

Usefulness of Four Different *Echinococcus granulosus* Recombinant Antigens for Serodiagnosis of Unilocular Hydatid Disease (UHD) and Postsurgical Follow-Up of Patients Treated for UHD[∇]

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Four different recombinant antigens derived from *Echinococcus granulosus*, designated B1t, B2t, E14t, and C317, were tested with enzyme-linked immunosorbent assays (ELISAs) for the detection of specific immunoglobulin G (IgG) in patients with unilocular hydatid disease (UHD). The results were compared to those obtained with hydatid fluid and were subjected to receiver operator characteristic analysis. The diagnostic performance of the above-listed proteins was defined with respect to their specificity, sensitivity, and predictive values (PV); the influence of cyst location; and usefulness in the follow-up of surgical treatment for UHD and in the determination of whether or not patients have been surgically cured of UHD. The best diagnostic results were obtained with the anti-B2t IgG ELISA, with 91.2% sensitivity, 93% specificity, and high positive and negative PV (89.4 and 94.2, respectively). In addition, this diagnostic tool proved to be useful for the follow-up of surgically treated UHD patients. The anti-B2t IgG ELISA may find an application in the serodiagnosis of UHD in clinical laboratories.

Human unilocular hydatid disease (UHD) is caused by the larvae of the tapeworm *Echinococcus granulosus*. The diagnosis of UHD usually is approached by applying an imaging technique. In 2003, the World Health Organization Informal Working Group on Echinococcosis (28) proposed the use of ultrasonography (US) as the imaging technique of choice in order to promote uniform standards of UHD diagnosis and follow-up. It also was pointed out that US images suspected of showing UHD should be examined by alternative diagnostic methods, such as serological techniques (28). In addition, for pulmonary UHD, US is unhelpful in most cases, unless the cysts are close to the pleural surface (12). Currently, the preferred UHD immunodiagnostic techniques used in clinical practice detect serum immunoglobulin G (IgG) against crude parasite extracts (mainly hydatid fluid [HF]), most commonly by hemagglutination and enzyme-linked immunosorbent assay (ELISA) (26). However, HF cannot be standardized and gives rise to relatively frequent false-positive and false-negative results (3, 15). Moreover, HF is not useful for the follow-up of UHD, since the levels of specific (IgG) antibodies against HF remain high over long periods of time after curing (19). Many authors have attempted to find alternative antigens to improve UHD serodiagnosis and follow-up. Thus, purified, recombinant antigens and synthetic peptides, mainly derived from two parasite molecules (antigen B [AgB] and antigen 5 [Ag5]), have been applied, with various levels of success. Specifically, antigens derived from AgB have been shown to be good can-

didates for UHD serodiagnosis (3). AgB is formed by several subunits (6), two of which (AgB1 and AgB2) have been produced as recombinant molecules. Both AgB1 and AgB2 have been tested for the detection of specific antibodies, although authors disagree about the diagnostic performance of these two subunits (11, 20, 27). Thus, the standardization of currently available antigen preparations and the characterization of new candidate antigens remain to be undertaken to improve UHD diagnosis.

In this study, we set up an ELISA to perform an extensive serological survey. With this ELISA, we first compared the diagnostic performance of the N-terminal-truncated recombinant B1 and B2 antigens. We also obtained and assayed for the first time the usefulness of two additional recombinant antigens (E14t [23, 25] and C317 [5]) as diagnostic molecules, and their usefulness was compared to that of HF by ELISA. In addition, a comparison of the performance of these antigens was made regarding cyst location. Finally, we attempt to define a new tool for the postsurgical follow-up and detection of disease for patients treated for UHD.

MATERIALS AND METHODS

Antigens. Crude HF, obtained from fertile hydatid cysts from sheep at a slaughterhouse, was a kind gift from S. Jiménez (Servicio de Seguridad Alimentaria y Sanidad Ambiental, Consejería de Salud de La Rioja, Spain). HF was centrifuged at 1,000 × *g* for 5 min, and the protein concentration in the supernatant was measured with the MicroBCA protein assay kit (Pierce). Both the supernatant and the sediment (hydatid sands) were stored at –80°C until use.

