A Microtus fortis protein, serum albumin, is a novel inhibitor of Schistosoma japonicum schistosomula

Rong Li1, Guo-Jun Wu1, De-Hui Xiong1, Qiang Gong1, Ruan-Jing Yu2, Wei-Xin Hui1/+  
1Molecular Biology Research Centre, School of Life Sciences 2Lab Animal Centre,  
Xiangya School of Medicine, Central South University, Changsha, Hunan, China

Schistosomiasis is an endemic parasite disease and praziquantel is the only drug currently in use to control this disease. Experimental and epidemiological evidence strongly suggests that Microtus fortis (MF) is a naturally resistant vertebrate host of Schistosoma japonicum. In the present study, we found that MF serum albumin (MF-albumin) and the conditioned medium of pCMN3.1-MF-albumin caused 46.2% and 38.7% schistosomula death rates in 96 h, respectively, which were significantly higher than that of the negative control (p < 0.05). We also found that mice injected with MF-albumin had a 43.5% reduction in worm burden and a 48.1% reduction in liver eggs per gram (p < 0.05) in comparison to the control animals. To characterise the mechanisms involved in clearance, schistosomula were incubated with fluorescein isothiocyanate-labelled MF-albumin and fluorescent enrichment effects were found in the gut lumen of schistosomula after 48 h of incubation. Next, digestive tract excretions from schistosomula were collected and the sensitivity of MF-albumin to digestive tract excretions was evaluated. The results indicated that schistosomula digestive tract excretions showed indigestibility of MF-albumin. The death of schistosomula could be partially attributed to the lack of digestion of MF-albumin by digestive tract excretions during the development of the schistosomula stage. Therefore, these data indicate the potential of MF-albumin as one of the major selective forces for schistosomiasis.

Key words: Schistosoma japonicum - Microtus fortis - serum albumin

Various efforts have been undertaken to enhance the effectiveness of anti-schistosome treatment, usually involving the participation of an antibody or the expression of cytokine genes or hormone-like proteins. Recent experimental evidence has suggested that the hormonal microenvironment within the host can influence not only the course of parasitic infection by modulating the immune system, but it can also be exploited to favour the establishment, growth and reproduction of the parasites (Escobedo et al. 2005). M. fortis has been shown to be a non-permissive host of S. japonicum in a complex host-parasite evolutionary relationship (Li et al. 1965). However, the anti-schistosome mechanism of M. fortis remains under investigation. He et al. (1999) discovered in vitro a specific innate anti-schistosome antibody from M. fortis. Additionally, studies from other labs reported that mice that received M. fortis serum by passive transfer could achieve protection against S. japonicum (Jiang et al. 2004). It was hypothesised that the anti-schistosome characteristics of M. fortis were due to an unknown, but heritable material. Previous studies from our lab found that the anti-schistosomula effects of M. fortis serum were stronger than those of the homogenate supernatants of the heart, liver, lung, spleen, kidney or muscle (Shen et al. 2002, Qin et al. 2004, Gong et al. 2010, Cheng et al. 2011). Thus, we focused on M. fortis serum, as it has been shown to contain a potential anti-schistosome substance. Additionally, to shed greater light on the anti-schistosome mechanism of M. fortis, we isolated and purified M. fortis serum proteins by Blue Sepharose chromatography to test their anti-schistosomula effects.
MATERIALS AND METHODS

Parasites and animals - Oncomelania hupensis snails infected with S. japonicum were purchased from the Jiangsu Institute of Parasitic Diseases, China. M. fortis in closed colonies, BALB/c mice weighing 18-20 g each and New Zealand white rabbits weighing 1-2 kg each were provided by the Lab Animal Centre of Central South University. The animal experiments were performed according to the protocols approved by the National Animal Care and Use Committee of China.

Serum preparation - Blood samples from M. fortis and mice were collected using an eye-detachment technique, while the New Zealand white rabbit blood samples were collected via heart puncture. The sera were separated by centrifugation and the sera of the rabbits and mice were then filtered (MILLEX® GP Filter Unit, 0.22 μm, Millipore). All of the sera were stored at -70°C until further use.

