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Immunization of mice with cells from juvenile worms of *Schistosoma japonicum* provides immunoprotection against schistosomiasis

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To validate the protective efficacy against schistosomiasis by immunization with cells from juvenile *Schistosoma japonicum* in a murine model and to analyze possible factors related to protection, in this study, two independent repeated vaccination trials were performed. After three subcutaneous vaccinations, in trial one, in the absence of adjuvant, primary juvenile worm cells (pJCs) from *S. japonicum* induced remarkable average reductions in worm burden (54.3%), liver eggs per gram (LEPG) load (59.8%) as well as egg granulomas size (66.5%) compared to PBS control group ($P<0.01$), which were significantly higher than those elicited by fractions of juvenile worm cells (JCFs) or fractions of juvenile worms (JWFs) ($P<0.05$). Non-cell components of worms (WNCs) showed no significant protection. In trial two, compared to PBS control group, significant protective effect was also observed for cultured juvenile worm cells (cJCs) from *S. japonicum* with 58.4% worm reduction and 68.1% LEPG reduction ($P<0.01$). However, cultured adult worms cells (cACs) showed significantly higher worm burden ($P<0.05$) and egg burden ($P<0.01$) when compared to cJCs. Immunological analysis of trial two revealed that cJCs engendered a Th1-biased mixed Th1/Th2 type of immune response while cACs elicited a Th2-type response. Our data indicated that immunization with both primary and cultured cells from *S. japonicum* juvenile worms provided high immunoprotection, for which the physical character of immunogens, stage-specific parasite and the type of immune response induced might be responsible, suggesting that vaccination with whole cells from *S. japonicum* larvae is a promising approach to produce protective immunity against schistosomiasis.

Schistosoma japonicum, juvenile worm cell, cultured cell, immunoprotection, immune response

Despite intensive long-term control programmes, Schistosomiasis japonica is still an important public health problem in China and the Philippines. The development of vaccines against schistosomiasis is now recognized as a priority to complement existing control measures, most notably praziquantel-based chemotherapy[1]. So far, while offering in some cases the highest levels of protection[2], the use of irradiated cercarial vaccine in humans is not a safe strategy due to the potential of pathological implications[3]. Many other vaccine strategies have also been pursued, including the use of attenuated larvae, killed schistosomula or soluble parasite extracts[2,4−7]. Additionally, DNA vaccines and purified or recombinant parasite antigens, including several promising defined *S. japonicum* candidate vaccines (e.g.

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Sj26GST, Sj97, SjTPI, SjFABPc, Sj23 and Sj16)[8] or *S. mansoni* candidate vaccines (e.g. Sm28GST, Sm97, SmTPI, Sm23, SmFABP, IrV5)[9] have also been tested. Most of these strategies have shown some degree of effectiveness in animal models but are far from satisfactory[1,10,11]. Possible interpretations for the fact are that the most potent vaccine candidates have not yet been identified, the immune responses induced by vaccination with single antigens may not be strong enough to combat this large and complex parasite or current genetic engineering techniques fail to express the immunogenic saccharide groups of protective glycoproteins such as Sm97 and Sm38. In contrast, the live attenuated vaccines induce immune responses against many antigenic components of the parasites, several of which may contribute to vaccine efficacy[2]. These suggest that it should be important and necessary to identify new effective vaccine antigens and to develop new schistosome vaccine strategies including multivalent antigen vaccines against schistosomiasis.

Over the past decades, non-immunological whole-cell vaccines have been successfully used in immunoprophylaxis and immunotherapy of tumors[12−14] or infectious diseases[15,16]. Recently, a whole-cell *Leishmania amazonensis* vaccine plus BCG adjuvant against cutaneous leishmaniasis has been applied in clinical Phase III study[17]. Additionally, piglets immunized with a whole-cell *Cysticercus cellulosae* vaccine conferred an outstanding protective efficacy against cysticercosis cellulosae with a worm reduction of more than 90%[18]. These suggest that it is feasible to develop a whole-cell vaccine. However, to our knowledge, few literatures discussed the possibility of using schistosomal cells as an immunogen against schistosomiasis.

