

AMCoR

Asahikawa Medical College Repository <http://amcor.asahikawa-med.ac.jp/>

Journal of Infectious Diseases (2006. Dec) 194(12):1783–1790.

Evaluation of purified *Taenia solium* glycoproteins and recombinant antigens in the serologic detection of human and swine cysticercosis

Sato, Marcello Otake ; Sako, Yasuhito ; Nakao, Minoru ;
Yamasaki, Hiroshi ; Nakaya, Kazuhiro ; Ito, Akira

Evaluation of Purified *Taenia solium* Glycoproteins and Recombinant Antigens in the Serologic Detection of Human and Swine Cysticercosis

Marcello Otake Sato,^{1,3a} Yasuhito Sako,^{1,a} Minoru Nakao,¹ Hiroshi Yamasaki,¹ Kazuhiro Nakaya,² and Akira Ito^{1,a}

¹ Department of Parasitology and ²Animal Laboratory for Medical Research, Asahikawa Medical College, Asahikawa, Japan; ³Laboratório de Parasitologia, Escola de Medicina Veterinária e Zootecnia, Universidade Federal do Tocantins, Araguaína-TO, Brazil

Cysticercosis caused by infection with embryonated eggs of *Taenia solium* is an important cause of neurological disease worldwide. On the basis of mitochondrial DNA analysis, *T. solium* is divided into 2 (African/American and Asian) genotypes. Glycoproteins (GPs) in cyst fluid purified from the 2 genotypes of *T. solium* were characterized and compared with the recombinant chimeric *T. solium*-Ag1V1/Ag2 protein (Rec-Ag1V1/Ag2) as serodiagnostic antigens. Immunoblot analysis revealed that banding patterns of GPs differed between the 2 genotypes because of posttranslation modification, especially glycosylation. The comparison of native GPs with Rec-Ag1V1/Ag2 by enzyme-linked immunosorbent assay demonstrated that there was no statistical difference in sensitivity. In addition, the conservation of the genes encoding Ag1V1 and Ag2 in *T. solium* worldwide was verified. These results indicate that Rec-Ag1V1/Ag2 has great potential for usefulness in serodiagnosis as an alternative to native antigens.

The larval stage of the pork tapeworm *Taenia solium* causes cysticercosis (CC). Humans become accidentally infected with *T. solium* by ingesting embryonated eggs excreted with feces from worm carriers who harbor the adult tapeworm in the intestine. The hatched embryos migrate throughout the body; invade skeletal muscle, subcutaneous tissue, the eyes, or the central nervous system (CNS; this is known as “neurocysticercosis”); and develop into cysticerci. This disease is one of the reemerging zoonoses worldwide, and it is the major etiological agent of epileptic seizures in areas of en-

demicity, making it a major public health problem in most developing countries [1–3].

The diagnosis of CC in developed countries is mainly based on clinical criteria and on computed tomography (CT) and nuclear magnetic resonance imaging results. The imaging techniques are useful for diagnosis but have the possibility of overlooking the infection when the number of parasites is low and/or the figures are not clear or not typical [4]. Moreover, these techniques are not suitable for the diagnosis of CC in areas where it is endemic, especially developing countries, because of the high cost. Therefore, the development of an immunodiagnostic test that detects specific antibodies in either serum or cerebrospinal fluid is an urgent issue because of the simplicity and reliability of such tests, especially in serum [5]. Glycoproteins (GPs) in fluid from *T. solium* cysts have widely been accepted for serodiagnostic purposes. Tsang et al. [6] characterized, using lentil-lectin chromatography, GPs in a crude extract of metacestodes that showed 7 major diagnostic bands of 10–50 kDa, and they demonstrated the usefulness of GPs for serodiagnosis based on immunoblot but not on ELISA. By contrast, we developed a simple

Received 11 July 2006; accepted 4 August 2006; electronically published 6 November 2006.

Presented in part: 11th International Congress of Parasitology, August 2006, Glasgow, Scotland (abstract 720).

Potential conflicts of interest: none reported.

Financial support: Japan Society for Promotion of Science (grants-in-aid for scientific research 14256001 and 17256002 to A.I.).

^a M.O.S., Y.S., and A.I. contributed equally to the work.

Reprints or correspondence: Prof. Akira Ito, Dept. of Parasitology, Asahikawa Medical College, Midorigaoka Higashi 2-1-1, Asahikawa-Hokkaido, Japan (akiraito@asahikawa-med.ac.jp).

The Journal of Infectious Diseases 2006;194:1783–90

© 2006 by the Infectious Diseases Society of America. All rights reserved. 0022-1899/2006/19412-0023\$15.00

method to purify GPs using preparative isoelectric-focusing electrophoresis (IEFE) that is applicable for both immunoblot and ELISA for humans, pigs, and dogs that [7–10]. In addition, we demonstrated the usefulness of the recombinant chimeric *T. solium*-Ag1V1/Ag2 protein (Rec-Ag1V1/Ag2), the backbone proteins of GPs used for serodiagnosis in humans and animals [8–10].