Schistosomula culture in vitro - Cercariae were collected from infected O. hupensis snails as described previously (Salafsky et al. 1988). Cercariae were washed four times with Dulbecco’s modified Eagle’s medium (DMEM) (Gibco, USA) containing 300 U/mL of penicillin G sodium and 300 μg/mL of streptomycin. After centrifugation at 322 x g for 10 min, schistosomula were transferred from cercariae by culturing in DMEM containing 100 U/mL of penicillin G sodium and 100 μg/mL of streptomycin at 37°C, 5% CO₂ with 25% New Zealand white rabbit sera for 24-48 h.

Schistosomula were next cultured in 48-well cell plates with 100 ± 20 schistosomula per well at 37°C and 5% CO₂. The viability of the incubated schistosomula was assessed directly with a light microscope (Olympus Co, Tokyo, Japan). Dead schistosomula showed a loss of motility, an increase in opacity and occasionally gross morphological changes in shape or obvious lesions on the tegument. Furthermore, there was no motion during a continuous 30 s observation. The viability assay was ensured by methylene blue (MB) uptake testing after observation with the unstained schistosomula considered dead and the stained ones alive (Gold & Flescher 2000).

Screening of anti-schistosomula-associated protein - To obtain an effective schistosomula protein from M. fortis serum, molecular sieve chromatography was performed with a Sephadex G50 column. Chromatographic separations were performed by high-performance liquid chromatography (HPLC) (Waters Company, USA). To confirm the anti-schistosome activity of HPLC separations further, eluted peaks were pooled, desalted and lyophilised after the separations were gradient-eluted with 50-80% methanol and was dialysed overnight against phosphate buffered saline (PBS) using Dalton membranes (8,000-14,000). After centrifugation at 8,050 g for 10 min, the supernatant was applied to the Blue Sepharose FF affinity chromatography column (4.8 cm x 40 cm) (Pharmacia) at a flow rate of 2 mL/min. The column was eluted with solution A, followed by solution B (0.05 M KH₂PO₄, 1.5 M KCl, pH 7.0). The elution peak was pooled and dialysed overnight at 4°C against PBS using Dalton membranes (8,000-14,000). The solution was then lyophilised, desalted and re-lyophilised as above. Finally, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis was conducted to ensure the purity of the M. fortis-albumin (Mf-albumin).

Anti-schistosomula experiment of Mf-albumin in vitro - The inhibitory effects of Mf-albumin on schistosomula were detected in vitro. After the live schistosomula were counted, samples corresponding to 1.25 M of Mf-albumin were added and then incubated at 37°C. M. fortis serum (40%, final concentration) was used as the positive control and mice serum albumin was used as the negative control. Each experiment was duplicated in three wells. Schistosomula were cultivated for up to 96 h and the residual live schistosomula were counted and the mortality rate of schistosomula was calculated. The death rate of schistosomula was evaluated by the following formula: death rate % = (total number of schistosomula - live number of schistosomula)/total number of schistosomula × 100.

Preparation of the conditioned medium - Full-length Mf-albumin genes (GenBank accession AY885264) were amplified from the total RNA of M. fortis livers by reverse transcription polymerase chain reaction (PCR) using the following PCR primers: 5'-TATTGGATCCACCATGGAGGGGCTTGTTTCGCCGAGA-3' and 5'-TTATCTTCGAGACAAACAGTTTGGACCCCTGCTAG-3' for Mf-albumin and 5'-TATTGGATCCACCATGAGGGGTGGTTTCCGCCAGA-3' and 5'-TATTCTCGAGAAGCAAAGTTTGGACCCCTGCTAG-3' for mouse serum albumin (Ms-albumin). The generated DNA fragment was cloned into plasmid pcDNA3.1 (+) and was transferred into the Escherichia coli strain DH5α. The recombinant plasmid (pcDNA3.1-albumin) was sequenced and transiently transfected into 293T cells using Lipofectamine™ 2000 (Invitrogen, USA). Twelve hours after DNA transfection, the cells were cultured with 2 mL of serum-free DMEM. Supernatant (conditioned medium) from each pool was collected 36 h later and was tested at a concentration of 50% for its anti-schistosomula potential.