In recent years, our group made much progress in *in vitro* cultivation of cells from *S. japonicum* larvae and adult worms by using defined culture medium although infinitely-proliferative cell lines have not yet been established (ref. [19] and our unpublished data), encouraging us to attempt to develop a novel approach against schistosomiasis by using schistosomal whole cells as an immunogen. Our initial work has preliminarily demonstrated that immunization of mice with primary cells from *S. japonicum* larvae could elicit an efficacious protection[20]. Herein, immunoprotection against schistosomiasis in Kunming murine strain model engendered by primary and cultured cells from *S. japonicum* larvae were validated and possible related factors were analyzed. The results showed that immunization with both primary and cultured schistosomulum cells induced significant protection, for which the physical character of immunogens, stage-specific parasite and the type of immune response induced might be responsible, suggesting that vaccination with whole cells from *S. japonicum* larvae is a promising approach to produce protective immunity against schistosomiasis.

1 Materials and methods

1.1 Parasites and animals

A mainland strain of *S. japonicum* was originally maintained in *Oncomelania hupensis* snails provided by the Hunan Institute of Schistosomiasis Control (Yueyang, Hunan Province, P.R. China). Female Kunming mice, 6−8 weeks of age, and New Zealand White rabbits were purchased from Central Southern University Animal Unit (Changsha, Hunan Province, P.R. China).

1.2 Preparation of immunogens

Sufficient *S. japonicum* 12 day-old juvenile worms were recovered from infected rabbits by perfusion of mesenteric blood vessels and washed repeatedly with sterilized phosphate-buffered saline (PBS) buffer containing antibiotics (1000 IU/mL penicillin G and 1000 μg/mL streptomycin sulfate) and 10 IU/mL heparin. The worms were slightly minced on a 300 mesh screen and the fragments were filtered after addition of a small quantity of PBS. The filtered solution containing cells and other molecules was used for preparation of the following immunogens. For preparation of fractions of juvenile worms (JWFs), part of the whole filtered solution was directly homogenized followed by 6 freeze-thaw cycles in liquid nitrogen and sonication (21 kHz at 6.5-μm amplitude for 120 s) on ice when cells in filtered solution were adjusted to 2×10^7/mL with PBS. The remaining filtered solution was centrifuged for 10 min at 600×*g* and 4℃. The supernatants were collected, adjusted to 1.6 mg/mL protein nitrogen (an equivalent protein concentration to that of 2×10^7/mL of JCs) with PBS by using the BCA Protein Assay Kit (Pierce, USA), according to the manufacturer's instructions and used as non-cell components of worms (WNCs). The collected cell pellets were divided into three parts after three washes with PBS. Cells of part one were adjusted to 2×10^7/mL for direct preparation of primary juvenile worm cells (JCs)

for immunization. Cells of part two were adjusted to 2×10^7/mL for preparation of fractions of juvenile cells (JCFs) by sequence homogenation, repeated freeze-thaw cycles and sonication as previously described. For cultivation, cells of part three were adjusted to a final density of 2×10^6/mL with a defined 1640-40 culture medium which mainly contained RPMI-1640 medium (HyClone, USA), fetal bovine serum (FBS, Gibco, USA) and several kinds of cytokines (R&D, USA) which could promote schistosomal cells growth. Adult worm cells (ACs) for culture were obtained from 30-day-old adult worms using the same protocol. The JCs and ACs for culture were transferred to a 50 mL culture flask (Corning, USA) and incubated under 5% CO_2 in a humidified incubator at 37℃. The JCs and ACs were cultured (details seen in ref. [19] and our unpublished data) until the cell numbers reached 8-fold of primary cells. The viability of cells was determined by acridine orange (AO, Sigma, USA) and ethidium bromide (EB, Sigma, USA) staining. Prepared immunogens were used for the 1st immunization immediately or routinely stored in liquid nitrogen until use for the 2nd and 3rd immunizations. Before immunization, all cells were adjusted to a concentration of 2×10^7/mL with PBS. All the above manipulations were performed under aseptic conditions.

