

Gene cloning, expression, and localization of antigen 5 in the life cycle of *Echinococcus granulosus*

Yuzhe Li · Hongxu Xu · Jiajia Chen · Wenjia Gan ·
Weihua Wu · Weiping Wu · Xuchu Hu

Received: 30 June 2011 / Accepted: 6 December 2011 / Published online: 27 December 2011
© Springer-Verlag 2011

Abstract Antigen 5 (Ag5) has been identified as a dominant component of cyst fluid of *Echinococcus granulosus* and is considered as a member of serine proteases family, which in other helminth, plays an important role in the egg hatch and larva invasion. However, whether Ag5 is expressed and secreted in all life stages is unknown. In this study, according to the sequence in GenBank, we cloned and sequenced the open reading frame (ORF) of Ag5 gene from the protoscolices of *E. granulosus* isolated from the sheep in Qinhai Province of China, and found several substitutions and a base insert and deletion in a short region near the stop code, leading to a frameshift mutation which is conserved with the homologue of other cestode. The ORF is 1,455 bp in length, encoding 484 amino acids with a secretory signal peptide. Bioinformatics analysis predicted several phosphorylation and

myristoylation sites and a N-glycosylation site and a species-specific linear B epitope in the protein. The ORF was cloned into the plasmid pET28a(+) vector and expressed in *Escherichia coli*. The recombinant protein was purified by affinity chromatography. Anti-rEgAg5 antiserum was prepared in rats and used to analyze the localization of Ag5 in protoscolex and adult worm by immunofluorescence technique. Results demonstrated that the Ag5 is strongly expressed in the tegument of protoscolex and the embryonic membrane of egg and surface of oncosphere; meanwhile, it is also weakly expressed in tegument of the adult. This study showed that Ag5 is expressed in all stages of life cycle, secreted from the surface of the worm and may be anchored in membrane by its myristoylation sites; these characteristics make it a candidate antigen for diagnosis and vaccine for both intermediate and definitive hosts.

Yuzhe Li and Hongxu Xu contributed equally to this article

Y. Li · J. Chen · W. Gan · W. Wu · X. Hu (✉)
Department of Parasitology, Zhongshan School of Medicine,
Sun Yat-sen University,
Guangzhou, Guangdong, China
e-mail: huxuchu@mail.sysu.edu.cn

X. Hu
Education Ministry Key Laboratory for Tropical Disease Control
Research, Sun Yat-sen University,
Guangzhou, Guangdong, China

H. Xu
Department of Laboratory Medicine,
The First Affiliated Hospital of Sun Yat-sen University,
Guangzhou, Guangdong, China

W. Wu
National Institute of Parasitic Disease,
Chinese Center for Disease Control and Prevention,
Shanghai, China

Introduction

Cystic echinococcosis (CE) is one of the most important parasitic zoonotic diseases caused by metacestodes of the tapeworm *Echinococcus granulosus* which brings great economic losses and disease burden in endemic region (Nasrieh et al. 2003). It distributes globally, particularly highly prevalent in the northwestern regions of China, where there are about 70 million population in danger and 0.6–1.3 million existing cases of human echinococcosis with more than several billion Yuan of the annual economic loss in husbandry (Bart et al. 2006; Kia et al. 2010; Neghina et al. 2011; Raether and Hanel 2003; Varcasia et al. 2008; Wang et al. 2008). Cystic hydatid disease is an important public health problem that hampers the social development of these endemic areas.

At present, except for health education and regular helminthicide for dogs, the prevention and control of human hydatid diseases mainly depend on chemotherapy for early infected patients and surgical treatment for the later patients (Ceballos et al. 2008; Craig and Larrieu 2006; Heath et al. 2006; Jamshidi et al. 2008). The Earlier diagnosed, the easier and more effective is chemotherapy, and the less is disease burden (Urrea-Paris et al. 1999, 2002). Therefore, early diagnosis is crucial for the treatment of hydatid patients; however, the early diagnosis is still a problem because the small cyst is difficult to be confirmed by imaging diagnosis. Immunological methods give us great wish, but none is certain for diagnosis of early active infection of *E. granulosus* (Gonlugur et al. 2005; Kalantari et al. 2010; Tawfeek et al. 2011; Wen et al. 2010). The key for prevention of hydatid disease is controlling infection of the intermediate host, livestock, and the definitive host, dog, because they mainly constitute the life cycle. Regular monthly administration of anthelmintic agents for dogs in endemic area is an effective but difficult task, and the drug treatment for livestock is more difficult and seldom enforced for its high cost. Therefore, vaccine for livestock bears the wish for prevention and control of animal hydatid disease (Torgerson 2009).

No matter for immunodiagnosis and vaccine, antigen screening is the bottleneck. Therefore, at present, identifying discrete antigen that could provide more sensitive and specific serological test and protective immunity has always being the focus of the studies on *E. granulosus*. Hydatid cyst fluid (HCF) has being applied as serological diagnostic antigen for a long time; however, the HCF is a complex of multiple antigens, including some common antigens of other helminthes which produce cross-reactions or non-specific reactions (Al-Yaman and Knobloch 1989; Poretti et al. 1999; Shepherd and McManus 1987; Wellinghausen and Kern 2001).