Protective experiment in mice with Mf-albumin - Thirty-six-week-old male BALB/c mice were infected with 40 S. japonicum cercariae percutaneously and were randomly distributed into three groups containing 10 mice each: one blank PBS group, one Ms-albumin control group and one Mf-albumin test group. On the first day, the blank group was administered 0.3 mL of PBS, the control group was administered 0.3 mL of Ms-albumin and the test group with 0.3 mL Mf-albumin, all via intravenous injection. On the third and sixth days after the first injection, two other injections were administered.
using the same doses as above. All of the mice were sacrificed on day 42 by cervical dislocation. *S. japonicum* adult worms in the hepatic and portomesenteric veins were recovered and counted. The number of eggs in the liver was estimated after the tissue was digested with 4% KOH. The worm burdens and liver eggs per gram (LEPG) were calculated. The percentage changes in worm and egg burden were evaluated by the following formula: % change = (mean number in infected controls - mean number in infected, treated mice)/mean number in infected control × 100.

**Isolation of digestive tract excretions from schistosomula and adult worms** - After schistosomula were harvested and washed three times with distilled water, they were placed in distilled water at room temperature for 20 min until the water became turbid with dark granular material from the parasites' digestive tracts. These excretions were collected, adjusted to a pH of 4.9 with 0.1 M citrate and then centrifuged at 8,050 g for 10 min, after which the supernatant was collected, lyophilised, sealed and kept at -70°C until further use (Chappell & Dresden 1986). Digestive tract excretions of adult worms were collected using the same method. Immediately before use, the powder was dissolved with PBS and the protein content was measured using bovine serum albumin as a standard (Lowry et al. 1951).

**On-site detection of Mf-albumin in schistosomula** - Labelling of protein was performed with fluorescein isothiocyanate (FITC) according to a conventional protocol. FITC-Mf-albumin was added to the schistosomula cultures and was incubated for 24 h and 48 h in vitro. FITC-labelled Ms-albumin, fluorescein (30 μg/mL) and schistosomula without treatment were used as controls. To ascertain the site of fluorescence, schistosomula were collected after 24 h and 48 h of incubation, washed with 0.01 M PBS (pH 7.0) three times and then were observed under an Olympus fluorescence microscope.

**Albumin digestion with digestive tract excretions in vitro** - Samples of 5 μg/μL Mf-albumin and digestive tract excretions were incubated for 0 h, 1 h, 2 h, 4 h, 8 h, 16 h and 24 h, respectively, at 37°C in citrate saline buffer solution, pH 4.0, in the presence of 20 mM cysteine. After digestion, the reaction was terminated by 100 μL of 0.5 M Tris buffer (pH 6.8, 10% SDS). Twenty microlitres of digestion products and 10 μL of loading buffer were run on 10% SDS-PAGE.

**Statistical analysis** - The differences between groups are presented as means ± standard deviation by one-way ANOVA, using SPSS statistical software version 13.0. A p-value < 0.05 was considered a significant difference.

**RESULTS**

**Schistosomula culture in vitro** - The tails of cercariae were detached in DMEM medium containing 25% fresh New Zealand white rabbit serum for 24-48 h and the transformation rate remained greater than 90% with a less than 10% blank mortality rate. Under light microscopy, the live schistosomula were very vivacious with semitransparent bodies, crawling unhindered on the bottom of culture plate, whereas the dead schistosomula showed ankylosis and immobility, increased opacity and obvious lesions on the tegument and they did not display MB staining (Fig. 1).

**Identification of the protein associated with the inhibitory effects on schistosomula** - The protein components of *M. fortis* serum were isolated by means of molecular sieve chromatography using a Sephadex G50 column and HPLC and the protein that induced resistance to schistosomula was acquired. The amino-terminal end was identified as DAHKSEIAHR by protein sequencing and BLAST showed that it had 85% homology with mouse albumin and was named *Mf*-albumin. At a protein concentration of 1 mg/mL, it induced a schistosomula mortality rate of 36.9% ($\chi^2 = 3.8594$, p < 0.05) in vitro, significantly greater than that of the negative control.

**Albumin purification** - Blue Sepharose FF was used to purify *M. fortis* and *Ms*-albumin. There were two main peaks in the Blue Sepharose FF affinity chromatography (data not shown). The albumin only existed at the second peak, theoretically. Thus, it was accumulated.

![Fig. 1: micrographic images of schistosomula: dead schistosomula showed an increased opacity and obvious lesions in the tegumental outer membrane. The live schistosomula took up the dye and stained into blue-black with orbicular tegumental outer membrane, whereas the dead ones have not taken up the dye and unstained. A: schistosomula cultured in Dulbecco’s modified Eagle’s medium (DMEM) without methylene blue; B: live (stained) and dead (unstained) schistosomula after introduction of methylene blue into the DMEM.](image-url)
and dialysed thoroughly with distilled water and then was placed in a -80°C ultra-low temperature freezer for at least 4 h. The purity of the albumin was detected by SDS-PAGE and the result showed a single monomeric protein migration at approximately 66 kDa (Fig. 2). The purified Mf serum albumin was subjected to N-terminal amino acid sequencing for further verification.