1.3 Immunization and challenge infection

Two independent vaccination trials were performed with 10 mice per group in trial 1 and 12 mice in trial 2. Each trial was repeated twice in the same way to confirm the reproducibility. The protocol followed in each trial is illustrated in Table 1. Mice were divided randomly and injected subcutaneously (s.c.) at each inguinal groove with different immunogens in 100 μL PBS (50 μL at each site) on days 0, 14, and 28. For all trials, all mice were challenged with 30±2 cercariae by abdominal skin penetration 4 weeks after the last booster.

1.4 Determination of protective effects

Six weeks post-infection, mice were sacrificed by aether inhalation and adult worms were perfused from the mesenteric veins. The recovered adult worms from each animal in each group were sexed and counted. At the end of the perfusion, the livers were removed for monitoring egg granulomas and processed for egg counts. Briefly, one gram of the liver tissue from each mouse in same group was homogenated and placed into 5 mL of 4% KOH at 37℃ on a rocking platform for 16 h and the number of eggs in three 200 μL aliquots was then counted under light microscopy and expressed as liver eggs per gram (LEPG). The worm reduction rate and the LEPG reduction rate were calculated according to the following formula: [(the average number of control group-the average number of test group)/ the average number of control group] ×100%.

In trial 1, for measurement of egg granuloma size, a fragment of ventral median lobe from each mouse liver was fixed in 10% methanol for 2 days, dehydrated in 90, 80, 70% alcohol, embedded in paraffin, and then cut into 10 μm sections, mounted on glass slides and then stained with haematoxylin-eosin for microscopic observation. The areas represented by squares under both egg granulomas and eggs were measured using Motic Images Advanced 3.2 image analysis system software (Motic China Group, Xiamen, China). Results were expressed as the average area square ($\mu m^2 \pm$ S.D.) from 50 granulomas per group of mice. A granuloma area was calculated with the formula: Granuloma area (μm^2) =

Table 1 Immunization scheme for trials 1 and 2

Group	No. of mice	Times of vaccination and dose of administration			Immunogens
		1st	2nd	3rd	
Trial 1					
Control	10	100 μL	100 μL	100 μL	PBS
WNCs	10	160 μg [a)]	160 μg [a)]	160 μg [a)]	non-cell components of worms
JWFs	10	2×10^6 [b)]	2×10^6 [b)]	2×10^6 [b)]	fractions of juvenile worms
JCFs	10	2×10^6 [c)]	2×10^6 [c)]	2×10^6 [c)]	fractions of juvenile cells
pJCs	10	2×10^6	2×10^6	2×10^6	primary juvenile worm cells
Trial 2					
Control	12	100 μL	100 μL	100 μL	PBS
cACs	12	2×10^6	2×10^6	2×10^6	cultured adult worm cells
cJCs	12	2×10^6	2×10^6	2×10^6	cultured juvenile worm cells

a) Equivalent to the protein amount of 2×10^6 pJCs; b) equivalent to the amount of 2×10^6 pJCs; c) equivalent to the amount of 2×10^6 pJCs and corresponding non-cell components.

Area of egg granuloma − Area of egg.