Four recombinant antigens were used in this study: the carboxy-terminal portions of secretory antigens B1 and B2 (B1t and B2t; see below) and the E14t (23, 25) and C317 (5) somatic proteins. The B1t and B2t antigens were obtained as follows. Total RNA from *E. granulosus* hydatid sands was extracted with the RNeasy protect mini kit (Qiagen, Spain) and reverse transcribed with a first-

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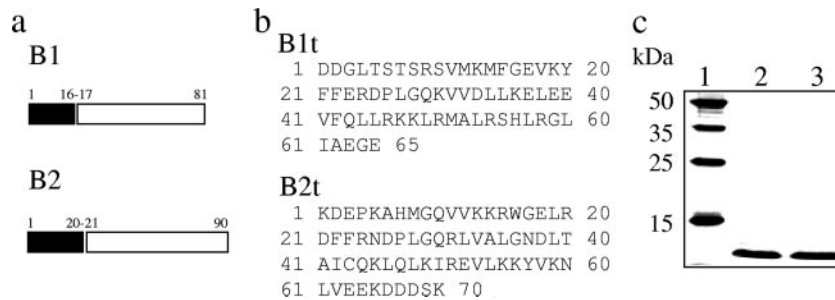


FIG. 1. Recombinant proteins B1t and B2t. (a) The coding regions of these proteins were obtained by RT-PCR of *E. granulosus* cDNA. Primers were based on the nucleotide sequences from antigens B1 and B2 available in GenBank (accession no. AF143813 and U15001, respectively) to amplify the sequence encoding the C-terminal portion of antigens B1 and B2 (white boxes) but not the coding region for the putative signal peptides from both sequences (black boxes). Numbers indicate amino acid positions. (b) The resulting RT-PCR products encoding the proteins B1t and B2t were subcloned in the pGEX-4T1 vector and expressed in *E. coli* BL21 CodonPlus RIL cells. (c) The purified proteins after thrombin cleavage are shown in a 15% acrylamide Coomassie blue-stained gel. Lane 1, molecular mass markers; lane 2, B1t protein; and lane 3, B2t protein. Relative molecular masses are shown to the left of the gel.

strand cDNA synthesis kit (Roche Farma, Spain). The resulting cDNA was used for PCR performed with the primer pairs B1Fwd (5'-GATGATGGCCTCACC TCG) plus B1Rev (5'-GTATTGGCACTCTATTC) and B2Fwd (5'-AAAGA TGAGCCAAAAGCAC) plus B2Rev (5'-AGGCAAATCATGTGTCGG), which were based on the nucleotide sequences from antigens B1 and B2 that are available at GenBank (accession no. AF143813 and U15001, respectively). Primers were designed to amplify the sequence encoding the C-terminal portion of antigens B1 and B2 (the last 65 and 70 amino acids of the full-length sequences, respectively) in order to eliminate the coding region for the putative signal peptides from both sequences (Fig. 1). These were predicted, with the SIG-Pred Internet tool (http://www.bioinformatics.leeds.ac.uk/prot_analysis/Signal.html), to be the most likely signal peptide in each sequence. The absence of signal peptide sequences prevents the potential secretion of the corresponding recombinant proteins during their expression in *Escherichia coli* (see below). PCR was performed for 30 cycles of 40 s at 94°C, 40 s at 48°C, and 1 min at 72°C and resulted in PCR products of 210 and 241 bp for B1t and B2t sequences, respectively. PCR products were electrophoresed in Tris-borate-EDTA (TBE), 1% agarose gels and were stained with ethidium bromide. Amplification products then were visualized in a UV transilluminator (UVP, Cambridge, United Kingdom) and purified with the StrataPrep DNA gel extraction kit (Stratagene, Spain). The purified fragments were cloned in the pCR 2.1 TOPO vector (Invitrogen, Spain), and the cloning reaction was used to transform *E. coli* DH5 α cells (Invitrogen). Plasmids extracted from the transformants with the StrataPrep plasmid miniprep kit (Stratagene) were automatically sequenced at the Automated Sequencing Service (Universidad de Salamanca), digested with EcoRI (Roche Farma), and electrophoresed, and the resulting products were subcloned in the pGEX-4T1 expression vector (GE Healthcare Lifesciences, Spain) with T4 DNA ligase (Roche Farma). The ligation products were used to transform the BL21 CodonPlus RIL *E. coli* strain (Stratagene). Plasmids extracted from the transformants were automatically sequenced as described above to check the open reading frames. Selected transformants were grown in 2 \times yeast extract tryptone (2 \times YT) medium overnight with gentle shaking. The resulting cultures were diluted 1:100 in 2 \times YT medium and grown for a further 3 h under the same conditions. Protein expression was induced with 0.1 mM isopropyl- β -D-thiogalactopyranoside (IPTG; Applied Biosystems, United Kingdom) for 3 h under the same conditions. The corresponding recombinant proteins were extracted from the resulting pellet cells, purified, and thrombin cleaved, as described elsewhere (27).

E14t recombinant protein was obtained as described elsewhere (25), and the C317 recombinant polypeptide (accession no. B1244032) was a kind gift from C. Fernández and E. Osinaga (Cátedra de Inmunología and Departamento de Inmunobiología, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay).