Inhibitory effects of Mf-albumin on schistosomula in vitro - To confirm the anti-schistosomula effects of Mf-albumin, Mf-albumin was added to cultured schistosomula at final concentrations of 0.31, 0.62, 1.25, 2.5, 5.0, 10.0 and 20.0 mg/mL, with Ms-albumin as a negative control and M. fortis serum as a positive control. We observed and calculated the death rate of schistosomula after 96 h of incubation. In addition to the morphological changes of the dead schistosomula previously described, we also observed that there were one or two large blebs on the bodies of schistosomula during the period of Mf-albumin treatment. The same change was observed in the positive control group, but no change was seen in the negative control group. Most of the schistosomula in the negative control group were vivacious with semi-transparent bodies and we observed them to be crawling on the bottom of the cell plate (Fig. 3). The results of the morphological observations and schistosomula death rate data showed that Mf-albumin had obvious inhibitory activity on schistosomula in vitro. Our results showed that when the concentration of Mf-albumin was 1.25 mg/mL, the death rate of schistosomula was 46.2% ($\chi^2 = 8.4703$, p < 0.05), significantly greater than that of the negative control. The histogram of the mortality rate for each group is presented in Fig. 4.

Effects of conditioned media on schistosomula - To verify further the anti-schistosome effects of Mf-albumin, conditioned medium containing Mf-albumin or Ms-albumin was added to schistosomula culture at a concentration of 50%. We observed and calculated the death rate of schistosomula at 96 h. Our results showed the average schistosomula-killing rate of pcDNA3.1/Mf-albumin conditioned medium was 38.7%, which was significantly higher than that of the negative control (Fig. 5).

Anti-schistosome effects of Mf-albumin in vivo - BALB/c mice were randomly divided into three groups and were infected with S. japonicum cercariae. Adult worms were quantified in these mice after heart perfusion 42 d after infection. The resulting worm burden and number of LEPG in BALB/c mice treated with Mf-albu-

---

**Fig. 2**: Sodium dodecyl sulfate polyacrylamide gel electrophoresis of Microtus fortis-albumin purified with Blue Sepharose FF affinity chromatography. Lane M: broad range protein molecular weight marker; 1: M. fortis serum albumin purified.

**Fig. 3**: Micrographic images of schistosomula cultured in 96 h: dead schistosomula showed an increased opacity and obvious lesions in the tegumental outer membrane. Sometimes the blebs appeared in their tegumental outer membrane (arrowed). A: schistosomula treated with 5 mg/mL Microtus fortis (Mf)-albumin; B: schistosomula treated with 10 mg/mL Mf-albumin; C: schistosomula treated with represented 40% M. fortis serum; D: schistosomula treated with 10 mg/mL mouse serum albumin.

**Fig. 4**: Inhibitory effect of Microtus fortis-albumin on schistosomula in vitro. This diagram showed that when the target protein concentration was equal or greater than 1.25 mg/mL, significant difference of schistosomula mortality rate was observed when compared with negative control and the dead rate of schistosomula raised markedly along with the rise of albumin concentration (asterisk means p < 0.05, compare with negative control). Results represented mean ± standard deviation for triplicates. N: negative control; P: positive control.
min and Ms-albumin are presented in Table I. BALB/c mice administered Mf-albumin (15 mg/animal) showed a significant worm burden reduction of 43.5% (p < 0.05) compared with Ms-albumin administration. Moreover, we also observed a highly significant reduction in LEPG in the Mf-albumin treated group, which had a 48.1% (p < 0.05) egg reduction. Additionally, there were no differences in the numbers of egg granuloma observed between the controls (data not shown). All of these results showed that Mf-albumin possessed significant inhibitory activity on S. japonicum in vivo.