Recently, we reported that (1) *T. solium* could be divided into 2 genotypes—African/American and Asian—on the basis of mtDNA analyses [11] and (2) banding patterns of GPs recognized in serum from patients with CC differed among fluid from *T. solium* cysts from persons living in geographically different regions [12]. These results raised the possibility that the origin of GPs might affect the quality of antigens and the sensitivity and/or specificity of serodiagnosis.

In the present study, we characterized GPs from the 2 genotypes of *T. solium* and compared the antigenicity of GPs from Asia, America, and Africa. Furthermore, we compared the usefulness of Rec-Ag1V1/Ag2 with native GPs as serodiagnostic antigens and verified the existence of the Ag1V1 and Ag2 genes in *T. solium* isolates from around the world.

MATERIALS AND METHODS

Animals. The animals used in our study were euthanized in accordance with the guidelines of the Ethics Committee, Asahikawa Medical College, Asahikawa, Japan.

Production of polyclonal and monoclonal antibodies to *T. solium* GPs. *T. solium* GPs to be used for the serodiagnosis of CC in humans and pigs were purified using IEFE [7]. Polyclonal antibodies against *T. solium* GPs were prepared by immunizing rabbits subcutaneously twice, with a 3-week interval between, with IEFE-purified GPs from South American (Ecuador) isolates that had been emulsified with Freund's complete adjuvant and boosted with the same GPs emulsified with Freund's incomplete adjuvant.

Monoclonal antibodies were produced using mouse NS-1 myeloma cells [13]. Briefly, female BALB/c mice, immunized by intraperitoneal (ip) injection of GPs emulsified in Freund's complete adjuvant, were boosted 3 weeks later with the same GPs emulsified with Freund's incomplete adjuvant. Three days before cell fusions, the mice were boosted ip with 50 μ g of antigens in PBS. Spleen cells from immunized mice were fused with NS-1 myeloma cells. ELISA using GPs was applied for the screening of antibody-secreting hybridomas. Hybridomas were selected and cloned by limited dilutions at least twice. From several clones producing antibodies to *T. solium* GPs, we chose clone 4F10, which showed reactivity to recombinant protein, Ag1V1 [9], on which to perform the study. Both polyclonal and monoclonal antibodies were purified using a Protein G Sepharose column (Amersham Biosciences) in accordance with the manufacturer's instructions.

Affinity purification of GPs from fluid from *T. solium* cysts.

Both polyclonal and monoclonal antibodies to GPs were coupled to an N-hydroxysuccinimide-activated column [13], in accordance with the manufacturer's instructions, and used for the purification of GPs from fluid from *T. solium* cysts. All cysts were collected from naturally infected pigs. Fluid from *T. solium* cysts was prepared from cysts from South America (Brazil), Africa (Tanzania), and Asia (China and Indonesia). After purification, GPs were dialyzed against PBS and kept at -20°C until use. Protein concentrations were determined using a bicinchoninic acid protein assay kit (Pierce).

SDS-PAGE and immunoblot analysis. Protein analysis by SDS-PAGE was performed using the method of Laemmli [14]. Proteins were solubilized with a SDS sample buffer (10 mmol/L Tris-HCl [pH 6.8] that contained 2% SDS, 5% 2-mercaptoethanol, and 10% glycerol) at 100°C for 5 min and separated electrophoretically in a 15.0% polyacrylamide gel. For immunoblot analysis, the separated proteins were transblotted onto a polyvinylidene difluoride (PVDF) membrane sheet (Millipore) [7]. The PVDF sheet was blocked with blocking solution (20 mmol/L Tris-HCl, 150 mmol/L NaCl, 1% casein, and 0.05% Tween 20 [pH 7.6]) and probed with antibodies and then with peroxidase-conjugated protein G (Cappel) [7]. Color development was performed using 3,3',5,5'-tetramethylbenzidine (Kirkegaard & Perry Laboratories).

Enzymatic deglycosylation. GPs purified with monoclonal and/or polyclonal antibodies were deglycosylated with 250 mU of peptide-N4-(N-acetylglucosaminyl) asparagine amidase (PNGase F; Sigma) at 37°C for 18 h, in accordance with the manufacturer's instructions.

Serum samples. A total of 90 serum samples—60 human and 30 pig—were used. Human serum samples were from South America (12 from Ecuador and 8 from Brazil), Africa (4 from Cameroon), and Asia (12 from Korea and 24 from China). All human serum samples were prepared using *T. solium* isolates from patients with CC who had a history of epilepsy and who were living in areas where *T. solium* is highly endemic and whose infection has been confirmed serologically using both GPs and Rec-Ag1V1/Ag2 and also by CT in China [15–18]. In Papua, Indonesia, there was no statistical difference in antibody responses to GPs and Rec-Ag1V1/Ag2 between persons suspected as having neurocysticercosis with a history of epilepsy and those with subcutaneous cysticercosis [16, 17]. Cysts resected from subcutaneous nodules were confirmed to be *T. solium* cysticerci by morphological testing and the presence of mtDNA [15–17, 19]. All pig serum samples were obtained from pigs naturally infected with and harboring *T. solium* cysticerci, except for those from Mexico, which were from experimentally infected pigs [8, 20]. Cysticerci from all pigs were confirmed to be *T. solium* by mtDNA analysis [21]. They were from Latin America (6 from