Among the *E. granulosus* antigens, antigen 5 (Ag5) is identified as one of the predominant component of HCF and a Con A binding lipoprotein with molecular weight of approximately 67 kDa, and is composed of two subunits of 38 and 22 kDa linked by disulfide bond. Different immunological techniques have been used to purify native Ag5 and the two subunits were isolated from the HCF for application in serodiagnosis of hydatid disease (Zhang et al. 1995). The two subunits are different in immunological characteristics. In cystic echinococcosis patients, the major antibody classes against Ag5 are IgG1, IgG4, and IgE. The IgG1 is of 100% sensitivity, but only of 70% specificity; the IgG4 is less sensitive, but of more specificity; the IgE is of 100% specificity and of 70% sensitivity. The IgG1 is against 38-kDa subunit and the IgG4 and IgE is against the 20-kDa subunit. The phosphorylcholine bound to the 38-kDa

subunit has been shown to be related to part of the cross-reactivity (Khabiri et al. 2006). Thus, the prokaryotic expressed recombinant protein may have a better performance in diagnosis for it will reduce the cross-reaction for its lacking the lipid modification.

The C-terminal 38-kDa subunit is closely related to trypsin of the serine protease family (Lorenzo et al. 2003). Helminth serine protease plays an important role in the eggs hatching and larvae invasion and is a potential vaccine candidate (Dzik 2006; Young et al. 1999). However, up to date, whether Ag5 is expressed in the two infective stages—protoscolex and egg is still unknown. EgAg5 has significant homology with the Trypsin-like protein of *Taenia solium* which exists in the excretory/secretory (E/S) antigens of oncosphere of *T. solium* and shows high level of activity in serine proteases (Zimic et al. 2007); therefore, we guess EgAg5 is also expressed in oncosphere or egg of *E. granulosus*.

The aim of this study is to clarify whether EgAg5 is expressed and localized in the surface of protoscolex and egg through immunohistochemistry. This article will describe the cloning, expression, identification, and localization of EgAg5.

Material and method

EgAg5 gene amplification and sequencing

Eg Ag5 gene was amplified from mRNA extracted from *E. granulosus* protoscolexes which were isolated from sheep in Qinghai Province of China and stored in liquid nitrogen by reverse transcript polymerase chain reaction (RT-PCR) using forward primer: AAAGGATCCGGCTTG GAGCT CACTCTCG and reverse primer: GCCAAGCTTGCTA TATGGTAGCCTTTTCGG which are designed basing on the sequence in GenBank (Access No:AY052477). PCR reaction consisted of 30 cycles of 94°C for 1 min, annealing at 60°C for 1 min, and 72°C for 1 min. The purified product of PCR was cloned in pCR2.1vector and transformed into DH5 α . The recombinant positive clone was picked up and sequenced.

Bioinformatics analysis

The EgAg5 sequence was compared with other homologues in GenBank by Blastx, its physicochemical properties were predicted by the online program ProtParam (<http://www.expasy.ch/tools/protparam.html>); program Motifscan (http://myhits.isb-sib.ch/cgi-bin/motif_scan) analyzed the function domain and modification sites; their linear B cell epitopes were predicted by DNASTAR package and BepiPred 1.0 Server (<http://www.cbs.dtu.dk/services/BepiPred/>), and cytotoxic T lymph cell (CTL) epitopes by NetCTL1.2 Server

(<http://www.cbs.dtu.dk/services/NetCTL/>). The distinct linear B cell epitopes were identified to apply in immunodiagnosis.

EgAg5 cloning, expression, and purification

The fragment coding the mature peptide of EgAg5 was cut from recombinant pCR2.1 plasmid by *Bam*H I and *Hind* III double enzyme digestion and subcloned into prokaryotic expression vector pET28a(+). The recombinant plasmid was transformed into host bacteria *Escherichia coli* BL21 (DE3). EgAg5 expression in BL21 was induced with optimal IPTG concentration and temperature and time. The insoluble fraction was solubilized in 6 M urea, 500 mM NaCl, 20 mM Tris/HCl, pH 8.0. The rEgAg5 was purified by His.Bind purification kit (Novagen) under denatured conditions according to the manufacturer's protocol. Eluted protein was renatured by stepwise elution of urea and dialyzed against PBS at last and adjusted its final concentration to 0.5 mg ml⁻¹.

Preparation of anti-rEgAg5 sera and collection of sera of CE patients

SD rat was injected subcutaneously with 200 µg recombinant protein emulsified with equal volume of complete Freund's adjuvant (Sigma) and followed by two boosts with half amount of antigen which was emulsified with incomplete Freund's adjuvant (Sigma) at 2-week intervals. Two weeks after the last vaccination, serum was collected from the rat, and the EgAg5-specific antibodies were detected by the enzyme-linked immunosorbent assay (ELISA). CE-positive serum samples were collected from patients in Qinghai Province *E. granulosus* endemic area who were diagnosed with B ultrasonic image, serum samples from healthy human without parasitic infection were used as negative control.

Western blot

Protein samples were subjected to SDS-PAGE (15% polyacrylamide gel) and subsequently transferred by electroblotting onto polyvinylidene difluoride membrane (Whatman). Then, the membranes were blocked with 5% (w/v) skim milk powder in PBS (pH 7.4) at 4°C over night, and subsequently incubated with the primary antibodies (serum from the rat immunized with the recombinant EgAg5, normal rat serum, patient serum, and healthy human sera 1:200 dilution), respectively at 37°C for 2 h. After washing and incubation with rabbit anti-rat immunoglobulin G conjugated horseradish peroxidase (HRP) (1:1,000 dilution) (Boster Co.) at 37°C for 1 h, the HRP was visualized by diaminobenzidine (Boster Co.) substrate solution.

Immunolocalization of EgAg5 at adult worm

An *E. granulosus* adult was fixed with acetone after twice washing gently with normal saline and then embedded with paraffin wax and sliced into 4-µm sections. After deparaffinization, freshly prepared 1% sodium borohydride in 1% disodium hydrogen phosphate monohydrate was applied to the sections three times for 10 min to quench the auto-fluorescence. Sections were then blocked with 90% healthy sheep sera (Boster Co.) and 1% BSA in PBS at 4°C for 2 h. The sections were subsequently incubated in the rat anti-EgAg5 serum (1:100 dilution) over night. Preimmune rat serum was employed as negative control. At last, the sections were incubated in Cy3 dye-conjugated goat anti-rat IgG (H+L; 1:2,000; molecular probes) (Byotime co.) for 1 h and 15 min. The sections were observed under fluorescence microscope. All the parameters and microscope settings used were maintained throughout the process.