Detection of the deposit site of Mf-albumin in schistosomula - Schistosomula culture was incubated with FITC-labelled proteins or other control proteins. The results, provided in Fig. 6, clearly showed that a fluores-

![Fig. 5: inhibitory effect of pcDNA3.1/Microtus fortis conditioned medium on schistosomula after 96 h of incubation. A: schistosomula cultured with control pcDNA3.1/mouse serum albumin (Ms-albumin) conditioned medium; B: schistosomula cultured with pcDNA3.1/M. fortis-albumin (Mf-albumin) conditioned medium; C: schistosomula cultured with pcDNA3.1/Amp conditioned medium (negative control); D: schistosomula cultured with 20% M. fortis sera (positive control) (more dead schistosomula were observed in Mf-albumin group than the negative control group in 96 h); E: histogram of schistosomula mortality rate after incubation with conditioned medium (p < 0.05 compared with negative control). Results represented mean ± standard deviation for triplicates.](image)

![Fig. 6: on-site of Microtus fortis-albumin (Mf-albumin) in schistosomula (24 h and 48 h post-treated). A1: treated with fluorescein isothiocyanate (FITC)-labelled Mf-albumin after 24 h incubation; A2: treated with FITC-labelled Mf-albumin after 48 h incubation; B1: treated with FITC-labelled mouse serum albumin (Ms-albumin) after 24 h incubation; B2: treated with FITC-labelled Ms-albumin after 48 h incubation; C1: fluorescein control after 24 h incubation; C2: fluorescein control after 48 h incubation; D1: blank control after 24 h incubation; D2: blank control after 48 h incubation.](image)

### TABLE

| Groups   | Worm burden (mean ± SE) | Worms reduction rate (%) | LEPG (mean ± SE) | Egg reduction rate (%) | p   |
|----------|-------------------------|--------------------------|------------------|------------------------|-----|
| PBS      | 24.9 ± 4.8              | -                        | 59.543 ± 6.887   | -                      | > 0.05 |
| Ms-albumin | 24.6 ± 4.8            | -                        | 59.510 ± 6.256   | -                      | > 0.05 |
| Mf-albumin | 13.9 ± 2.6             | 43.5                     | 30.900 ± 3.298   | 48.1                   | < 0.05 |

Worm burden and egg reduction rate were calculated relative to phosphate buffered saline (PBS) control. Worm burden and liver eggs per gram (LEPG) between PBS and mouse serum albumin (Ms-albumin) groups were not statistically different (p > 0.05). Worm burden and LEPG of Mf-albumin group were statistically different from those of PBS and Ms-albumin groups (p < 0.05). SE: standard error.
cent enrichment effect could be found in the gut lumen of schistosomula after incubation with FITC-Mf-albumin for 24 h. After 48 h of incubation, the fluorescent enrichment effect became even stronger in the gut of schistosomula by fluorescence microscopy. However, there was no distinct fluorescence enrichment in schistosomula that were treated with the FITC-labelled Ms-albumin, fluorescence and the blank control.

Digestion of Mf-albumin by digestive tract excretions from schistosomula - Digestive tract excretions obtained from schistosomula were used to evaluate the ability of the digestion processes of M. fortis and mice albumin. The digestion of the potentially digestible fraction could be detected by SDS-PAGE. The results showed that evident digestion could be observed when Ms-albumin was incubated with digestive tract excretions of schistosomula for only 2 h, but no evident digestion of Mf-albumin was detected until co-incubation for 24 h (Fig. 7).

DISCUSSION

Schistosomiasis is a fairly prevalent communicable disease in the tropics and subtropics. It is well known that M. fortis possesses natural resistance to S. japonicum and this characteristic could be steadily inherited. Host resistance to parasites can be behavioural, physiological, mechanical or immunological. In addition, there can be variations in host resistance and parasite infectivity within a species or across different species (Schwarzzenbach et al. 2005). This resistance can be based on the genetic makeup of the animals. However, the degree of resistance, which can be a plastic response to environmental variation in the natural population, is not well understood (Nagel et al. 2010). Some studies have shown that environmental variation might increase ischaemia-modified albumin concentrations, which are a form of human serum albumin (HSA) (Bar-Or et al. 2001). In addition, there has also been evidence of possible genetic variants of HSA, particularly structural changes at the N-terminus, which abolish transition metal binding (Sheat et al. 1991, Bhagavan et al. 2003, Lippi et al. 2005). Therefore, we hypothesised that a genetic difference in albumin between M. fortis and mice would result in functional disparity between them, giving Mf-albumin stronger anti-schistosome potential.