The concentrations of the B1t, B2t, E14t, and C317 recombinant proteins were measured with the MicroBCA protein assay kit (Pierce). The purity and integrity of the recombinant proteins and the HF were checked in 15% polyacrylamide gels stained with Coomassie blue. All proteins were stored at -80°C until use.

Sera. A total of 265 sera were collected and classified as follows: 102 sera from surgically confirmed UHD patients, 68 sera from patients with other parasitic diseases (10 alveolar echinococcosis, 20 schistosomiasis, 10 teniosis, 12 cysticercosis, 8 dirofilariosis, 4 fasciolosis, and 4 mixed infections [fasciolosis and schistosomiasis]) confirmed by parasitological and/or serological diagnosis, and 95

sera from healthy donors from the Canary Islands, an area free of UHD. From the 102 UHD sera, the cyst location was known for 100 patients; thus, this group of sera was divided into 86 samples from liver and 14 from lung hydatid cyst patients.

Finally, 71 sera from 35 UHD patients subjected to and cured by surgical intervention were recovered to monitor the level of specific UHD antibodies for 11 years. These sera were classified into six groups as follows: before surgery ($n = 35$), 6 months after surgery ($n = 10$), 1 year after surgery ($n = 7$), 3 years after surgery ($n = 8$), 7 years after surgery ($n = 5$), and 11 years after surgery ($n = 6$). All patients were followed up by using an imaging technique (US) for 11 years.

ELISA. The ELISA technique for the detection of total IgG for the comparison of the diagnostic performance of the four recombinant antigens and the HF was performed as follows. Ninety-six-well polystyrene plates (Corning, Spain) were incubated at 4°C overnight with 100 μ l/well B1t, B2t, E14t, C317 (0.5 μ g/ml), or HF (5 μ g/ml) in carbonate buffer (pH 9.6). Plates then were washed six times with phosphate-buffered saline (PBS) (pH 7.4) and 0.05% Tween 20 (washing buffer) and incubated for 1.5 h at 37°C with 200 μ l washing buffer plus 1% bovine serum albumin (BSA; Sigma Aldrich, Spain) (blocking buffer). Sera then were added in duplicate (100 μ l/well) at 1:200 dilutions (or 1:100 for the C317 antigen) in blocking buffer, and the plates were incubated for 1 h at 37°C. After the plates were washed as described above, the secondary antibody (peroxidase-labeled rabbit anti-human IgG; Sigma Aldrich) was added (100 μ l/well) at a 1:2,000 dilution in blocking buffer, and the plates were incubated for 1 h at 37°C. After the plates were washed as described above, the reaction was developed in the dark with 100 μ l/well of citrate buffer, pH 5, plus orthophenylene diamine (0.28 mg/ml; Sigma Aldrich) and hydrogen peroxide (0.4 μ l/ml; Sigma Aldrich). The reaction was stopped with 50 μ l/well of 3 N sulfuric acid, and plates were read at 492 nm in an ELISA reader (EAR 400 FT; SLT Lab Instruments, Germany).

Statistics. The serological index was calculated for each optical density and was used to establish a common cutoff for all of the ELISAs performed for each antigen by using the following formula: $[(NC - S)/(NC - PC)] \times 100$, where NC and PC represent the negative and positive controls, respectively, and S stands for each serum.

Receiver operator characteristic (ROC) analysis was used to determine the diagnostic value of each antigen (10). The levels of sensitivity were plotted against the levels of 1 minus the specificity at each cutoff point on an ROC curve. The threshold (cutoff) values used for the four antigens were those giving the highest percentage of sensitivity and specificity for each antigen. The area under the ROC curve (AUC) was used to define the discriminatory values of the antigens (between subjects with the disease and those without it). The nonparametric chi-square test was used to test differences between AUCs. Positive and negative predictive values were calculated as described elsewhere (17).

All statistical calculations were done with the Epidat-3.1 statistics program, available at <http://dxsp.sergas.es/default.asp>.

RESULTS

Comparative performance of B1t and B2t recombinant subunits in ELISA. Owing to the disagreement among several