Immunolocalization of EgAg5 at protoscolices of *E. granulosus*

Freshly collected protoscolices were fixed in paraform overnight. Then intact samples were washed 30 min with PBS in an Eppendorf tube at room temperature. After removing the supernatant, the worms were blocked with 100 µl normal goat serum overnight at 4°C. Then the worms were washed with PBS for three times (5 min each) and incubated with anti-EgAg5 serum diluted 1:100 in 5% BSA-PBS for 2 h at room temperature. Serum from non-immunized rat was used as a negative control. After three washes with PBS, the parasites were incubated in the dark with the secondary antibody Cy3-conjugated goat anti-rat IgG (BioTime) diluted 1:2,000 in 0.1% BSA-PBS for 1 h and 15 min. They were then washed three times with PBS, transferred to a clean glass slide and examined immediately by fluorescence microscope. All the parameters and microscope settings used were maintained throughout the process.

Results

The EgAg5 sequence variation and its characteristics

The EgAg5 sequence (GenBank access No.JF970202) cloned from the *E. granulosus* isolated from sheep in endemic area in Qinghai Province was sequenced, the open read frame is of 1,455 bp in length, encoding 484 amino acids, with the calculated molecular weight of 54,874.8 Da, with theoretical pI of 6.36. The instability index (II) is computed to be 55.78, classifying the protein as unstable. The leading sequence of twenty amino acids is predicted as

signal peptide. Motifscan program predicts that there are serine protease, trypsin domain from aa₁₈₉ to aa₄₈₄ and several potential phosphorylation and myristoylation sites and a N-glycosylation site in the putative protein.

The sequence is somewhat different from that deposited in GenBank. There are several base substitutions, but only the substitution of C₁₂₂₅ to “T” changes Arg₄₀₉ into Cys₄₀₉, others belong to synonymous mutation; particularly there is a single base insertion of A₈₉₃ and deletion of C₉₃₇ which produce a partial frameshift mutation (Fig. 1). However, comparing with the homologue in *T. solium*, this region in our sequence is completely conserved, probably there are mistakes in the EgAg5 sequence deposited in GenBank. Although the EgAg5 is conservative protein in cestodes, there is no homolog found in trematodes and nematodes, only with very low homology found with some serine proteinase in other helminth. EgAg5 has rich B and T cell epitopes, and with no homologues in hosts, it may be good antigen. Although EgAg5 homologues are very conservative among genus, the mutational region from aa_{159–168} which constitutes a linear B cell epitope that may distinguish echinococcosis and taeniasis and cysticercosis (Fig. 2).

Expression and purification of recombinant protein

The mature peptide coding sequence was successfully cloned into the expression vector pET28a (+), and the recombinant protein was expressed thoroughly in the form of inclusion body that can resolve in 6 M urea solution. Purified protein shows a band between 66.2 and 45.0 kDa molecular marker in 12% SDS-PAGE (Fig. 3), which accords with the calculated molecular weight of 54.85 kDa of the recombinant protein.

Western-blot analysis

Western-blot demonstrated the combinant EgAg5 could be recognized by sera from immunized rat and patient infected with *E. granulosus*, and not by sera from healthy rat and healthy person (Fig. 4). The results indicated that the rEgAg5 has immunogenicity and antigenicity.

Immunolocalization of EgAg5 at protoscolices and adult

The analysis of immunolocalization by using rat anti-EgAg5 serum showed that EgAg5 concentrated at the embryonic

Fig. 1 The coding region of EgAg5 and its characteristics. The letters in gray represents the signal peptide. The bold underlined letters in nucleotide sequence are the substitution mutation sites; the bold italic letters is the changed amino acids. Letter with shadow shows the inserted nucleotide “A”; letters with frame represent a site with a deletion “C” between the two nucleotides; the amino acid sequences in frame represent linear B cell epitopes and those underlined are T cell epitopes