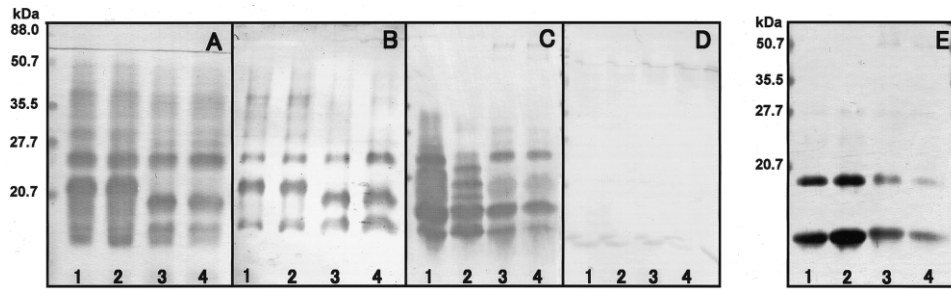


Figure 1. Immunoblots of *Taenia solium* glycoproteins (GPs) purified by affinity chromatography using 4F10 monoclonal antibody. Panels correspond to native GPs against polyclonal rabbit serum (A), monoclonal antibody 4F10 (B), serum from a patient with cysticercosis (C), and serum from a healthy person (D). E, GPs after treatment with *N*-glycosidase using 4F10 monoclonal antibody as a probe. The lanes are GPs from Africa (Tanzania, lane 1), South America (Brazil, lane 2), and Asia (China, lane 3; and Indonesia, lane 4).

Ecuador and 4 from Mexico), Africa (10 from Tanzania), and Asia (10 from Indonesia).

ELISA. ELISA plates (Nunc-Immuno plate MaxiSorp Surface; Nalge Nunc International) were coated with 0.1 μg of antigens. The wells were blocked with 300 μL of blocking solution for 1–2 h at 37°C. After the wells had been washed twice with PBS that contained 0.05% Tween 20 (PBST), 100 μL of serum samples diluted 1:100 in blocking solution was added and incubated for 1 h at 37°C. The wells were washed 5 times with PBST and incubated with 100 μL of protein G conjugated with peroxidase (Cappel) for 1 h at 37°C; they were then washed 5 times with PBST. After incubation with 100 μL of substrate (0.4 mmol/L 2,2'-azino-di-[3-ethyl-benzthiazoline sulfonate] in 0.1 mol/L citric acid buffer [pH 4.7]) for 30 min at room temperature, the optical density at 405 nm of each well was determined using an ELISA plate reader (model 450; Bio-Rad Laboratories) [7]. Serum samples giving optical density values at 405 nm that were greater than the mean \pm 3 SD for normal human or pig control samples ($n = 15$) were considered to be seropositive.

Preparation of Rec-Ag1V1/Ag2. Rec-Ag1V1/Ag2 was expressed in a bacteria system as described elsewhere [9], with some modification. Briefly, sequential polymerase chain reaction (PCR) mutagenesis was performed. PCR products amplified from both Ag1V1 cDNA with primers *EcoRI*Ag1V1 forward and Ag1V1/Gly/Ag2 reverse and for Ag2 cDNA with primers *Bam*HI Ag2 reverse and Ag1V1/Gly/Ag2 forward, were further amplified with primers *EcoRI*Ag1V1 forward and *Bam*HI Ag2 reverse so that the Ag1V1/Ag2 chimeric gene could be obtained. The primer sequences used were 5'-GGGAATTCGAGAAAAA-CAAACCGAAGTGT-3' (*EcoRI*Ag1V1 forward) and 5'-CTGAGAACAACAACTGCTGGAGGTGGAGGTGGAAAGGAACT-AAACCAGAG-3' (Ag1V1/Gly/Ag2 reverse); and 5'-TTGGATCCTTAAGCAGCTTCGTTCTTGAG-3' (*Bam*RHI Ag2 reverse) and 5'-CTCTGGTTTAGTTTCCCTTCCACCTCCACCTCCAGCAGTTTTGTTCTTCAG-3' (Ag1V1/Gly/Ag2 forward). The PCR products were digested with *EcoRI* and *Bam*HI and cloned

into the bacterial expression vector pTWIN-1 (New England Biolabs) to produce a fusion protein with chitin-binding domain/mini-inteins. The orientation of the inserted DNA was confirmed by sequencing. The cloned plasmid was transfected into *Escherichia coli* strain ER2566. The expression of Rec-Ag1V1/Ag2 was induced by the addition of 0.5 mmol/L isopropyl- β -D-thiogalactoside to the culture. The expressed recombinant proteins were purified using a chitin column, in accordance with the manufacturer's instructions.