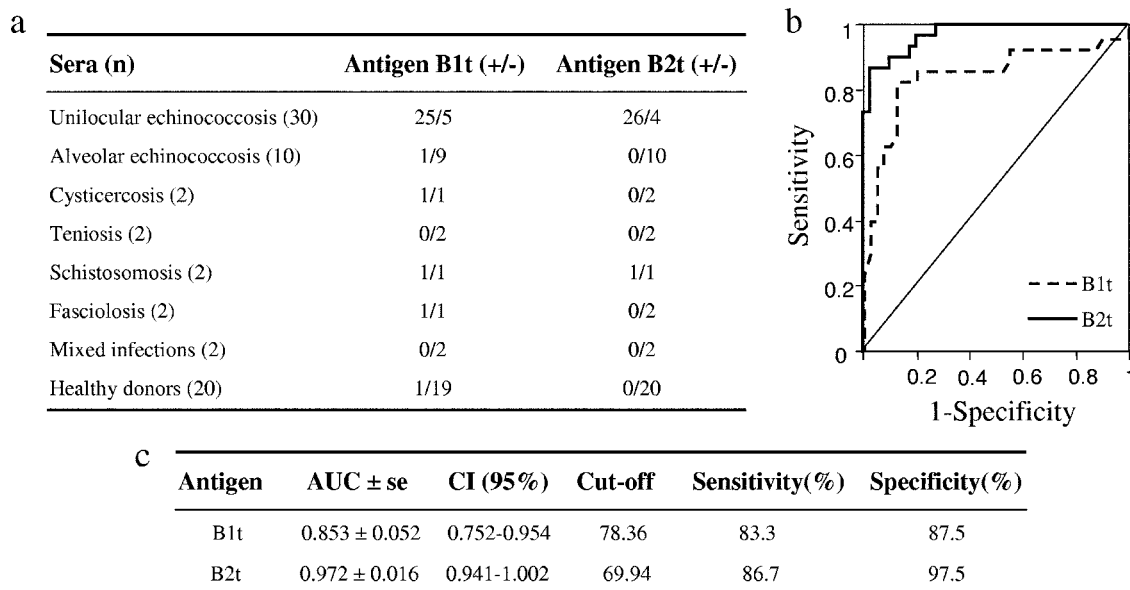


FIG. 2. Comparative analysis between the diagnostic performances of B1t and B2t recombinant proteins in IgG ELISA with a panel of defined sera. (a) The number of positive and negative (+/-) sera against B1t or B2t antigen. (b) ROC curves used to determine AUC and cutoff values for the B1t and B2t antigens. (c) The calculated AUCs ± standard errors (se), confidence intervals (CI), cutoffs, sensitivity, and specificity for each antigen are shown.

authors regarding the comparative diagnostic performance of the B1 and B2 recombinant antigen subunits (11, 20, 27) and to the differences in the sequence composition between the B1t and B2t antigens (Fig. 1) and those used by the above-mentioned authors, we first compared the diagnostic usefulness of B1t and B2t in an IgG ELISA using a panel of selected sera (Fig. 2). Our results showed that the B2t antigen afforded better AUC, sensitivity (86.7%), and specificity (97.5%) values than those for the B1t recombinant protein (83.3% sensitivity and 87.5% specificity) (Fig. 2). For this reason, we discarded the B1t antigen in ensuing experiments. We discarded the B1t antigen also because of the limitations in the volume of each serum available and the low efficiency in B1t production obtained under our experimental conditions.

Usefulness of B2t, E14t, and C317 recombinant antigens for the serodiagnosis of hydatid disease. Two additional recombinant proteins, E14t and C317, were tested individually and compared in ELISA to the B2t and HF antigens, since the combination of new recombinant antigens with the B2t molecule could improve the overall sensitivity and specificity of the serological test; for this comparison, a defined panel of sera was used (Fig. 3). The best AUC value was obtained with antigen B2t (0.949), with consecutively decreasing AUC values for the HF (0.898), C317 (0.714), and E14t (0.611) antigens (Fig. 3). Comparison of the AUCs from the four above-mentioned antigens by using the nonparametric chi-square test revealed significant differences ($P < 0.05$) among them, demonstrating that these differences were due solely to the antigens used in the study and not to other parameters. The antigen B2t also gave rise to the best sensitivity (91.2%), specificity (93%), and positive and negative predictive values (89.4 and 94.2%, respectively) (Fig. 3).

Finally, the sera giving positive ELISA scores with the C317 and E14t antigens also were positive in the B2t ELISA.

Comparison of levels of specific antibodies against the HF, B2t, E14t, and C317 antigens in patients with liver or lung hydatid cysts. Two AUCs from the respective ROC curves corresponding to the liver and lung were calculated for each antigen (HF, B2t, E14t, and C317), representing the comparative usefulness of the above-mentioned antigens for the detection of UHD in patients depending on the cyst location (liver or lung). The AUCs for the four antigens were slightly higher for patients with lung cysts than for those obtained for patients with liver cysts (Fig. 4). A comparison of the liver and lung AUCs from each antigen by using the nonparametric chi-square test did not uncover significant differences ($P < 0.05$) between them (Fig. 4).