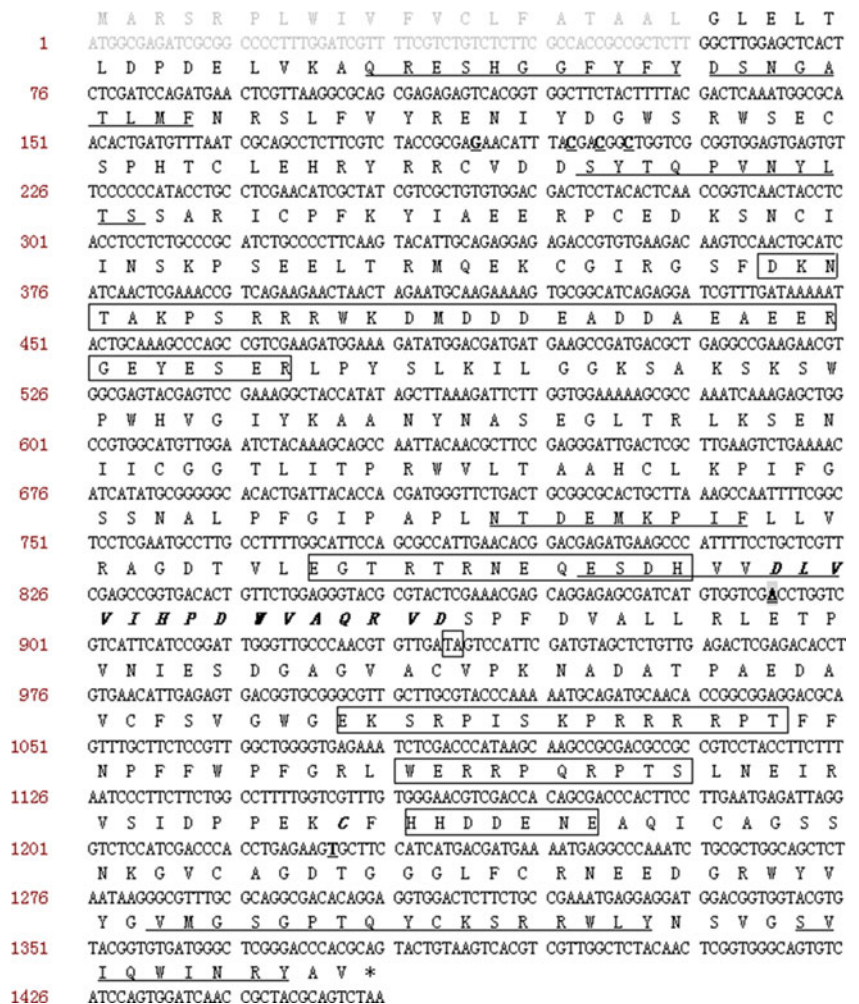
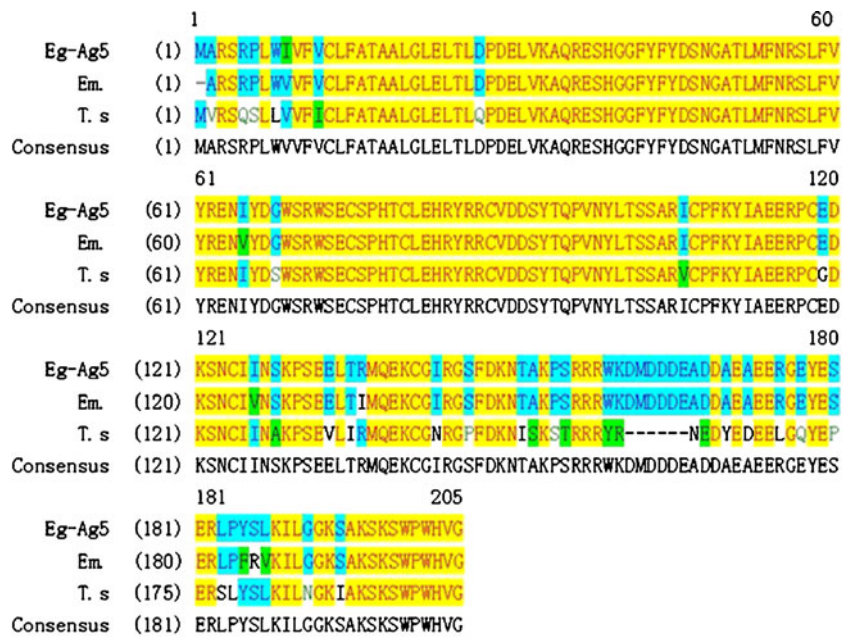


Fig. 2 Alignment of partial EgAg5 with its homologs from other taeniidae parasite. Em, *Echinococcus multilocularis* (GenBank accession no. DR748904.1); *T. s.*, *T. solium* (GenBank accession no. ADP89566.1). There is a deletion mutation region in *T. s.* homolog responding to the conserved strong B cellular epitope of “WKDMDDDEAD” of the two echinococcus sequences



membrane of egg and the surface of oncosphere in gravid proglottid, protoscolex and slightly expressed in the tegument of adult. Nearly no staining was observed in adult and protoscolex when incubated with pre-immune rat serum (Fig. 5).

Discussion

Ag5 was firstly paid intensive attention for its application in immunodiagnosis of echinococcosis, and its easy and large

scale of preparation from the cyst liquid by ConA affinity-chromatography (Bout et al. 1979; Shepherd and McManus 1987). Up to the present, most researches on its structure and function were based on the native protein and there are fewer attempts to prepare the recombinant protein. Apart from the antibody responses against Ag5 and its application in immunodiagnosis, little is known about its expression and secretory route at different developmental stages.

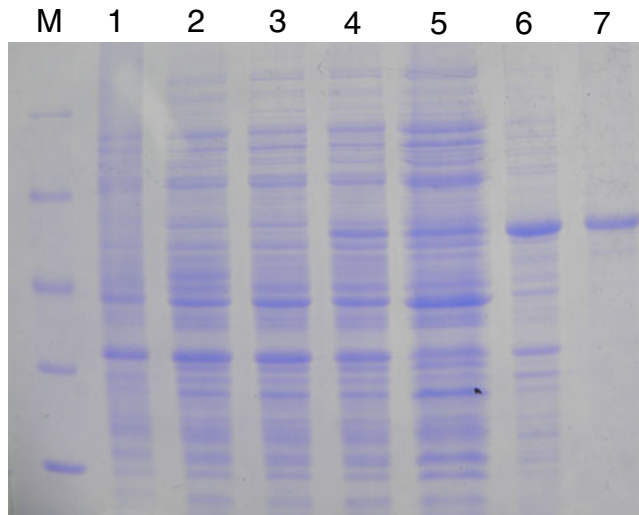


Fig. 3 Expression and purification of rEgAg5 in *E. coli* BL21/DE3. *M*, protein molecular weight markers; *lanes 1* and *2*, the lysate of bacteria transformed with pET-28a vector before and after IPTG induction; *lanes 3* and *4*, the lysate of pET-28a-EgAg5 transformants before and after IPTG induction; *lanes 5* and *6*, the supernatant and precipitation of the lysate of pET-28a-EgAg5 transformants after IPTG induction; *lane 7*, purified rEgAg5 protein