Amplification of Ag1V1 and Ag2 genes by PCR. A total of 7 isolates of *T. solium* cysticerci from South America (Brazil), Africa (Tanzania, Cameroon, and South Africa), and Asia (Indonesia, India, and China), collected from 1996 to 2001, were examined. All cysticerci samples were obtained from muscles of domesticated pigs by ourselves or our collaborators. Genomic DNA was extracted from a single cysticercus using a DNeasy Tissue Kit (Qiagen) and used as the template.

Two sets of PCR primers were used for amplification. The primer sequences used were 5'-CTCGCTCTCACTGTATT-CGT-3' (Ag1V1 forward) and 5'-TTGACAAGTTAAGCAGT-TTT-3' (Ag1V1 reverse); and 5'-CTCGCTCTCAGTGTTCGT-3' (Ag2 forward), and 5'-TTGACAAGTTAAGCAGCTTC-3' (Ag2 reverse). PCR was performed with high-fidelity enzyme PrimeSTAR DNA polymerase (Takara Bio) in a final volume of 50 μL that contained 0.2 $\mu\text{mol/L}$ each primer, 0.2 mmol/L each dNTP, 100 ng of genomic DNA, and 1.25 U of DNA polymerase. The amplicons were purified using the NucleoSpin ExTract kit and directly sequenced as described below. In some cases, the amplicons were purified and subcloned into the pGEM-T vector after the addition of adenine to the ends of PCR products.

DNA sequencing. Nucleotide sequences were determined using the Thermo Sequenase dye terminator cycle sequencing premix kit (Amersham Biosciences) and an automated DNA sequencer (Applied Biosystems model 377; Perkin Elmer).

Statistical analyses. Pearson's correlation coefficient was applied to calculate correlation coefficients for the respective