1.5 Measurement of specific anti-JC/AC antibodies

In trial 2, following immunization, sera of 12 mice from each vaccinated or control group were collected from the tail veins one day before the second, third immunization and challenge infection. The level of anti-JC (for JCs immunized mice and PBS control mice) or anti- AC (for ACs immunized mice and PBS control mice) IgG and subclasses IgG2a and IgG1 in the serum from each mouse were determined using indirect ELISA. Briefly, soluble juvenile worm antigens (SJWA) or soluble adult worm antigens (SAWA) were obtained from juvenile worms or adult worms by homogenization followed by sonication (21 kHz at 6.5-μm amplitude for 120 s) and centrifugation for 1 h at 10,000×*g* and 4℃. 96-well micro-plates (Nunc, Denmark) were coated with 1 μg/well of SJWA or SAWA in 0.05 mol/L carbonate-bicarbonate buffer, pH 9.6 overnight at 4℃. Plates were next blocked, washed, incubated with 100 μL of test sera diluted in PBST (1:100) at 37℃ for 2 h, and washed four times. Then 100 μL of horseradish peroxidase (HRP)-labeled goat anti-mouse total IgG (Bio-Rad, CA) diluted 1∶4000, IgG1 (Bio-Rad) diluted 1∶4000 or IgG2a (Bio-Rad) diluted 1∶5000 in PBST was added. TMB (New England Biolabs, USA) substrate incubations were carried out for 15 min at ambient temperature followed by termination of color development with 50 μL of 5% H_2SO_4. The plates were read at 490 nm in an ELISA reader (Bio-Rad, CA).

1.6 Cytokine analysis

In trial 2, splenocytes were isolated from macerated spleens of four individual mice immunized with cJCs, cACs or PBS before challenge infection and washed twice with sterile PBS. After washes, the cells, in RPMI 1640 medium (Hyclone, USA) supplemented with 10% FBS (Gibco, USA), 100 IU/mL of penicillin G sodium and 100 μg/mL of streptomycin sulfate, were divided into 1.5×10^6 cells per well. Splenocytes were maintained in culture with medium alone or stimulated with concanavalin A (ConA, Sigma, USA) (5 μg/mL) for all groups, SJWA (20 μg/mL) for JCs and PBS group or SAWA (20 μg/mL) for ACs and PBS group. The 96-well plates (Nunc) were maintained in an incubator at 37℃ with 5% CO_2. Culture supernatants were collected after 24 h of ConA stimulation, and 48 h (IL-4) or 72 h (IFN-γ) of SJWA or SAWA stimulation for cytokine analysis. The assays for measurement of IL-4 and IFN-γ were performed using an ELISA kit (Endogen, USA) according to the manufacturer's directions. The plates were read at 450 nm in an ELISA reader (Bio-Rad, CA). Cytokines were quantified based on interpolation of the standard curve. Cytokine measurement was performed twice.

1.7 Statistics analysis

Data were expressed as mean ± standard deviation and analyzed statistically by Student's *t*-test, using SPSS12.0 statistic software. The level of significance of hypothesis testing was $P<0.05$.

2 Results

2.1 Worm reduction and hepatic egg reduction

Protection studies were performed and repeated in trials 1 and 2, respectively and the results are shown in Table 2. In trial 1, the animals immunized with primary JCs without adjuvant conferred the highest levels of protection with average reductions in worm burden (54.3%) and egg counts (59.8%) ($P<0.01$) among all test groups, while WNCs showed no protection ($P>0.05$). JCFs and JWFs engendered similar partial protection levels but significantly lower than those provided by pJCs ($P<0.05$). In trial 2, mice immunized with cultured JCs produced significant decreases in worm burden (58.4%) and egg counts (68.1%) ($P<0.01$), while cACs showed significantly higher worm burden ($P<0.05$) and egg load ($P<0.01$) when compared to cJCs.

2.2 Anti-hepatic egg granuloma formation effects

Using a Motic Images Advanced 3.2 analysis software the differences in hepatic granuloma sizes among the experiment groups in trial 1 were compared. The data are shown in Table 3. The pJCs vaccinated group showed the highest mean areas reduction of hepatic egg among the test groups and significantly higher than JCFs and JWFs vaccinated groups ($P<0.05$), whereas WNCs group revealed no areas reduction of hepatic egg granulomas when compared to PBS control group ($P>0.05$). There was no significant difference in mean granuloma areas between JCFs and JWFs vaccinated groups ($P>0.05$).

