6	38.90%	30.14%	22.91%	1.31:1.00
P7	42.96%	26.63%	19.85%	1.34:1.00
P8	26.28%	6.77%	6.51%	1.03:1.00

animal models with schistosome infection, it has been observed that IFN-γ can suppress granuloma formation *in vivo*, and decrease the size of pulmonary granulomas and the extent of hepatic fibrosis [27,28]. The Th1-type epitopes of Sm28GST, which was recommended as a vaccine candidate by the World Health Organization, and others, such as a major egg antigen SmP38 [29,30], have been studied widely. However, only a small number of *S. japonicum* antigens were studied for Th1-type epitope identification. Liu *et al.* selected and identified a Th1-type

epitope of 12 aa from *S. japonicum* egg antigen, which can effectively stimulate lymphocytes to secrete high levels of IFN-γ and IL-2 [31]. The 28 kDa glutathione-S-transferase is an anti-oxidation enzyme in *Schistosoma*, and the monoclonal antibody against the molecule was related to anti-egg viability [32]. Shi *et al.* immunized sheep with recombinant Sj28GST molecules, which gave the immunized animals partial protection against the parasite infection, with significant egg reduction in dejecta, liver and intestines. However, the Th1-type epitope from Sj28GST remains to be elucidated.

In the present study, for the development of multiple Th1-type epitope vaccines to protect the vaccinated animals from *S. japonicum* infection, and decrease the pathology reaction elicited by this parasite, Th1-type epitopes from Sj28GST were predicted with software and identified by experiments. Nine putative candidate peptides were tested, of which P6 was found to be a dominant Th1-type epitope.

The kinds and levels of cytokines in the hosts infected with schistosomes are important markers of immune response status. The results obtained from ELISA showed that peptides P1, P5, P6, P7, P8 and P9 stimulated splenic cells to secret high levels of IFN-γ and could also make the stimulated cells release high amounts of IL-2. The proportion of IFN-γ-producing cells was higher than that of IL-4-secreting cells when driven by the peptides P1, P5, P6 and P7. This means that they induced a dominant Th1 response. Of the nine candidate peptides, P6 possessed the strongest Th1-type stimulation activity to the immunized C57BL/6 mice, demonstrating that it is an effective Th1-type epitope.

Epitopes P1, P5 and P6 share nine amino acids, and they all effectively stimulated proliferation of splenic lymphocytes and drove splenic lymphocytes to secrete IFN-γ with different levels. In our experiments, the immunological response induced by P6 was the strongest and drove the sensitized spleen cells to secrete high levels of IFN-γ while the immunological responses induced by P1 and P5 were weaker. Five amino acids (HNMMG) at the C-terminal of P6 were different from those of P1 and P5, which suggests that the five amino acids are more important for inducing the Th1-type response.

The homogeneity of both nucleotide and amino acid sequences between Sj28GST and Sm28GST are about 77% [33]. Peptide 190–211 aa from Sm28GST has been identified as an effective T cell epitope. Thus, the corresponding peptide (190–211 aa) from Sj28GST was named P9 and selected as a candidate peptide. However, the P9-induced Th1-type immune response was not as strong as that produced by peptide 190–211 aa from Sm28GST. Amino acid sequence comparison shows that seven amino acids are different between these two molecules. These seven amino acids probably contributed to the peptide P9 not showing strong Th1-type epitope activity. The predicted epitopes P7 and P8 shared some amino acids with epitope P9, but they could not induce strong Th1 polarization.

Identification of T cell epitopes by the overlapping synthetic peptide method is a common method that decreases the possibility of missed epitopes, but lots of peptides need to be synthesized, at a high cost. Computational prediction has already become a familiar and useful tool for selecting T cell epitopes from immunologically relevant proteins, as well as for the further development of information about different epitopes. Epitopes are selected by prediction with software, which saves the expense of synthetic peptides and working time. In our study, the T cell epitopes from Sj28GST were predicted by software SYFPEITHI and GUOTIF and nine peptide candidates were selected for experimental identification. The results showed that peptides P1, P5 and P6 are effective Th1-type epitopes. Thus, it is indicated that a combination of epitope prediction and experimental identification is a rapid and effective method. However, reliable epitope prediction is still only available for a limited number of organisms and alleles because almost no information is available about the corresponding peptide specificities. With the accumulation of relevant knowledge, the accuracy of prediction with software will be higher and higher.

In conclusion, the present study identified the immunodominant Th1-type epitope in the Sj28GST molecule and showed that peptide P6 of the Sj28GST antigen can prime and stimulate IFN-γ- and IL-2-producing Th1 cells. This result is highly suggestive for future development of a mini-gene vaccine consisting of multiple Th1-type epitopes in the hope of controlling schistosomiasis japonica.

Acknowledgement

We are in debt to Dr. Zhong-Li SHENG in Jiangsu Provincial Cancer Hospital (Nanjing, China) for help with the flow cytometry.

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