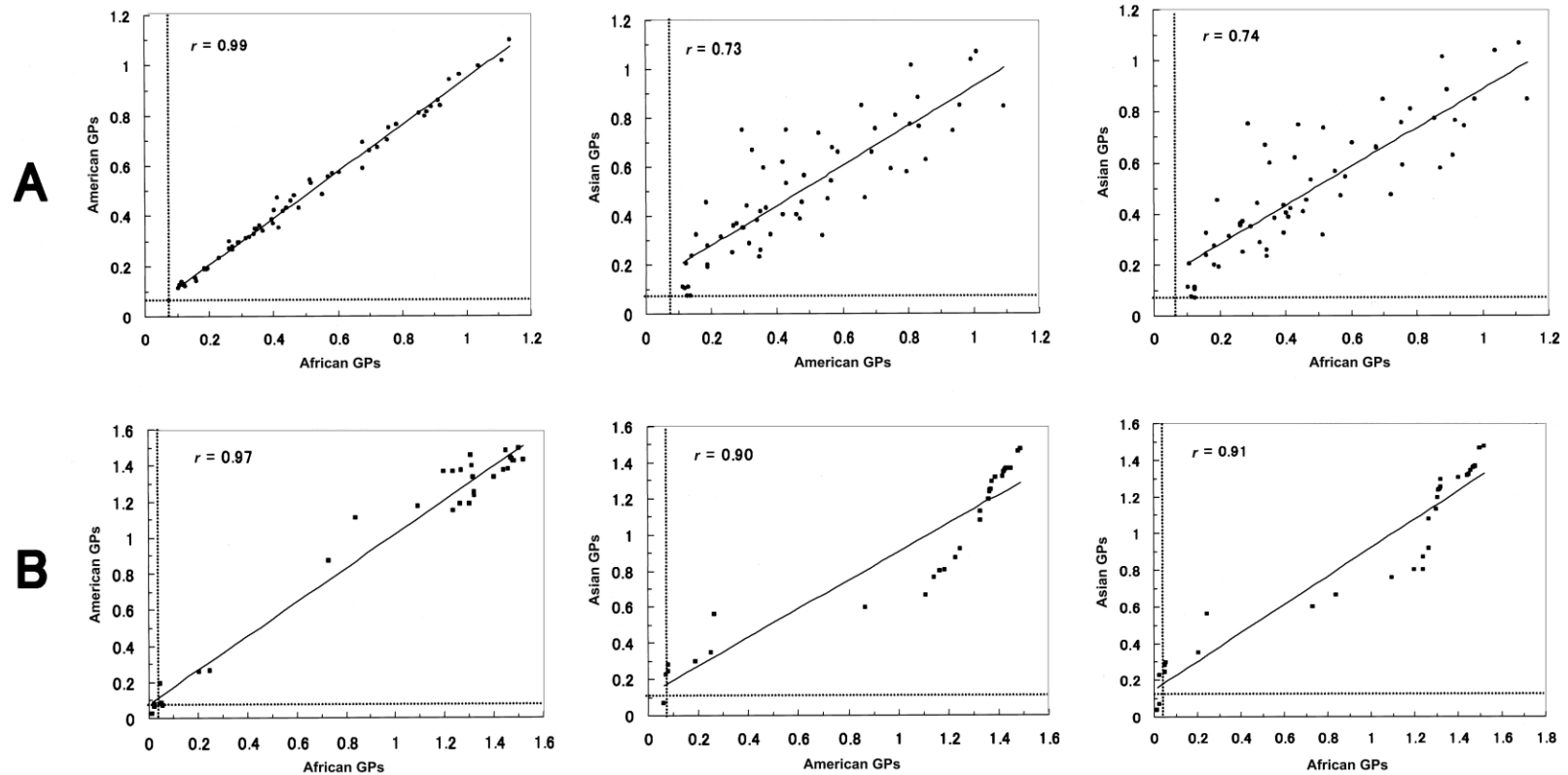


Figure 2. Direct comparative analysis of absorbance values obtained using African, American, and Asian glycoproteins (GPs) and serum from humans (A) and pigs (B). The charts show the scatter plots of the direct comparison between GPs as follows: America vs. Africa, America vs. Asia, and Africa vs. Asia. Pearson's r values are shown on the left top of each chart. The dotted line represents the estimated cutoff for the antigen.

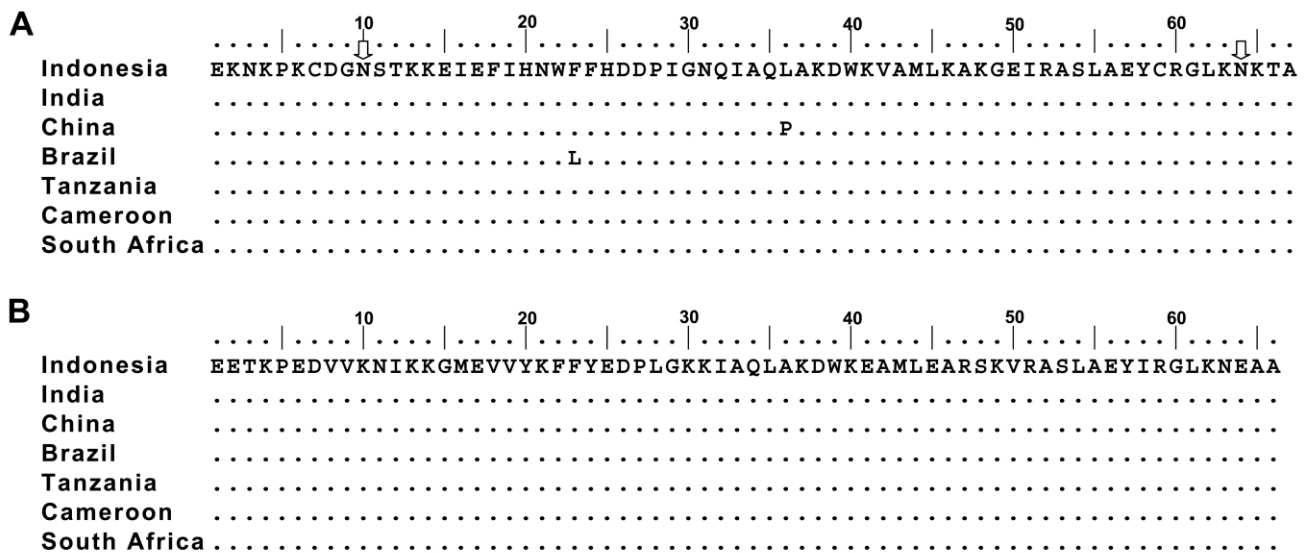


Figure 3. Ag1V1 (A) and Ag2 (B) amino acid sequence alignment for *Taenia solium* from different geographic areas. Arrows indicate putative glycosylation points.

absorbance values. The serological data obtained with native glycoproteins and Ag1V1/Ag2 protein were tested by Pearson's χ^2 test.

RESULTS

Immunoblot analyses of affinity-purified GPs. Using polyclonal and/or monoclonal antibodies against GPs and fluid from *T. solium* cysts isolated from different geographic areas, GPs were affinity purified and analyzed by immunoblotting under reducing conditions. The molecular sizes of GPs recognized as several bands were 8–50 kDa (figure 1). There were critical differences in banding patterns between African/American and Asian genotypes, especially when they were probed with polyclonal and monoclonal antibodies. The 22-kDa band in GPs from the African/American genotype was not detected in GPs from the Asian genotype, which instead showed an 18-kDa band. The difference in GP pattern between the 2 genotypes was also confirmed when serum from patients with CC was used. The banding patterns of GPs purified using polyclonal antibodies were the same as those of GPs purified using monoclonal antibody (data not shown).

The effect of glycosylation on this feature was investigated by PNGase F treatments. Enzymatic treatment of GPs was accompanied by a reduction in the number and molecular size of the antigens: only 2 bands of 8 and 16 kDa were recognized by the monoclonal antibody (figure 1E). In addition, there was no difference in banding patterns between GPs from the 2 genotypes after treatment.