2.3 Antibody responses to cJCs or cACs

In trial 2, since cJCs vaccination elicited significantly higher protective level than cACs immunization, we

Table 2 Protection levels induced by various immunogens in trial 1 and trial 2

Group	No. of mice	Worm burden recovery△ (mean ± S.D.)	Worm burden reduction▽ (%)	LEPG ($\times 10^3$)△ (mean ± S.D.)	LEPG reduction▽ (%)
Trial 1					
Contol	10,10	16.2 ± 5.9	–	19.9 ± 1.7	–
WNCs	10,9▲	14.9 ± 2.8	8.0	20.6 ± 2.8	–3.5
JWFs	9▲,10	10.6 ± 3.6 a,c)	34.6	14.2 ± 3.3 a,c)	28.6
JCFs	10,10	11.1 ± 2.2 a,c)	31.5	12.6 ± 2.3 a,c)	36.7
pJCs	10,9▲	7.4 ± 3.1 b,d,e,f)	54.3	8.0 ± 3.6 b,d,e,f)	59.8
Trial 2					
Control	8,8	20.2 ± 7.9	–	26.0 ± 3.7	–
cACs	8,8	15.5 ± 6.3 a)	23.3	27.2 ± 2.4	–4.6
cJCs	8,8	8.4 ± 3.2 b,g)	58.4	8.3 ± 2.5 b,h)	68.1

▲, One mouse died before being killed; △, mean of two repeated trials, mean ± S.D; ▽, worm burden or LEPG reduction compared to PBS control. a) vs. PBS control (trial 1 or trial 2), $P<0.05$; b) vs. PBS control (trial 1 or trial 2), $P<0.01$; c) vs. WNCs, $P<0.05$; d) vs. WNCs, $P<0.01$; e) vs. JWFs, $P<0.05$; f) vs. JCFs $P<0.05$; g) vs. cACs, $P<0.05$; h) vs. cACs, $P<0.01$. Control: PBS; WNCs: non-cell components of worms; JWFs: fractions of juvenile worms; JCFs: fractions of juvenile worm cells; pJCs: primary juvenile worm cells; cACs: cultured adult worm cells; cJCs: cultured juvenile worm cells.

Table 3 Mean areas of granulomas on liver slides from animals in trial 1

Group	Mean areas of granulomas△ (μm^2)	Reduction▽ (%)
Contol	143826 ± 31459	–
WNCs	163902 ± 38366	−13.9
JWFs	104705 ± 33126 a,c)	27.2
JCFs	92192 ± 36499 a,c)	35.9
pJCs	48182 ± 23115 b,c,d,e)	66.5

△, Mean of two repeated trials, mean ± S.D; ▽, compared to PBS control. a) vs. PBS control, $P<0.05$; b) vs. PBS control, $P<0.01$; c) vs. WNCs, $P<0.01$; d) vs. JWFs, $P<0.05$; e) vs. JCFs, $P<0.05$. Control: PBS; WNCs: non-cell components of worms; JWFs: fractions of juvenile worms; JCFs: fractions of juvenile worm cells; pJCs: primary juvenile worm cells.

further analyzed the immune responses induced in these animals following immunization. Two trial groups showed high titers of IgG that appeared one day before the second immunization compared to control groups. The highest level of total anti-JC/AC IgG antibodies was observed in JCs group before challenge and in Acs group after the second immunization, respectively (Figure 1). The levels of subclasses IgG1 and IgG2a were also analyzed in order to determine the type of immune response induced after vaccination. Mice immunized with both cultured JCs and cultured ACs showed statistically significant higher levels of IgG1 and IgG2a compared to the control group. However, the animals immunized with JCs produced higher levels of IgG2a compared to IgG1 (IgG2a/IgG1=2.62) (Figure 2(a)), while mice immunized with ACs generated an increased level of specific IgG1 when compared to IgG2a (IgG2a/IgG1=0.41) (Figure 2(b)). The IgG2a/IgG1 profile observed revealed that JCs immunization might induce a Th1-biased mixed Th1/Th2 type of immune response while Th2 type of immune response is dominant in ACs immunized mice.