Usefulness of the B2t recombinant antigen for the follow-up of hydatid patients. The usefulness of the B2t recombinant antigen for the follow-up of patients cured by surgical cyst removal was studied using ELISA with a group of 35 patients. The results of this ELISA were compared to the ELISA results obtained with HF using the same sera. The 35 sera proved to be positive (100%) for antibodies against both HF and B2t antigens just before surgery (Fig. 5). During the follow-up period (up to 11 years), the number of sera positive for antibodies against the B2t molecule began to decrease 1 year after surgery, with 25 and 0% of the sera being B2t positive at 3 years and 7 years after surgery, respectively (Fig. 5). In contrast, IgG antibodies against HF persisted in 100% of patients at 7 years after surgery, decreasing to 67% of patients at 11 years after surgery (Fig. 5).

DISCUSSION

The diagnosis and follow-up of patients with UHD by means of serology have limited support in clinical practice, despite the advent of several recombinant and peptide antigens that po-

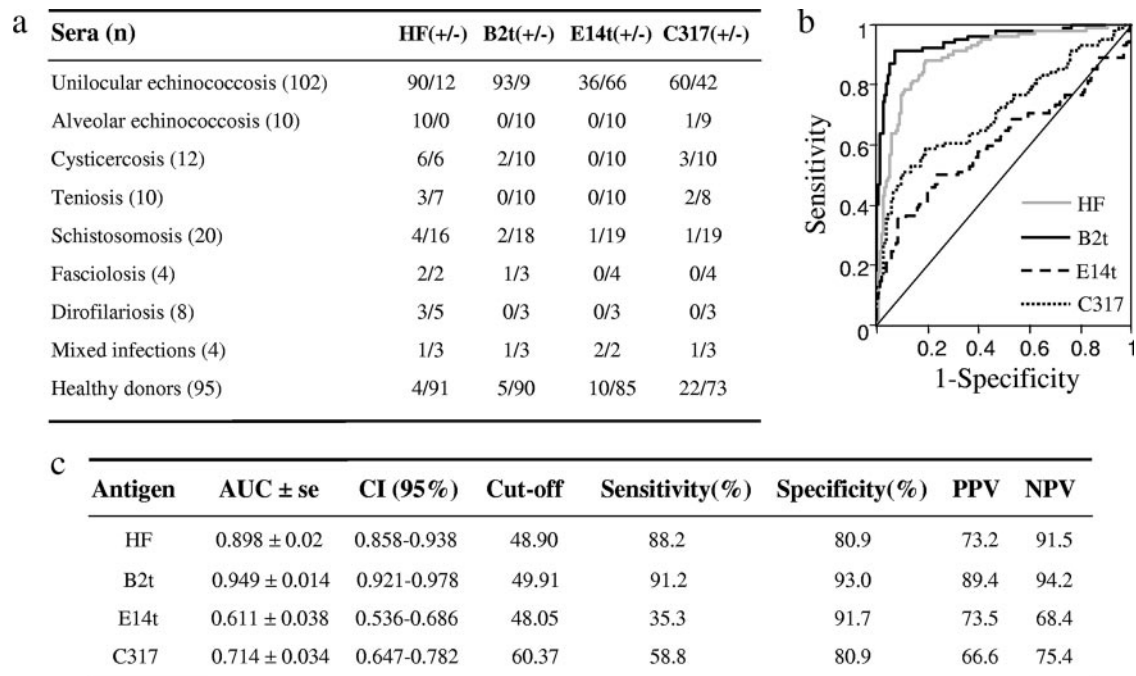


FIG. 3. Applicability of B2t, E14t, and C317 recombinant proteins for the diagnosis of UHD by IgG ELISA compared to that of HF with a panel of defined sera. (a) Number of positive and negative (+/-) sera against HF, B2t, E14t, and C317 antigens. (b) ROC curves used to determine AUC and cutoff values for the HF, B2t, E14t, and C317 antigens. (c) The calculated AUCs \pm standard errors (se), confidence intervals (CI), cutoffs, sensitivity, and specificity for each antigen are shown. Positive and negative predictive values (PPV and NPV, respectively) also are shown.

tentially are useful for the detection of specific antibodies. This is due mainly to the lack of standardization of both the antigens and the techniques applied. The characterization of new antigens that might improve the serodiagnosis of UHD is also an important task.

This study reports an assessment of two recombinant subunits of the antigen B, B1t and B2t, and the use of two new recombinant antigens, E14t and C317, in an ELISA for the detection of specific antibodies in UHD patients. For this study, we selected a well-characterized and extensive panel of sera that included sera from patients with UHD ($n = 102$) confirmed by surgery; from patients with different parasitic diseases ($n = 68$), especially those related to *E. granulosus* at the antigen composition level (alveolar hydatid disease, cysticercosis, teniosis, schistosomosis, fasciolosis, and nematodes) (7, 18, 30); and healthy donors ($n = 95$). This latter group of individuals was chosen from an area free of *E. granulosus*, because healthy donors from areas in which *E. granulosus* is endemic could give rise to false-positive results from individuals who have been in contact with the parasite without developing the disease (32).