Serodiagnostic evaluations of GPs using ELISA. ELISA tests using serum from patients seropositive for both Rec-

Ag1V1/Ag2 and GPs and from pigs that had been either naturally or experimentally infected in Africa, Latin America, and Asia were performed for the evaluation of the diagnostic values of GPs purified from the 2 genotypes of *T. solium*. The reactivities of serum samples with GPs from South America, Africa, and Asia were compared using ELISA with calculation of correlation coefficients for the respective absorbance values (figure 2). In this experiment, GPs purified from fluid from *T. solium* cysts prepared in China were used as Asian GPs, because GPs purified from those prepared in Papua, Indonesia, were not suitable for use as ELISA antigens because of high background. It was speculated that this was due to the poor conditions for preparation (very hot weather). The correlation rate for African and American GPs was higher (human, $r = 0.99$; pig, $r = 0.97$) than that for African and Asian GPs ($r = 0.74$ and $r = 0.91$) or American and Asian GPs ($r = 0.73$ and $r = 0.91$), but all values were found to have high correlations. When the reactivities of Asian GPs was compared with those of African or American GPs, several serum samples had low levels of reactivity to one antigen but high levels to the other. These results indicated that there were small antigenic differences in GPs between the 2 genotypes. However, all human serum samples and >87% of pig serum samples showed positive reactivities to purified GPs from Africa, South America, and Asia, and there were no differences in diagnostic sensitivities.

A comparison of GPs with Rec-Ag1V1/Ag2 was performed. All correlation coefficients for Rec-Ag1V1/Ag2 and GPs were high ($r > 0.63$). In humans, 4 of 60 serum samples were judged to be seronegative by Ag1V1/Ag2 testing (table 1). Two of these serum samples were near the cutoff applied for GPs. Never-

Table 1. ELISA results of cysticercosis in humans and pigs using glycoproteins (from America, Africa, and Asia) and recombinant Ag1V1/Ag2.

Serum samples	Glycoproteins			Recombinant Ag1V1/Ag2
	American	African	Asian	
Human				
Positive	60	60	60	56
Negative	0	0	0	4
Total	60	60	60	60
Pig				
Positive	27	26	29	26
Negative	3	4	1	4
Total	30	30	30	30

NOTE. Data are no. of samples.

theless, there was no statistical difference in diagnostic sensitivities between Rec-Ag1V1/Ag2 and GPs ($P > .1$). In pigs, 4 of 30 serum samples were judged to be seronegative, similar to those of native antigens (table 1).

Cloning and characterization of the Ag1V1 and Ag2 genes.

The monoclonal antibody used for the purification of GPs recognized recombinant Ag1V1 protein. There was no difference in sensitivity between Rec-Ag1V1/Ag2 and GPs, which indicated that all GPs contained protein identical or similar to Ag1V1 and Ag2 and that the protein backbones, not the carbohydrate components, of GPs were sufficient for serodiagnostic purposes. To clarify this issue, the *Ag1V1* and *Ag2* genes were amplified from *T. solium* isolates worldwide and compared. Primers specific to the *Ag1V1* and *Ag2* genes generated DNA fragments of 379 and 361 bp, respectively. The sequence data were deposited in Genbank (accession numbers AB263419–AB263432). Two introns were identified in each genomic clone (data not shown). The comparisons of Ag1V1 and Ag2 mature protein sequences with predicted mature protein sequences obtained in the present study revealed complete identities, except for Ag1V1 of Brazilian origin (1 substitution; F²³→L²³) and for Ag1V1 of Chinese origin (1 substitution; L³⁶→P³⁶) (figure 3).

DISCUSSION

GPs of 10–26 kDa in fluid from *T. solium* cysts have widely been accepted for serodiagnostic purposes. These GPs consist of a very closely related family of 8-kDa proteins having 0–3 predicted *N*-linked glycosylation sites [9, 22–24]. In the present study, we demonstrated that banding patterns of purified GPs recognized by polyclonal and monoclonal antibodies and serum from patients with CC showed critical differences between the 2 genotypes of *T. solium*, and, furthermore, that these differences in the size of GPs were caused by different posttranslational modifications, especially glycosylation.

Plancarte et al. [25] performed the characterization of *T. solium* GPs and determined the glycan portion of GPs. Analyses of the glycan portion of GP identified mannose, *N*-acetyl-D-glucosamine, and galactose in *N*-linked GP but not *N*-acetyl-galactosamine, *N*-acetylneuraminic acid, fucose, or sialic acid that were found in *N*- and *O*-linked GPs. The fact that the protein backbone has *N*-linked glycosylation sites but no evidence of *O*-linked glycosylation in GPs suggests that *N*-linked carbohydrates consist of glycoforms with differences in molecular mass, structure, and compositions between the 2 genotypes of *T. solium* that were the cause of the different banding patterns. There was no difference in GPs purified by polyclonal and monoclonal antibodies. It may be explained by the fact that GPs form a 120-kDa protein complex in fluid from *T. solium* cysts [26].

The deglycosylation by enzyme treatment resulted in the formation of 2 bands of 8 and 16 kDa, as described elsewhere [27]. The 8-kDa band was thought to correspond to the backbone protein, and the 16-kDa band might be an incomplete deglycosylated form, antigenically related protein, or homodimer and/or heterodimer of the 8-kDa protein described as likely being *Echinococcus* antigen B, which is closely related to *T. solium* GPs by unknown mechanisms [28, 29].