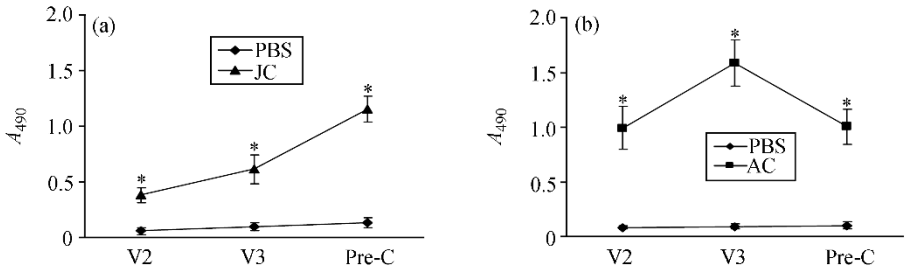

Figure 1 Kinetics of total anti-JC IgG in cJCs immunized mice or anti-AC in cJCs mmunized mice in trial 2. Sera of mice from each vaccinated or control group were collected one day before the vaccination 2 (V2), vaccination 3 (V3) and challenge infection (Pre-C) and assayed by ELISA for measurement of specific anti-JC IgG in cJCs immunized mice and PBS control mice (a) or anti-AC IgG in cACs immunized mice and PBS control mice (b). The results are presented as mean ± S.D. for each group ($n = 12$). Statistically significant difference compared to the PBS control groups is denoted by an asterisk (*) ($P<0.05$). The data are representative of two repeated trials.

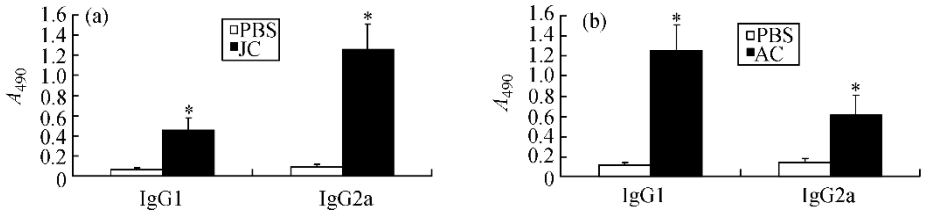

Figure 2 Levels of anti-JC IgG1 and IgG2a isotypes in cJCs immunized mice or anti-AC IgG1 and IgG2a in cACs immunized mice in trial 2. Before challenge infection, specific anti-JC IgG isotypes in cJCs immunized mice and PBS control mice (a) or anti-AC IgG isotypes in cACs immunized mice and PBS control mice (b) were determined by ELISA. The results are presented as mean ± S.D. for each group (n = 12). Statistically significant increase on isotype level of vaccinated mice compared to PBS control group is denoted by an asterisk (*) (P<0.05). The data are representative of two repeated trials.

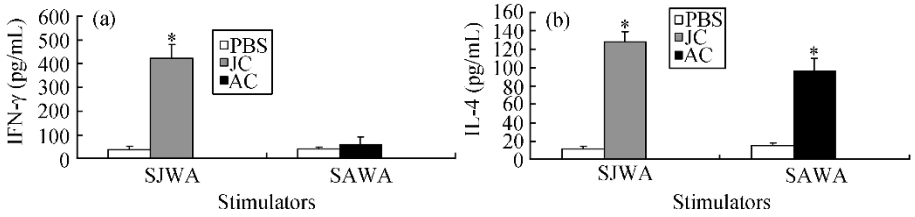

Figure 3 Cytokine profile of cJCs immunized mice or cACs immunized mice in trial 2. Before challenge infection, four mice per group were killed and splenocytes were isolated and assayed for IFN-γ (a) or IL-4 (b) production in response to ConA, medium alone (for all groups, data not shown), SJWA (for cJCs group and PBS group) or SAWA (for cACs group and PBS group). The results are presented as mean ± S.D. for each group (n = 4). Statistically significant difference compared to the PBS control groups is denoted by an asterisk (*) (P<0.05). The data are representative of two repeated trials.