Laboratory studies have shown that M. fortis sera possessed anti-schistosome activity in vitro and in vivo. In the present investigation, we showed that extract of albumin from M. fortis sera exhibited anti-schistosomal activity both in vitro and in vivo. It is well known that serum albumin is the most abundant protein in the circulatory system and it possesses many important physiological functions that contribute to colloid osmotic blood pressure and aid in the transport, distribution and metabolism of many endogenous and exogenous substances. In addition, it was reported that albumin might play an important role in innate immunity (Fassi et al. 1993, Brummer et al. 1999, Ahluwalia et al. 2001). Giles and Czuprynski (2003) showed that the albumin from several mammalian species had obvious inhibitory activity against Blastomyces dermatitidis, while albumin from rat serum failed to inhibit B. dermatitidis growth in vivo. Takami et al. (1992) indicated that maleylated HSA inhibited human immunodeficiency virus-1 infection and syncytia formation via binding to target cells with high affinity in vitro. Milojetic et al. (2009) found that HSA could inhibit Aβ fibrillation through a "monomer-competitor" mechanism by nuclear magnetic resonance and intrinsic albumin fluorescence experiments. Recently, a series of studies suggested that fluid resuscitation with albumin was associated with higher mortality in patients (Schierhout & Roberts 1998, Vincent et al. 2005, Myburgh et al. 2007, Wheeler et al. 2011). All of these data indicated that, apart from the basic functions, albumin possesses an immunological effect related to the innate immunity of living things.

M. fortis is a non-permissive host, the serum of which has been found to possess innate resistance to S. japonicum in vitro and in vivo. In this study, we isolated and purified M. fortis albumin. The inhibitory activity of the albumin to schistosomula was examined in vitro and in vivo. Schistosomula culture treated with 1.25 mg/mL of Mf-albumin caused a significantly higher schistosomula death rate when compared to the negative controls. The mortality rate of schistosomula approached 100% when the Mf-albumin concentration was increased to 10 mg/mL. Mf-albumin was administered to mice infected with cercariae by intravenous injection through the tail vein. After perfusion of the mice 42 d after infection, we found that mice injected with Mf-albumin had 43.5% worm burden reduction and 48.1% LEPG reduction compared with the control group. All of the results showed that Mf-albumin possessed significant inhibitory activity against schistosomula.

Until now, the possible mechanism of Mf-albumin anti-schistosome could be the only non-permissive host of schistosome among mammalians in China (Li et al. 1965). When infected, the growth and development of S. japonicum are hindered and no adult worms and eggs could be found in both wild and laboratory-bred M. fortis.
(He et al. 1995). In this study, FITC-labelled Mf-albumin accumulated in the gut lumen of schistosomula after incubation for 24 h and 48 h. Thus, Mf-albumin must be transported intact and be metabolised slowly by schistosomula. However, little is known about the mechanisms of protein transport in schistosomula. We speculated that the anti-schistosomal activity would be accompanied by a series of proteins involved in signal transduction and protein trafficking; these proteins might activate or strengthen the immune system and induce anti-schistosome effects during different stages of the schistosome life cycle in the vertebrate host. Recent experimental evidence has shown that hormonal-like proteins can regulate a variety of cellular and physiological functions of schistosomula in establishment, growth and reproduction, such as adrenal steroid hormones and testosterone (Barrabes et al. 1986, Fallon et al. 1998, Morales-Montor et al. 2001, Remoué et al. 2002). In combination with the results of FITC-labelled Mf-albumin accumulated in the intestines of schistosomula, it is possible that Mf-albumin could influence the microenvironment or be ingested by the digestive tract excretions of schistosomula. Thus, we presumed Mf-albumin might play an important role in resistance to schistosome, by acting as a kind of a stressor.

To explore further possible mechanisms of Mf-albumin against S. japonicum, we collected digestive tract excretions of schistosomula to test their digestion differences from M. fortis and mice serum albumin in vitro. The results showed that Mf-albumin was more difficult to be digested by the digestive tract excretions of schistosomula, compared with Ms-albumin. When infected, the growth and development of S. japonicum were hindered and no adult worms or eggs could be found in either wild or laboratory-bred M. fortis (He et al. 1995). Therefore, we presumed that Mf-albumin might be an important factor of the anti-schistosomula effects of M. fortis during S. japonicum development. In combination with the results of FITC-labelled Mf-albumin accumulated in the intestines of S. japonicum, it also provided insight that digestive tract excretions could be used for rapid immunodiagnosis, even during early stages of infection.