Despite the high correlation in respective absorbance values obtained from each comparison set (figure 2), the correlation coefficient value for African and American GPs was slightly higher than those for African and Asian or American and Asian GPs. mtDNA analyses revealed that *T. solium* could be divided into the 2 genotypes, African/American and Asian [11, 12]. Furthermore, the purified GPs could be differentiated into 2 banding patterns corresponding to the 2 known genotypes [11]. The higher correlation coefficient for the African/American genotype might have resulted from both GPs having been purified from *T. solium* belonging to the same genotype. In other words, the origin of the fluid from *T. solium* cysts may have affected the antigenicity of GPs. In fact, reactivity of serum from American patients to African/American GPs was higher than that of Asian patients, and the inverse phenomenon was also observed (data not shown). There are 2 possibilities to explain this feature: (1) the different sugar moieties create different banding patterns with different antigenicity and (2) different expression patterns of 8-kDa backbone proteins have different epitopes recognized by host immune responses, given that the 8-kDa protein is a multigene family and various 8-kDa protein genes have been identified in genomic and cDNA of *T. solium* [23]. There was no distinction in sensitivity between GPs derived from the 2 genotypes. The antigenicity of carbohydrate components on GPs and the expression patterns of backbone proteins remain for further studies.

Four of 60 human serum samples showed negative reactions

to Rec-Ag1V1/Ag2 protein (table 1). Because the native proteins were highly glycosylated, the potential contribution of the carbohydrate moiety to the antibody response might be considered. Obregon-Henao et al. [27] demonstrated diminished antibody reactivity for the native antigen after deglycosylation. However, our previous [9] and present data, using Rec-Ag1V1/Ag2, and the data of Hancock et al. [23], using a synthetic peptide of the same molecule, indicated that antibodies to peptide, not carbohydrates, exist in amounts in patient serum sufficient for the detection of *T. solium* infection. Alternatively, because both Ag1V1 and Ag2 are part of GPs expressed in fluid from *T. solium* cysts, the pattern of host immune responses might differ by individual, as was observed by Hancock et al. [23]. Nevertheless, there was no statistical difference in sensitivity between Rec-Ag1V1/Ag2 and GPs. This indicates that Rec-Ag1V1/Ag2 is a valuable target protein for serodiagnosis in humans and pigs. The facts that both Ag1V1 and Ag2 exist in *T. solium* worldwide without crucial variation and that it is easy to manage the quality and quantity of recombinant antigen, in contrast to native GPs, which we need to look for and/or maintain infected animals for, also support the usefulness of Rec-Ag1V1/Ag2.

To our knowledge, this is the first report of the characteristics of GPs from the 2 genotypes of *T. solium* worldwide and comparison of native GPs with recombinant chimeric protein as serodiagnostic antigens. Thus, the present work may contribute to the prophylaxis of the complex taeniasis-cysticercosis as advanced tools for detection of CC in humans and pigs with expectation of reducing the risk of infection and, consequently, the spreading of the disease in humans and pigs.

Acknowledgments

We thank W. Benitez-Ortiz (Ecuador), A. A. Kassuku (Tanzania), R. Moyoiu-Somo (Cameroon), T. Krecek (South Africa), A. Plancarte (Mexico), T. Ikejima (China), S. Y. Cho (Korea), and S. S. Margono and T. Wandra (Indonesia) for providing confirmed and/or highly suspected serum samples and for their technical assistance in the preparation of fluid from *T. solium* cysts.