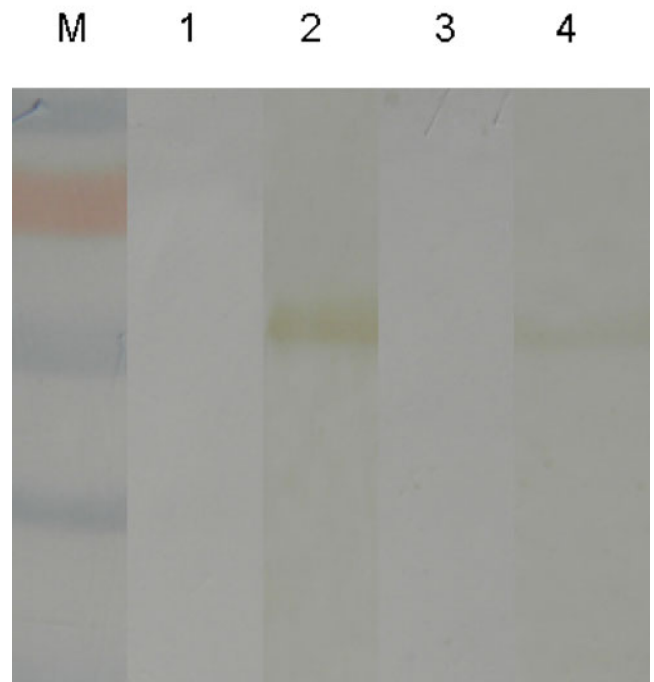
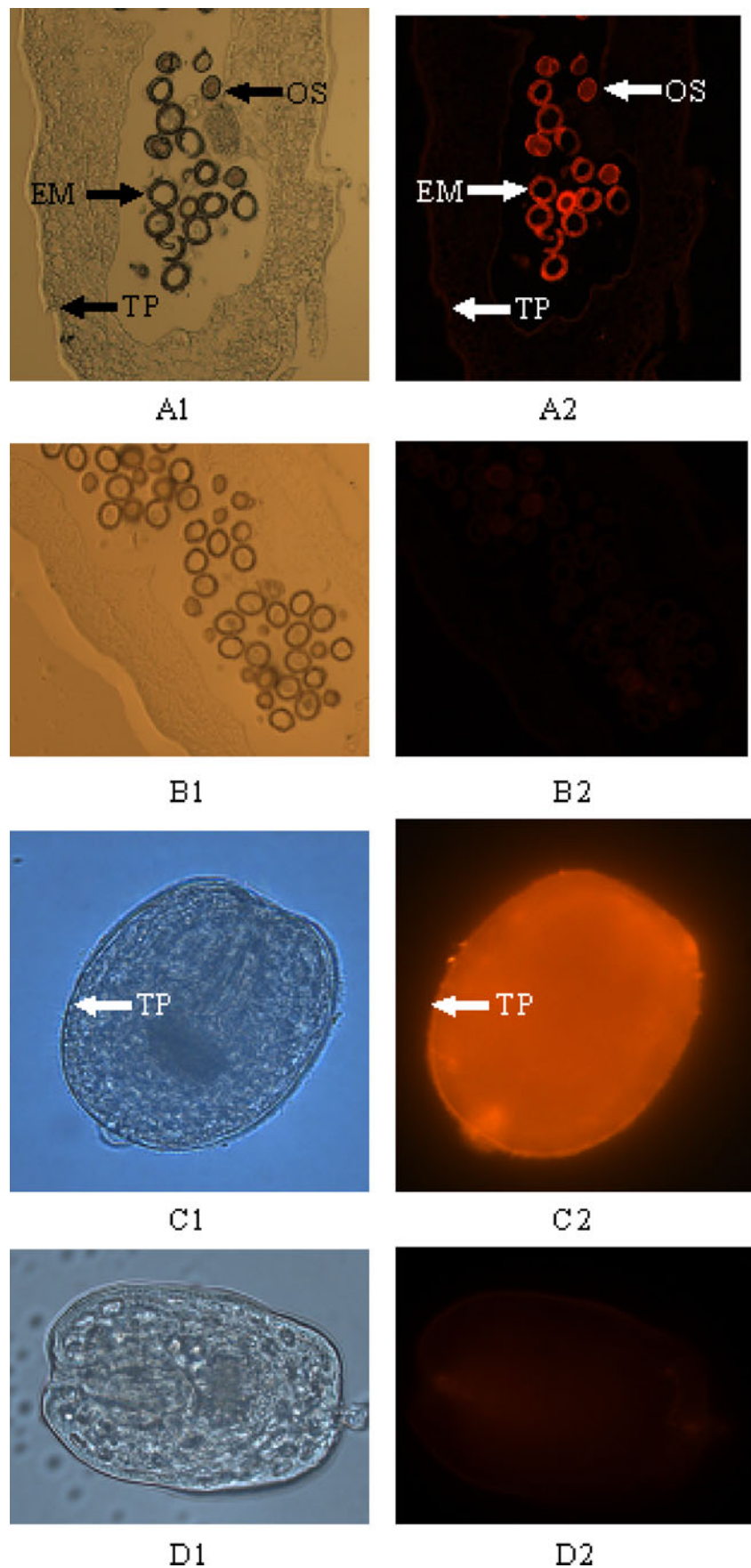


Fig. 4 Western blot analysis of *EgAg5*. *M*, protein molecular weight marker; *1* and *2*, with healthy and immune rat serum, respectively; *3* and *4* with healthy person and the CE patient serum, respectively