It is worthwhile to point out that when we studied the inhibitory effects of Mf-albumin on schistosomula in vitro, we observed some blebs appearing in the tegumental outer membrane. The appearance of these blebs was reported and was suggested to be the result of the action of complement (McLaren et al. 1978). However, in our in vitro experimental system, there was no complement or the complement was inactivated. Thus, we suggest that the presence of Mf-albumin created an environment that was not suitable for the survival of schistosomula because of the obvious morphological changes to the tegument of the schistosomula.

In summary, we screened proteins of M. fortis serum by molecular sieve chromatography, HPLC and Blue Sepharose FF and identified Mf-albumin as having anti-schistosomula effects, both in vitro and in vivo. We preliminarily explored the possible mechanism of Mf-albumin anti-schistosomula and concluded that Mf-albumin was an effective protein against schistosome.

ACKNOWLEDGEMENTS
To Saiqun Luo, Qingren Zeng, Shunke Zhang, Jingjing Chu and Gang Cheng, for providing technical assistance and suggestions.

REFERENCES
Ahlulwalia M, Brummer E, Sridhar S, Singh R, Stevens DA 2001. Isolation and characterization of an anticytotoxic protein in human cerebrospinal fluid. J Med Microbiol 50: 83-89.
Bar-Or D, Rael LT, Lau EP, Rao NK, Thomas GW, Winkler JV, Yukl RL, Kingston RG, Curtis CG 2001. An analog of the human albumin N-terminus (Asp-Ala-His-Lys) prevents formation of copper-induced reactive oxygen species. Biochem Biophys Res Commun 284: 856-862.
Barrabes A, Goma-Mouanda J, Reynouard F, Combescot C 1986. 17b estradiol receptors in Schistosoma mansoni. Contribution to the explanation of the protective power of this hormone in Schistosoma mansoni bilharziasis in the mouse. Preliminary study. Ann Parasitol Hum Comp 61: 637-641.
Bhagavan NV, Lai EM, Rios PA 2003. Evaluation of human serum albumin covalent binding assay for the assessment of myocardial ischemia and myocardial infarction. Clin Chem 49: 581-585.
Brummer E, Drasin T, Adler JD 1999. Antifungal activity of cerebrospinal fluid against Cryptococcus neoformans and Candida species. Med Mycol 37: 339-344.
Chappell CL, Dresden MH 1986. Schistosoma mansoni: proteinase activity of “hemoglobinase” from the digestive tract of adult worms. Exp Parasitol 61: 160-167.
Cheng G, Gong Q, Gai N, Xiong DH, Yu YJ, Zeng QR, Hu WX 2011. Karyopherin alpha 2 (KPN2A) is associated with the natural resistance to Schistosoma japonicum infection in Microtus fortis. Biomed Pharmacother 65: 230-237.
Escobedo G, Roberts CW, Carrero JC, Morales-Montor J 2005. Parasite regulation by host hormones: an old mechanism of host exploitation? Trends Parasitol 21: 588-593.
Fallon PG, Richardson EJ, Jones FM, Dunne DW 1998. Dehydroepiandrosterone sulfate treatment of mice modulates infection with Schistosoma mansoni. Clin Diag Lab Immunol 5: 251-253.
Fassi FO, Mohanty J, Elazhary Y 1993. Bovine serum albumin inhibition of respiratory syncytial virus on MDBK cells. Vet Res 24: 488-493.
Giles S, Czuprynski C 2003. Novel role for albumin in innate immunity: serum albumin inhibits the growth of Blastomyces dermatitidis yeast form in vitro. Infect Immun 71: 6648-6652.
Gold D, Flescher E 2000. Influence of mechanical tail-detachment techniques of schistosome cercariae on the production, viability and infectivity of resultant schistosomula: a comparative study. Parasitol Res 86: 570-572.
Gong Q, Cheng G, Qin ZQ, Xiong DH, Yu YJ, Zeng QR 2010. Identification of the resistance of a novel molecule heat shock protein 90a (HSP90a) in Microtus fortis to Schistosoma japonicum infection. Acta Trop 115: 220-226.
He HB, Zuo IZ, Liu BS 1995. Comparison of infection with Schistosoma japonicum between wild and laboratory bred Microtus fortis. J Pract Parasitic Dis 3: 72-74.
He Y, Luo X, Zhang X, Yu X, Lin J, Li Y, Li Y, Liu S 1999. Immunological characteristics of natural resistance in Microtus fortis to infection with Schistosoma japonicum. Chin Med J (Engl) 112: 649-654.
He YX, Salafsky B, Ramaswancy K 2001. Host-parasite relationship of Schistosoma japonicum in mammalian hosts. Trends Parasitol 17: 320-324.