After setting up the ELISA technique with the recombinant antigens, we first compared the diagnostic performance of the B1t antigen to that of the B2t antigen. This comparison was undertaken because of the equivocal results obtained by other authors, which pointed to a conflict with regard to the values of sensitivity and specificity attained when the B1t or the B2t antigen, as well as defined peptides derived from the above-mentioned sequences, were used in the ELISA. Similarly to the results of Rott et al. (20) and Virginio et al. (27), our results showed that the B2 subunit provides better diagnostic perfor-

mance than the B1t antigen. Those authors found values of 84 to 93.1% for sensitivity and 98 to 99.5% for specificity for the B2t antigen and 55 to 84.5% for sensitivity and 80 to 91.2% for specificity for the B1 polypeptide. In contrast, Lorenzo et al. (11) found higher sensitivity and specificity values for the B1 recombinant antigen (72.9 and 89.5%, respectively) than for B2t (66.7 and 71.2%, respectively). These results match those obtained with the corresponding derived peptides (3). Surprisingly, Lorenzo et al. (11) attributed the best diagnostic performance to HF instead of the B1 and B2 antigens, especially with defined sera from specific geographical areas. These differences could be partially attributed to the panel of sera used by each research group, but they also could be attributed to slight sequence variations in the recombinant proteins that could affect their antigenicity. Thus, the lack of the signal peptide sequence in the B1t and B2t recombinant proteins could enhance the diagnostic value of the B2t subunit compared to that of the B1t antigen, making the present results an advancement over those of previous work.

We therefore chose the B2t antigen for subsequent experiments. Nevertheless, the diagnostic values achieved with this antigen could be improved, e.g., through its combination with new antigens that react with otherwise false-negative sera. Consequently, we tested two additional antigens in our ELISA, E14t and C317, located in *E. granulosus* protoscoleces (24) and the cyst germinal layer (5), respectively; both represent biologically well-characterized somatic parasite antigens. Under our ELISA conditions, both the E14t and the C317 molecules provided relatively low specificities and sensitivities compared to the respective values for the HF and B2t antigens. Furthermore, E14t- and C317-positive sera also were found to be