2.4 Cytokine profiles produced by spleen cells of mice immunized with cJCs or cACs

In trial 2, before challenge infection, IFN-γ or IL-4 was detected in splenocyte culture supernatants from cJCs or cACs vaccinated or control mice. In cJCs vaccinated mice, statistically significant higher levels of IFN-γ or IL-4 were detected compared to the control group (Figure 3(a)). In cACs vaccinated mice, statistically significant higher levels of IL-4 compared to the control group but low levels of IFN-γ were detected (Figure 3(b)).These results indicate that cJCs vaccination induces a mixed Th1/Th2 type while cACs vaccination induces a Th2 type of immune response.

3 Discussion

Although evidence has pointed to the potential immunological importance of antigens from worm tegument and excretion[21,22], there is equally undeniable evidence for the importance of concealed, cytosolic antigens[23,24]. In fact, the parasite's longevity suggests that worm tegument and excretion fail to induce a strong and fatal immune attack against schistosomes due to a highly evolved relationship with the host. Moreover, of six *S. mansoni* candidate antigens, only one (Sm23) has since been shown to be truly exposed on the surface of labeled worms[25]. Therefore, schistosome cells, containing complex native antigens but generally embedded under the surface membrane of worm, are expected to be capable of inducing protective immunity after exposure to immune system of host though some potential efficacious molecules are yet unknown.

In trial 1, without any adjuvant, JCFs immunization conferred partial protection similar to JWCs immunization while WNCs immunization failed to induce significant protection. It is unclear why WNCs immunization offered less protection. Our previous work has shown that WNCs could be recognized by *S. japonicum* infection sera while JCs almost not (data not shown), suggesting WNCs are mainly composed of surface membranes and worm excretory-secretory (ES) components. Therefore, one possible reason for less protection provided by WNCs is that schistosomulum surface antigens (WNCs) contain not only some protective antigens such as Sj23 but also polyose or glycoprotein antigens capable of inducing the production of blocking antibodies[26] which may weaken the effect elicited by protective antigens. Most importantly, among all test groups, pJCs elicited the highest decreases in worm burden, liver egg and egg granuloma size that were all significantly higher

than those induced by JCFs or JWFs (P<0.05), indicating that JCs immunization is protective against challenge infection as well as anti-pathology. Vaccinated with equal quality and quantity of immunogens, why did animals immunized with pJCs offer greater protection than those with JCFs? One possible explanation is that specific physical character of JCs contributes to augmenting the immune response to antigen. It is generally accepted that particulate antigens are more immunogenic than soluble antigens[27,28]. Our previous studies also revealed that primary *S. japonicum* juvenile or adult cells induced significantly higher titers of specific antibodies than corresponding fractions of cells (data not shown). As an intact cell, JCs possess physical character of particulate antigen and are more efficacious than JCFs. On the other hand, some important protective antigens in JCFs or JWFs preparation, in the absence of protease inhibitors, might be devitalized due to homogenization, repeated freeze-thaw cycles and sonication. For example, Sm97 (paramyosin) is not stable enough to easily breakdown into its two major degradation products of 95 and 78 kDa[29].