Fig. 5 Immunolocalization of EgAg5 at protoscolices and adult worm of *E. granulosus*. Fluorescence microscopy images (left) and corresponding optical images (right) of are shown. **a** and **b**, EgAg5 is shown intensively on the embryonic membrane (*EM*) and surface of oncosphere (*OS*) in eggs and weakly on the tegument (*TP*) of the adult worm with immune and preimmune rat serum respectively; **c** and **d**, EgAg5 was shown strongly in the surface of protoscolex control with immune and preimmune rat serum under fluorescence, respectively



According to the sequence deposited in GenBank, we amplified the coding sequence from the mRNA extracted from the *E. granulosus* protoscolices isolated from sheep of Qinhai Province of China by RT-PCR. Sequencing the product discovered it is somewhat different from the sequence in GenBank, with several base replacements as well as one base insert and deletion which produces a short region of shift-frame mutation. Sequence alignment with the homologue from other taeniidae, *T. solium* shows that the frame-shift region in our sequence is highly conservative. Therefore, we think that the single base insert and deletion of the sequence in GenBank may come from the mistake of original sequencing.

Most of the previous studies of EgAg5 were focused on its antibody responses and the applications in diagnosis (Liu et al. 1993b). Although antigen B (AgB) and Ag5 are the predominant antigens of HCF, using four monoclonal antibodies against Ag5 and AgB to detect the circulating antigens (CAg) in sera of human patients with *E. granulosus* infection, the combined detection rate for CAg was only 19% (Liu et al. 1993a, b). It is due to the thick cyst wall that blocks the release of these antigens or the released antigens are neutralized by the antibodies. Therefore, the sensitive diagnostic marker of cystic hydatid disease should be the specific antibody instead of the antigen.

In mice secondary infection model with protoscolices, the anti-Ag5 IgG antibodies were negligible up to 2 weeks, and appeared a small increase till 16 weeks post infection (p.i.) when there was a big increase and persisted afterward. While the antibodies against crude protoscolices antigens were evident in 3–5 days p.i., increased steadily until 16 weeks and then maintained a high level (Liu et al. 1992). No antibody response in early stage easily lead to the deduction that the Ag5 is not expressed in the challenging protoscolices. However, our study demonstrated that Ag5 is highly expressed in the tegumental surface membrane of protoscolices, as the antibody is directly binding to Ag5 protein attached on the surface protoscolices fixed with paraform which maintains the integrality of the plasma membrane and forbids the entry of the antibodies into the protoscolices. The secreted EgAg5 attachment to surface of tegumental membrane can be explained by the myristoylation modification, one N-myristoylation site Gly21 has been predicted by online program Scanprosite (<http://www.expasy.ch/tools/scanprosite/>). Beside localization in protoscolices surface, EgAg is also highly expressed in the embryonic membrane of egg and surface of oncosphere, it means there should be antibody response against Ag5 in early infection of CE patient. This paradox is interesting to the immunological characteristics of Ag5. In rats immunized with rAg5, the titer is very low in spite of two boosts in the early 6 weeks but acute increase appeared after the third boosts (data is not shown), this phenomenon indicates Ag5 is able

to inhibit immune response at the initiating stage. Ag5 is highly similar to members of the trypsin family (Lorenzo et al. 2003)—a serine protease plays an important role in the cestode eggs hatching and larvae invasion of helminth parasites (Dzik 2006; Young et al. 1999). This early immunological unresponsiveness suggests it may play a role in immune evasion which is associated with its serine protease activity, the mechanisms deserve further studies.

E. granulosus grows slowly in human, the patients are ordinarily infected in childhood and manifested space-occupying lesion by the enlarged cyst in adult or the old age. In the early years of infection, *E. granulosus* is more sensitive to chemotherapy, but the cyst is too small to be diagnosed by imaging examination. When the cyst develops enough large to be easily diagnosed, it has become difficult to be cured by drugs and most of CE patients had to depend on surgical resection which brings large operation risk and medical burden (Garcia-Llamazares et al. 1998).

Due to the imaging examination is not competent for early diagnosis; the sensitive, specific immunodiagnostic assay is urgently desired (Moro et al. 2005). As the IgG or its subclasses cannot distinguish the active or abortive infection exactly, the IgG is not the ideal indicator for active infection of *E. granulosus*. The IgE against hydatid cyst fluid (HCF) is of high specificity and relatively high sensitivity in CE patients (Sjolander et al. 1989). In spite that the Ag5-specific IgE could only be detected in 70.1% confirmed CE patients, the specificity was 100% (Khabiri et al. 2006). Furthermore, 3 to 14 days after the primary exposure to protoscolices, the protoscolices-specific IgE begins to rise and persists so long as the live protoscolices exists (Riley et al. 1986). In CE patients who received treatment, when the parasite is killed completely, the IgE rapidly decrease to negative level (Vuitton 2004). Therefore, the specific IgE can be considered as a marker of active infection of *E. granulosus* (Wellinghausen and Kern 2001) and can be applied in early diagnosis of CE patients.

Detection of *E. granulosus* in definitive host dog is of importance in the epidemiological surveys and the implementation of hydatid control programmes in endemic areas (Ersfeld et al. 1997). At present, arecoline purgation is the most commonly used method for detecting canine Echinococcosis. While, it is not a perfect method with low sensitivity of only 64% for *E. granulosus* infected experimental dogs (Lahmar et al. 2007). Meanwhile, it is time consuming, biohazardous, and requires trained personnel. The development of sensitive and specific diagnostic antigen for the detection of canine Echinococcosis is important for surveillance of hydatid control programmes. The development of diagnosis of dog *E. granulosus* infection is detecting coproantigens, predominantly the excretory–secretory products from proglottids by ELISA using specific monoclonal or polyclonal antibodies (Allan et al. 1992). In spite of some monoclonal

antibodies that have been used in the immunodiagnosis, their target antigen molecule is unknown and the specificity is the main issue, for other cestodes, such as *Taenia elliptica* are always existing in the intestine of dogs. In addition, the components of excretory–secretory antigens coming from these parasites are very complex, if antibody using to detect *E. granulosus* is against a common antigen of cestode, the false-positive result will occur. Considering Ag5 is highly expressed in eggs, it may be a coproantigen for detecting canine *E. granulosus* infection. Meanwhile, there are epitopes in EgAg5 different from other taeniidae, such as *T. solium*, this will be favorable to discriminate the *E. granulosus* from other intestinal cestodes.