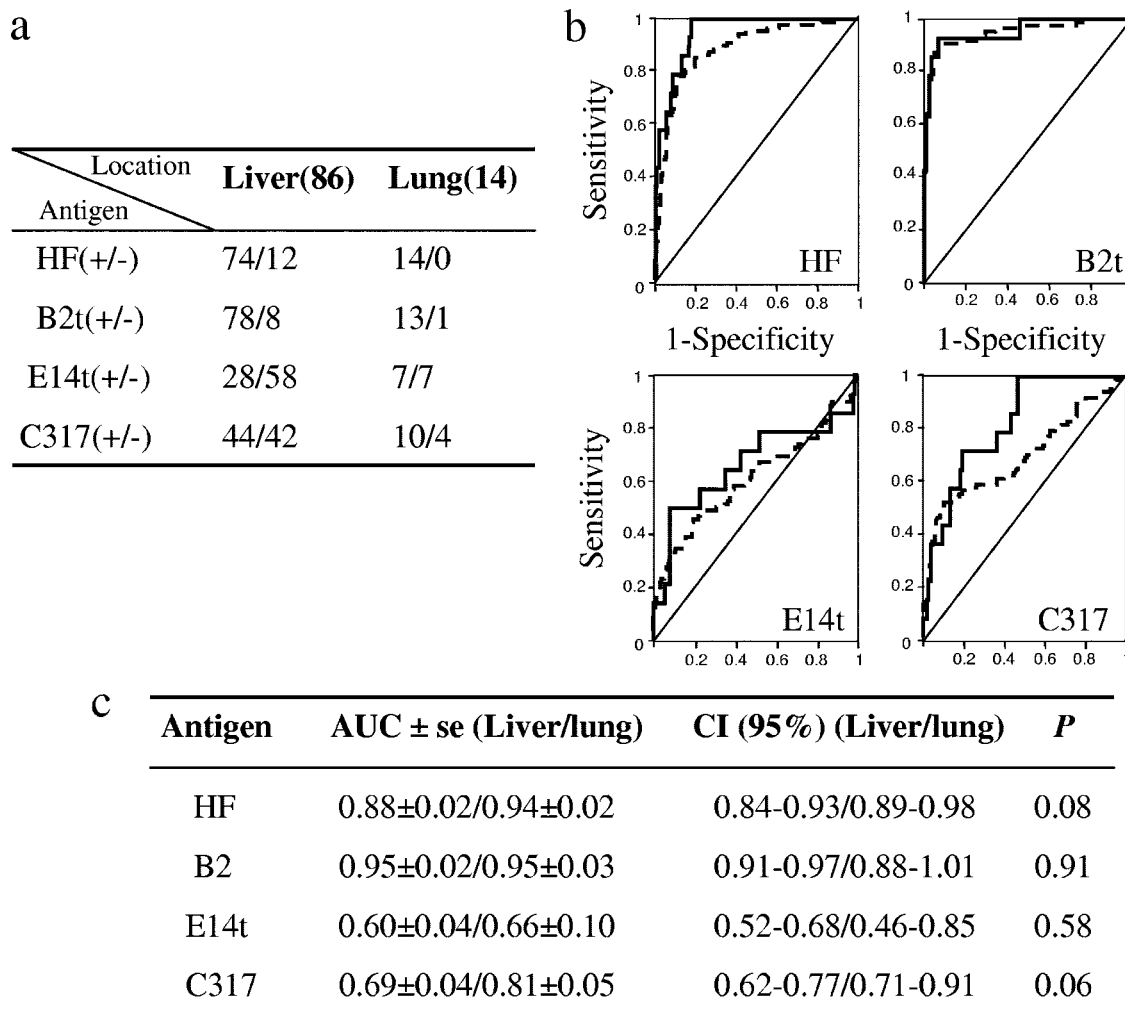


FIG. 4. Influence of cyst location on the diagnostic performance of HF, B2t, E14t, and C317 antigens by IgG ELISA with a panel of defined sera. (a) Number of positive and negative (+/-) sera against HF, B2t, E14t, and C317 antigens. (b) ROC curves used to determine AUC and cutoff values for the HF, B2t, E14t, and C317 antigens in the detection of liver (dashed lines) or lung (black lines) cysts. (c) The calculated AUCs ± standard errors (se), confidence intervals (CI), and statistical significances (P) are shown. Differences are considered statistically significant for P values of <0.05.

reactive against the Bt2 antigen and hence did not add any further diagnostic advantage above that of the Bt2 antigen. The B2t antigen showed the best scores for sensitivity (91.2%), specificity (93%), positive predictive (89.4%) values, and negative predictive (94.2%) values, followed by the HF, C317, and Et14 antigens. Additionally, the statistical analysis confirmed that the diagnostic differences among the four antigens tested here were due solely to their own characteristics and not to other factors (e.g., the panel of sera used). It is noteworthy that the B2t antigen did not cross-react with sera from patients with *E. multilocularis*. This could be an additional advantage over the HF and other *E. granulosus* antigens, especially in areas of the world in which *E. granulosus* and *E. multilocularis* coexist. Nevertheless, a higher number of sera should be tested to draw a statistical conclusion.

Several authors have tested different somatic proteins for the detection of specific antibodies in sera from UHD patients. Among them, the best characterized are malate dehydrogenase

(EgcMDH), assessed with an ELISA (11, 27), and rEpC1-GST, tested in an immunoblot assay (9). Regarding the EgcMDH antigen, the results described (95.1% sensitivity and 89.7% specificity [27]) are no better than those reported here for the B2t antigen. In addition, a high variability in specificity and sensitivity compared to the results for the EgcMDH antigen has been observed by other authors who used the same antigen in an ELISA (71.8% sensitivity and 62.2% specificity [11]). Similar results have been described for the vast majority of somatic molecules applied in UHD ELISA (3). The rEpC1-GST antigen, in contrast, seems to have better performance than the B antigen (92.2% sensitivity for rEpC1-GST versus 84.5% sensitivity for B antigen), although the comparison was done by immunoblotting and the B antigen represented a purified fraction of native HF (9); such factors could influence the comparative data obtained by those authors. Thus, we, like other authors, failed to observe that the newly tested somatic antigens improved the results obtained with the B (B2t) re-