To further evaluate the protective efficacy induced by cultured JCs and whether the level of protection is related to stage-specific antigens from schistosoma cells, trial 2 was carried out. The results showed that, just like primary JCs, cultured JCs also induced similarly high reductions in worm burdens and egg burdens while cACs showed significantly higher worm burden (P<0.05) and egg load (P<0.01) when compared to cJCs, which suggests that the level of protection is related to stage-specific antigens from schistosoma cells. With the same physical character, why did JCs induce significantly greater protection than ACs? One possible interpretation for this is that specific antigens to 12-day larvae cells are capable of stimulating higher protective immune responses. Previous studies have demonstrated that *ex vivo* lung-stage larvae, delivered to mice intradermally, can stimulate high levels of protective immunity[30]. Moreover, antigens from lung-stage larvae of *Schistosoma mansoni* are potent inducers of Th1-cell responses[31]. Additionally, death of the irradiated cercaria at the lung-stage of migration indicates that antigens unique to the adult worm (e.g. molecules associated with bloodfeeding or oviposition) can be discounted as important inducers of protection[32]. Because lung schistosomula have a relatively small biomass and are not easily recovered from the host, in this study, in order to get more cells for immunization and cultivation we used cells from 12-day old liver-stage (very closed to lung-stage) schistosomula and demonstrated that a property of the cells from the liver-stage also has the ability to engender a protective immune response. Finally, in trial 2, cACs immunization engendered 23.3% of worm reduction but no egg reduction (−4.6%). This phenomenon could be related to the possible raise of fecundity in the parasite population when they are less numerous[33].

Another possibility to higher protection induced by JCs may be related to the type of immune response induced. In the attenuated cercaria vaccine model, IFN-γ is involved in the protective response induced by immunization[34]. However, more data from vaccination of B cell deficient mice[35] and double-cytokine knockout mice[36] suggest that both humoral and cellular immune responses are involved in the protective immunity. Thus, any candidate vaccine for schistosomiasis should activate cell-mediated and humoral immune effector arms[36]. Generally, in a murine model, IFN-γ and IgG2a are the characteristic of cellular immune response (Th1 type), while IgG1 and IL-4 are indicative of humoral immune response (Th2 type)[37]. In the present study, after three immunizations, mice immunized with pJCs induced the significant production of anti-JC total antibodies IgG, IgG1 and IgG2a subclass compared to those immunized with PBS, with higher levels of specific IgG2a than IgG1 (Ig2a/IgG1=2.62), conjunction with significant synthesis of both IFN-γ and IL-4, which is indicative of a Th1-biased mixed Th1/Th2 type of immune response. However, mice vaccinated with ACs revealed statistically significant production of anti-AC total antibodies IgG, IgG1 and IgG2a subclass (Ig2a/IgG1=0.41), high levels of IL-4 but low levels of IFN-γ, suggesting that Th2 type of immune response is dominant in ACs immunized mice. The different type of immune response in JCs and ACs immunized mice might account for the result that JCs elicited higher protection than ACs. The reason why JC antigens induce a Th1-bias mixed Th1/Th2 response while AC antigens elicit a Th2 response is unclear. Differences in their biochemical composition (e.g. degree of glycosylation) may cause differential activation of APCs and accessory cells (e.g. macrophages, dendritic cells, neutrophils, natural killer (NK) cells, mast cells and γδ-T cells) leading to the re-

lease of various cytokines. The local cytokine milieu is particularly important for the development of one Th subset over another[38].

In summary, this study revealed a high level of protection against schistosomiasis by using schistosomal larvae cells as an immunogen in a murine model. This animal model against schistosomiasis may establish a foundation for discovery of new candidate antigens by screening cDNA or phage peptide library and elucidation of immune effector mechanisms against schistosomiasis. Practical application of this cell vaccine for human and/or animals is promising and maybe mostly depends on the establishment of a continuous schistosome cell line. Our present work is to further optimize the culture condition, to discover specific marker molecules on the surface of schistosome cells for separation and culture of cells, and to establish continuous schistosome cell lines by introduction of immortalization gene into cells or development of the cultivation of schistosome stem cells. Moreover, it is necessary to apply a proper adjuvant and optimize the immunization delivery for achievement of higher levels of protection.

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