Intensive expression in surface of egg and protoscolex which are infective stages for intermediate and definitive hosts indicates Ag5 may be a candidate of vaccine. Furthermore, Ag5 is a strong allergen as it provokes specific IgE in hydatid patients (Takahashi et al. 2006). If it remains the allergenic property in definitive hosts, it is a ideal vaccine candidate for dogs, because only the vaccine that induces IgE production can lead to effective protective immunity to nearly all the intestinal helminths (Torgerson 2009; Zhang et al. 2006; Khabiri et al. 2006; Torgerson 2009; Zhang et al. 2006).

In conclusion, EgAg5 is expressed in eggs and protoscolices, the two infective stages to human and dogs respectively. Considering its characteristics of trypsin and the localization in the surface of the eggs and protoscolices, we speculate that Ag5 has the value in diagnosis and vaccine development for Echinococcosis and it is a potential immunomodulatory agent.

Acknowledgments This study was supported by grants from the national Science and Technology Infrastructure Program (No. 2006BAI06B06). We grateful to Ms. Junying Ma of institute of Qinghai Province endemics control for providing *E. granulosus*-infected patients sera.

References

Allan JC, Craig PS, Garcia NJ, Mencos F, Liu D, Wang Y, Wen H, Zhou P, Stringer R, Rogan M et al (1992) Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. *Parasitology* 104(Pt 2):347–356

Al-Yaman FM, Knobloch J (1989) Isolation and partial characterization of species-specific and cross-reactive antigens of *Echinococcus granulosus* cyst fluid. *Mol Biochem Parasitol* 37:101–107

Bart JM, Morariu S, Knapp J, Ilie MS, Pitulescu M, Anghel A, Cosoroaba I, Piarroux R (2006) Genetic typing of *Echinococcus granulosus* in Romania. *Parasitol Res* 98:130–137

Bout D, Carlier Y, Capron A (1979) Immunodiagnosis of hydatidosis using monospecific immune serum anti Ag5. *Biomedicine* 31:214–215

Ceballos L, Elisondo C, Moreno L, Dopchiz M, Sanchez BS, Denegri G, Alvarez L, Lanusse C (2008) Albendazole treatment in cystic echinococcosis: pharmacokinetics and clinical efficacy of two different aqueous formulations. *Parasitol Res* 103:355–362

Craig PS, Larrieu E (2006) Control of cystic echinococcosis/hydatidosis: 1863–2002. *Adv Parasitol* 61:443–508

Dzik JM (2006) Molecules released by helminth parasites involved in host colonization. *Acta Biochim Pol* 53:33–64

Ersfeld K, Gasser RB, Craig PS (1997) The immunodiagnostic potential of *Echinococcus granulosus* adult-worm antigens in human cystic echinococcosis. *Parasitol Res* 83:90–92

Garcia-Llamazares JL, Alvarez-de-Felipe AI, Redondo-Cardena PA, Prieto-Fernandez JG (1998) *Echinococcus granulosus*: membrane permeability of secondary hydatid cysts to albendazole sulfoxide. *Parasitol Res* 84:417–420

Gonlugur U, Ozelik S, Gonlugur TE, Celiksoz A (2005) The role of Casoni's skin test and indirect haemagglutination test in the diagnosis of hydatid disease. *Parasitol Res* 97:395–398

Heath D, Yang W, Li T, Xiao Y, Chen X, Huang Y, Yang Y, Wang Q, Qiu J (2006) Control of hydatidosis. *Parasitol Int* 55(Suppl): S247–S252

Jamshidi M, Mohraz M, Zangeneh M, Jamshidi A (2008) The effect of combination therapy with albendazole and praziquantel on hydatid cyst treatment. *Parasitol Res* 103:195–199

Kalantari E, Bandehpour M, Pazoki R, Taghipoor-Lailabadi N, Khazan H, Mosaffa N, Nazari-pouya MR, Kazemi B (2010) Application of recombinant *Echinococcus granulosus* antigen B to ELISA kits for diagnosing hydatidosis. *Parasitol Res* 106:847–851

Khabiri AR, Bagheri F, Assmar M, Siavashi MR (2006) Analysis of specific IgE and IgG subclass antibodies for diagnosis of *Echinococcus granulosus*. *Parasite Immunol* 28:357–362

Kia EB, Rahimi H, Sharbatkhori M, Talebi A, Fasihi HM, Mirhendi H (2010) Genotype identification of human cystic echinococcosis in Isfahan, central Iran. *Parasitol Res* 107:757–760

Lahmar S, Lahmar S, Boufana B, Bradshaw H, Craig PS (2007) Screening for *Echinococcus granulosus* in dogs: comparison between arecoline purgation, coproELISA and coproPCR with necropsy in pre-patent infections. *Vet Parasitol* 144:287–292

Liu D, Lightowers MW, Rickard MD (1992) Examination of murine antibody response to secondary hydatidosis using ELISA and immunoelectrophoresis. *Parasite Immunol* 14:239–248