References

- Schantz PM, Wilkins PP, Tsang VCW. Immigrants, imaging and immunoblots: the emergence of neurocysticercosis as a significant public health problem. In: Scheld WM, Craig WA, Hughes JM, eds. Emerging infections 2. Washington, DC: ASM Press, 1998:213–42.
- Ito A, Yamasaki H, Nakao M, et al. Multiple genotypes of *Taenia solium*—ramifications for diagnosis, treatment and control. *Acta Trop* 2003; 87:95–101.
- Taeniasis/cysticercosis and echinococcosis with focus on Asia and the Pacific: proceedings of the 5th international symposium, Asahikawa, Japan, 2005. *Parasitol Int* 2006; 55(Suppl):S1–308.
- Takayanagui OM, Odashima NS. Clinical aspects of neurocysticercosis. *Parasitol Int* 2006; 55:S111–5.
- Ito A, Craig PS. Immunodiagnostic and molecular approaches for the detection of taeniid cestode infections. *Trends Parasitol* 2003; 19:377–81.
- Tsang VC, Brand JA, Boyer AE. An enzyme-linked immunoelectro-transfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). *J Infect Dis* 1989; 159:50–9.
- Ito A, Plancarte A, Ma L, et al. Novel antigens for neurocysticercosis: simple method for preparation and evaluation for serodiagnosis. *Am J Trop Med Hyg* 1998; 59:291–4.
- Sato MO, Yamasaki H, Sako Y, et al. Evaluation of tongue inspection and serology for diagnosis of *Taenia solium* cysticercosis in swine: usefulness of ELISA using purified glycoproteins and recombinant antigen. *Vet Parasitol* 2003; 111:309–22.
- Sako Y, Nakao M, Ikejima T, Piao XZ, Nakaya K, Ito A. Molecular characterization and diagnostic value of *Taenia solium* low-molecular-weight antigen genes. *J Clin Microbiol* 2000; 38:4439–44.
- Ito A, Putra MI, Subahar R, et al. Dogs as alternative intermediate hosts of *Taenia solium* in Papua (Irian Jaya), Indonesia confirmed by highly specific ELISA and immunoblot using native and recombinant antigens and mitochondrial DNA analysis. *J Helminthol* 2002; 76: 311–4.
- Nakao M, Okamoto M, Sako Y, Yamasaki H, Nakaya K, Ito A. A phylogenetic hypothesis for the distribution of two genotypes of the pig tapeworm *Taenia solium* worldwide. *Parasitology* 2002; 124:657–62.
- Ito A, Nakao M, Okamoto M, Sako Y, Yamasaki H. Mitochondrial DNA of *Taenia solium*: from basic to applied science. In: Singh G, Prabhakar S, eds. *Taenia solium* from basic to clinical science. Wallingford, UK: CABI Publishing, 2002:47–55.
- Harlow E, Lane DP. Antibodies: a laboratory manual. Woodbury, NY: Cold Spring Harbor Laboratory, 1988.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227:680–5.
- Ito A, Nakao M, Wandra T. Human taeniasis and cysticercosis in Asia. *Lancet* 2003; 362:1918–20.
- Ito A, Wandra T, Yamasaki H, et al. Cysticercosis/taeniasis in Asia and the Pacific. *Vector Borne Zoonotic Dis* 2004; 4:95–107.
- Wandra T, Ito A, Yamasaki H, Suroso T, Margono SS. *Taenia solium* cysticercosis, Irian Jaya, Indonesia. *Emerg Infect Dis* 2003; 9:884–5.
- Ikejima T, Piao ZX, Sako Y, et al. Evaluation of clinical and serological data from *Taenia solium* cysticercosis patients in eastern Inner Mongolia Autonomous Region, China. *Trans R Soc Trop Med Hyg* 2005; 99:625–30.
- Wandra T, Subahar R, Simanjuntak GM, et al. Resurgence of cases of epileptic seizures and burns associated with cysticercosis in Assolougaima, Jayawijaya, Irian Jaya, Indonesia, 1991–95. *Trans R Soc Trop Med Hyg* 2000; 94:46–50.
- Santamaria E, Plancarte A, Aluja AS. The experimental infection of pigs with different numbers of *Taenia solium* eggs: immune responses and efficacy of establishment. *J Parasitol* 2002; 88:69–73.
- Yamasaki H, Allan JC, Sato MO, et al. DNA differential diagnosis of taeniasis and cysticercosis by multiplex PCR. *J Clin Microbiol* 2004; 42:548–53.
- Sako Y, Nakao M, Nakaya K, Yamasaki H, Ito A. Recombinant antigens for cysticercosis and echinococcosis. *Parasitol Int* 2006; 55:S69–73.
- Hancock K, Khan A, Williams FB, et al. Characterization of the 8-kilodalton antigens of *Taenia solium* metacestodes and evaluation of their use in an enzyme-linked immunosorbent assay for serodiagnosis. *J Clin Microbiol* 2003; 41:2577–86.
- Chung J-Y, Bahk YY, Huh S, Kang S-Y, Kong Y, Cho S-Y. A recombinant 10-kDa protein of *Taenia solium* metacestodes specific to active neurocysticercosis. *J Infect Dis* 1999; 180:1307–15.
- Plancarte A, Hirota C, Martinez-Ocana J, Mendoza-Hernandez G, Zen-

- teno E, Flisser A. Characterization of GP39-42 and GP24 antigens from *Taenia solium* cysticerci and of their antigenic GP10 subunit. *Parasitol Res* **1999**;85:680-4.
26. Lee EG, Bae YA, Jeong JY, et al. Proteomic analysis of a 120kDa protein complex in cyst fluid of *Taenia solium* metacestodes and preliminary evaluation of its value for the serodiagnosis of neurocysticercosis. *Parasitology* **2005**;131:867-79.
27. Obregon-Henao A, Gil DL, Gomez DI, Sanzon F, Teale JM, Restrepo BI. The role of N-linked carbohydrates in the antigenicity of *Taenia solium* metacestode glycoproteins of 12, 16 and 18 kD. *Mol Biochem Parasitol* **2001**;114:209-15.
28. González G, Nieto A, Fernández C, Örn A, Wernstedt C, Hellman U. Two different 8kDa monomers are involved in the oligomeric organization of the native *Echinococcus granulosus* antigen B. *Parasite Immunol* **1996**;18:587-96.
29. Mamuti W, Sako Y, Xiao N, et al. *Echinococcus multilocularis*: developmental stage-specific expression of antigen B 8-kDa-subunits. *Exp Parasitol* **2006**;113:75-82.