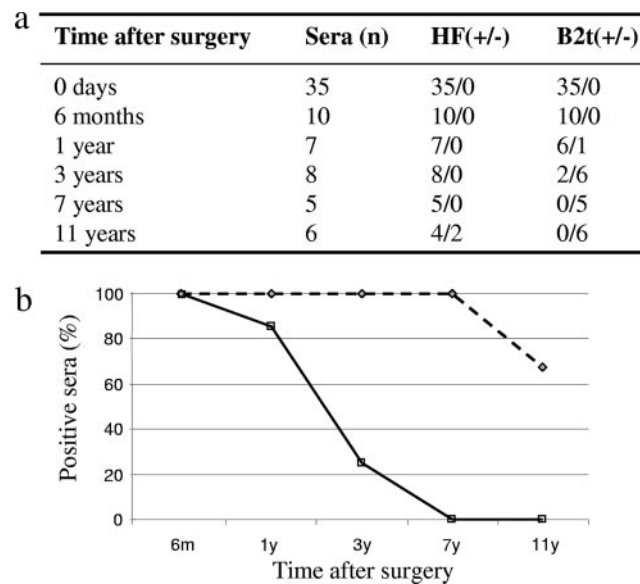


FIG. 5. Comparative performance of HF and B2t antigens in IgG ELISA for the follow-up of the treatment of UHD and for determining whether the patients are cured of UHD. (a) Time intervals after surgery, number of sera for each time interval, and number of positive and negative (+/-) sera against the HF and B2t antigens at each time interval. (b) Percentage of positive sera against HF (dotted line) and B2t (black line) antigens at each time interval after surgery. m, months; y, years.

combinant antigen. The low sensitivities reported here for the E14t and C317 antigens could be due to the lack of recognition of the native antigens by sera of UHD patients. These results and those obtained by others suggest that somatic antigens that are not directly in contact with the host would not be useful for the serodiagnosis of UHD.

Regarding cyst location and specific antibody levels, it has been reported that the intensity of the immunological response to hydatid cysts may differ depending on the anatomical location of the parasite. In general, it has been postulated that the location of cysts in the liver will elicit higher antibody levels than those in the lung (1), and this will affect the sensitivity of a given serological test. Nevertheless, it has not been clearly stated whether these differences would be due to the antigen used in the test or to other factors (e.g., technique, antibody isotype, etc.). In this respect, it has been reported that IgE detection gives rise to lower sensitivity rates for pulmonary cysts than for hepatic lesions regardless of the technique applied for diagnosis (13, 14, 31). The opposite results are obtained when the detected isotype is IgM (13). Additionally, by using two different antigens (HF and native antigen B), the detection of total IgG by ELISA has been shown to be location independent (13, 14, 22). This is in agreement with our own results, since we failed to find significant differences in antibody levels resulting from cyst location with any of the antigens used during our study. Thus, the detection of specific IgGs in UHD patients by ELISA should provide similar results, regardless of cyst location, independently of the antigen used for the diagnosis.

The risk of recurrence of human UHD after surgery varies from 3 to 16% (29), but today's imaging methods are not fully functional for the detection of new cysts, whether or not a patient has been cured, or small changes in cyst morphology (19). In addition, serological testing has its own drawbacks,

because specific antibodies may persist in patients' sera for several years after recovery, especially those against HF (16). Thus, the follow-up of UHD patients and the determination of whether or not they have been cured also remain a problem to be solved. In this work, we assessed the usefulness of long-term serological monitoring by the detection of antibody (IgG) against the B2t antigen (and compared it to the usefulness of long-term monitoring by the detection of IgG against anti-HF) to determine whether or not patients were cured of UHD. A series of 35 patients was studied, all of whom had undergone surgical treatment and had been subjected to an imaging follow-up lasting 11 years. Our findings confirm that levels of HF-specific IgGs remained elevated in the majority of cured patients throughout the study period. In contrast, levels of anti-B2t-specific IgG began to decrease 1 year after surgery, with all patients being negative for this antigen between 3 and 7 years after being cured.

The results obtained by other authors have shown that IgG is the most common antibody isotype in UHD patients, normally persisting over prolonged time periods after they are cured (14, 19, 31). The levels of additional antibody isotypes, such as IgE, IgA, and IgM, seem to decline 2 to 4 years after the cure, as reported elsewhere (2, 4, 8, 21). Unfortunately, only a limited number of UHD patients show detectable levels of these isotype antibodies (21). In addition, these changes in antibody levels have been demonstrated both with crude and purified native antigens but not with recombinant or peptide-derived parasite proteins. Only one *E. granulosus*-derived recombinant antigen has been tested for its usefulness in the follow-up of UHD patients, and unfortunately the tests were unsuccessful, since specific antibody levels against this recombinant protein (rEpC1-GST) persisted 6 years after the cure (9). Thus, our results demonstrate for the first time that a defined *E. granulosus* recombinant antigen, B2t, could be use-

ful for determining whether or not patients have been cured of UHD, although its usefulness in the follow-up of pharmacologically treated patients needs to be further investigated.

In conclusion, we have defined a new serodiagnostic tool, B2t ELISA, for the detection of specific IgG with high specificity and sensitivity; this tool is potentially applicable for the follow-up of the treatment of UHD and for determining whether or not patients have been cured of UHD.

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