Liu D, Rickard MD, Lightowers MW (1993a) Analysis of taeniid antigens using monoclonal antibodies to *Echinococcus granulosus* antigen 5 and antigen B. *Parasitol Res* 79:82–85

Liu D, Rickard MD, Lightowers MW (1993b) Assessment of monoclonal antibodies to *Echinococcus granulosus* antigen 5 and antigen B for detection of human hydatid circulating antigens. *Parasitology* 106(Pt 1):75–81

Lorenzo C, Salinas G, Brugnini A, Wernstedt C, Hellman U, Gonzalez-Sapienza G (2003) *Echinococcus granulosus* antigen 5 is closely related to proteases of the trypsin family. *Biochem J* 369:191–198

Moro PL, Garcia HH, Gonzales AE, Bonilla JJ, Verastegui M, Gilman RH (2005) Screening for cystic echinococcosis in an endemic region of Peru using portable ultrasonography and the enzyme-linked immunoelectrotransfer blot (EITB) assay. *Parasitol Res* 96:242–246

Nasrieh MA, Abdel-Hafez SK, Kamhawi SA, Craig PS, Schantz PM (2003) Cystic echinococcosis in Jordan: socioeconomic evaluation and risk factors. *Parasitol Res* 90:456–466

Neghina R, Neghina AM, Marincu I, Iacobiciu I (2011) Epidemiology and history of human parasitic diseases in Romania. *Parasitol Res* 108:1333–1346

Poretti D, Felleisen E, Grimm F, Pfister M, Teuscher F, Zuercher C, Reichen J, Gottstein B (1999) Differential immunodiagnosis between cystic hydatid disease and other cross-reactive pathologies. *Am J Trop Med Hyg* 60:193–198

Raether W, Hanel H (2003) Epidemiology, clinical manifestations and diagnosis of zoonotic cestode infections: an update. *Parasitol Res* 91:412–438

- Riley EM, Dixon JB, Jenkins P, Ross G (1986) *Echinococcus granulosus* infection in mice: host responses during primary and secondary infection. *Parasitology* 92(Pt 2):391–403
- Shepherd JC, McManus DP (1987) Specific and cross-reactive antigens of *Echinococcus granulosus* hydatid cyst fluid. *Mol Biochem Parasitol* 25:143–154
- Sjolander A, Guisantes JA, Torres-Rodriguez JM, Schroder H (1989) The diagnosis of human hydatidosis by measurement of specific IgE antibody by enzyme immunoassay. *Scand J Infect Dis* 21:213–218
- Takahashi H, Nemoto T, Yoshida T, Honda H, Hasegawa T (2006) Cancer diagnosis marker extraction for soft tissue sarcomas based on gene expression profiling data by using projective adaptive resonance theory (PART) filtering method. *BMC Bioinformatics* 7:399
- Tawfeek GM, Elwakil HS, El-Hoseiny L, Thabet HS, Sarhan RM, Awad NS, Anwar WA (2011) Comparative analysis of the diagnostic performance of crude sheep hydatid cyst fluid, purified antigen B and its subunit (12 Kda), assessed by ELISA, in the diagnosis of human cystic echinococcosis. *Parasitol Res* 108:371–376
- Torgerson PR (2009) Dogs, vaccines and Echinococcus. *Trends Parasitol* 25:57–58
- Urrea-Paris MA, Moreno MJ, Casado N, Rodriguez-Caabeiro F (1999) *Echinococcus granulosus*: praziquantel treatment against the metacestode stage. *Parasitol Res* 85:999–1006
- Urrea-Paris MA, Moreno MJ, Casado N, Rodriguez-Caabeiro F (2002) Relationship between the efficacy of praziquantel treatment and the cystic differentiation in vivo of *Echinococcus granulosus* metacestode. *Parasitol Res* 88:26–31
- Varcasia A, Garippa G, Pipia AP, Scala A, Brianti E, Giannetto S, Battelli G, Poglayen G, Micagni G (2008) Cystic echinococcosis in equids in Italy. *Parasitol Res* 102:815–818
- Vuitton DA (2004) Echinococcosis and allergy. *Clin Rev Allergy Immunol* 26:93–104
- Wang Z, Wang X, Liu X (2008) Echinococcosis in China, a review of the epidemiology of *Echinococcus* spp. *Ecohealth* 5:115–126
- Wellinghausen N, Kern P (2001) A new ImmunoCAP assay for detection of *Echinococcus multilocularis*-specific IgE. *Acta Trop* 79:123–127
- Wen H, Aji T, Shao YM (2010) Diagnosis and management against the complications of human cystic echinococcosis. *Front Med China* 4:394–398
- Young AR, Mancuso N, Bowles VM (1999) Biochemical aspects of egg hatch in endo- and ectoparasites: potential for rational drug design. *Int J Parasitol* 29:861–867
- Zhang LH, Leggatt GR, McManus DP (1995) Further characterization of the 38 kDa antigen from *Echinococcus granulosus* (hydatid disease) cyst fluid: evidence for antigenic heterogeneity and reactivity with anti-P1 antibodies. *Parasite Immunol* 17:287–296
- Zhang W, Zhang Z, Shi B, Li J, You H, Tulson G, Dang X, Song Y, Yimiti T, Wang J, Jones MK, McManus DP (2006) Vaccination of dogs against *Echinococcus granulosus*, the cause of cystic hydatid disease in humans. *J Infect Dis* 194:966–974
- Zimic MJ, Infantes J, Lopez C, Velasquez J, Farfan M, Pajuelo M, Sheen P, Verastegui M, Gonzalez A, Garcia HH, Gilman RH (2007) Comparison of the peptidase activity in the oncosphere excretory/secretory products of *Taenia solium* and *Taenia saginata*. *J Parasitol* 93:727–734