Jiang SF, Wei MX, Lin JJ, Pan CE, Li H, Cao L, He YY, Fu ZQ 2004. Study on protection against Schistosoma japonicum induced by passively transferred sera from Microtus fortis in the mouse. Chin J Parasit Dis Con 17: 298-300.

Li SK, Zhu ZL, Jin BR 1965. Uninfectibility to Schistosoma japonicum of Microtus fortis. Acta Parasitol Sinica 2: 103.

Lippi G, Brocco G, Salvagno GL, Montagnana M, Dima F, Guidi GC 2005. High-workload, endurance training may increase serum ischemia-modified albumin concentrations. Clin Chem Lab Med 43: 741-744.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ 1951. Protein measurement with the folin phenol reagent. J Biol Chem 193: 265-275.

McLaren DJ, Ramalho-Pinto FJ, Smithers SR 1978. Ultrastructural evidence for complement and antibody-dependent damage to schistosomula of Schistosoma mansoni by rat eosinophils in vitro. Parasitology 77: 313-324.

Milojevic J, Radiatis A, Melacini G 2009. Human serum albumin inhibits Aβ fibrillation through a “monomer-competitor” mechanism. Biophys J 97: 2585-2594.

Morales-Montor J, Mohamed F, Ghaleb AM, Baiq S, Hallal-Calleros C, Damian RT 2001. In vitro effects of hypothalamic-pituitary-adrenal axis (HPA) hormones on Schistosoma mansoni. J Parasitol 87: 1132-1139.

Myburgh J, Cooper DJ, Finfer S 2007. Saline or albumin for fluid resuscitation in patients with traumatic brain injury. New Engl J Med 357: 874-884.

Nagel L, Robb T, Forbes MR 2010. Inter-annual variation in prevalence and intensity of mite parasitism relates to appearance and expression of damselfly resistance. BMC Ecol 10: 5.

Qin ZQ, Hu WX, Wu GJ, Xu B, Shen QX, Gong Q, Wu C 2004. Construction of gene pool of Microtus fortis and screening resistance-associated genes to infection of Schistosoma japonicum. Life Sci Space Res 8: 333-338.

Remoué F, Mani JC, Puginière M, Schacht AM, Capron A, Riveau G 2002. Functional specific binding of testosterone to Schistosoma haematobium 28-kilodalton glutathione S-transferase. Infect Immun 70: 601-605.

Salafsky B, Fuscom AC, Whitey K, Nowicki D, Ellenberger B 1988. Schistosoma mansoni: analysis of cercarial transformation methods. Exp Parasitol 67: 116-127.

Schierhout G, Roberts I 1998. Fluid resuscitation with colloid or crystalloidal solutions in critically ill patients: a systematic review of randomised trials. BMJ 316: 961.

Schwarzenbach GA, Hosken DJ, Ward PL 2005. Sex and immunity in the yellow dung fly Scathophaga stercoraria. J Evol Biol 18: 455-463.

Shen QX, Hu WX, Xu B 2002. Killing effect of schistosomula of Schistosoma japonicum by tissues/organs of Microtus fortis in vitro. Bull Hunan Med Univ 27: 198-200.

Takami M, Sone T, Mizumoto K, Kino K, Tsunoo H 1992. Maleylated human serum albumin inhibits HIV-1 infection in vitro. Biochim Biophys Acta 1180: 180-186.

Vincent JL, Sakr YK, Reinhart CL, Sprung HG, Ranieri VM 2005. Is albumin administration in the acutely ill associated with increased mortality? Results of the SOAP study. Crit Care 9: 745-754.

Wheeler DS, Giuliano Jr JS, Lahni PM, Denenberg A, Wong HR, Zinarelli 2011. The immunomodulatory effects of albumin in vitro and in vivo. Adv Pharmacol Sci 2011: 691928.

Wu G, Zhang R, Yang YQ 1962. Laboratory infects Microtus fortis with Schistosoma japonicum. Acta Parasitol 1: 189-190.