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Specific Recognition of Hydatid Cyst Antigens by Serum IgG, IgE, and IgA Using Western Blot

Younes Sbihi, Dirk Janssen, and Antonio Osuna*

Instituto de Biotecnología, Universidad de Granada, Granada, Spain

Diagnosis of hydatid disease in humans relies on the detection of specific antibodies against antigens of the metacestode from *Echinococcus granulosus*. The specificity and sensitivity of current immunological techniques based on specific serum IgG rely on the way antigens are purified. We used Western immunoblotting to detect specific IgG, IgE, and IgA antibodies in serum from patients with hydatid disease using either crude antigen preparations (total hydatid fluid), purified fractions enriched in Antigens 5 and B, and glycoproteins from hydatid fluid. Depending on whether crude HF or purified antigen fractions were used, IgG and IgE recognized specifically low-to-medium MW bands between 12 and 42 kDa. IgA recognized specifically 110 kDa band in crude hydatid fluid and in the glycoprotein fraction of hydatid fluid, and a 42 kDa band in all antigen samples used. Besides the advantage of detecting specific IgA in crude hydatid fluid, these results offer the possibility of simplifying future immunological tests if specific secretory IgA can be similarly detected. J. Clin. Lab. Anal. 11:154–157, 1997.

Key words: *Echinococcus granulosus*; hydatid disease; Western blotting; IgE; IgG; IgA; antigen

INTRODUCTION

Hydatidosis is a zoonosis caused by the larval stage of the tapeworm *Echinococcus granulosus*. The unilocular metacestode, or hydatid cyst, parasitizes a variety of mammals in a large series of anatomical sites such as the lungs, liver, heart, and brains. Humans are normally dead-end hosts, with an estimated global incidence of hydatidosis of >100,000 cases per year. In endemic areas, the surgical rate is >10 million in a population per year. Although acute complications can arise due to anaphylactic responses after cyst rupture, many infections are asymptomatic. Clinical complaints in humans are generally related to the pressure of isolated cysts growing against organs. Patients are often subjected to a morphological examination by image techniques such as computerized tomography or nuclear magnetic resonance that gives information on the location of cysts. However, to differentiate hydatidosis from other cystic lesions or tumors, the clinical diagnosis relies on immunological techniques (1,2).

Whereas raised specific antibody titers in patients with hydatid disease are routinely assayed by techniques such as indirect haemagglutination or latex agglutination, immunoelectrophoresis, and indirect fluorescent antibody tests (3), the importance of defined diagnostic antigens such as antigens 5 and B becomes evident through ELISA and Western blotting assays (4). We recently showed that certain methods to purify parasite antigens can strongly improve the sensitivity and specificity of these assays (5). Nevertheless, although there is a general agreement on the need for antigens that enable specific and sensitive diagnosis of hydatidosis, the specific antibodies that are screened for are mostly of the IgG class. In fact, specific antibodies in all major classes of immunoglobulin, i.e., IgM, IgG, IgA, and IgE, have been demonstrated in sera from humans with hydatid disease (6,7), but so far there is little information on which antigens are recognized by the different Ig classes. In this report, compare qualitatively the specific recognition of hydatid antigens, obtained after different purification methods, by IgE, IgG, and IgA, and we show that this approach may improve and simplify immunological techniques for a routine practice.

MATERIALS AND METHODS

Parasitic Material

Hydatid cyst fluid (HF) was obtained through local slaughterhouses in Spain from infected sheep liver. Freshly isolated hydatid fluid was centrifuged (4,000 g, 30 min), the supernatant was removed and supplemented with NaN_3, (1 g/l) and EDTA (5 mM), and stored at -20°C before use.

*Correspondence to: Antonio Osuna, Instutito de Biotecnología, Universidad de Granada, Campus Fuentenueva s/n, 18071 Granada, Spain.

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Antigen Preparation

Fractions of HF that were enriched in antigens 5 and B (further labelled as fraction *Ag5/B*) and fractions containing predominantly glycoproteins (labelled *GP*) were prepared as previously described (5). In short, fraction *Ag5/B* was obtained following Rogan et al. (8) based on the method of Oriol et al (9): HF was dialysed against 5 mM acetate buffer (pH 5.0) and centrifuged at 48,000 g for 30 min. The precipitate was dissolved in 0.2 M phosphate buffer (pH 8.0) and boiled for 15 min. After centrifugation, the supernatant was removed and depleted from host antibodies by passing it through a protein G column (Pharmacia LKB, Uppsala, Sweden), following the manufacturer's instructions. The *GP* fraction of HF was obtained by diluting HF (1/1) in a Tris buffer (Tris-HCl 0.02 M, NaCl 0.5 M, pH 7.4) and passing it through a Con A-Sepharose column (Pharmacia) according to Kennedy et al. (10). Bound fractions were eluted with the same buffer but supplemented with methyl α-D-mannopyranoside, and concentrated by dialysis and lyophilization. Protein concentrations of HF, fraction *Ag5/B* and fraction *GP* were measured by micro-Lowry (11).

Sera

Sera from patients with cysticercosis (4) confirmed by clinical symptoms and serology, as well as 11 sera from patients with surgically hepatic hydatid disease, were obtained from Virgen de la Nieves Hospital (Granada, Spain). Nine other sera from hydatidosis patients with identical cyst locations were obtained from Ibn Sina Hospital (Rabat, Morocco). These cases were aged 20–40 years. Control sera were from healthy volunteers resident in Spain, showed no abnormality on medical examination, and had no antibody to *E. granulosus* as assayed by enzyme-linked immunosorbent assay (ELISA).

Western Immunoblotting

Samples of hydatid antigen (0.1 µg) were subjected to discontinuous SDS-PAGE in 12.5% homogeneous polyacrylamide gels (PhastGel™) using the PhastSystem (Phamacia LKB). The antigens were transferred from the unstained gel to nitrocellulose membranes (Schleicher & Schuell, Keene, NH) by means of the PhastTransfer (Pharmacia LKB) following standardized procedures (12). Efficiency of transfer was checked by staining strips of blotted antigen with Amido Black (0.01%). Membranes with blotted antigen were cut into strips and blocked for 3 hours at room temperature in PBS with Tween 20 (0.2%) and gelatine (0.4%). Next, the strips were washed (PBS with 0.1% of Tween 20) and incubated for 2 hours at room temperature with test sera diluted 1:50 previously determined (5). After three washings, the strips were further incubated with peroxidase labelled antibodies against human IgG (Fc specific, Sigma, Lot number: 114H4804) and human IgA (α-chain specific, Sigma, Lot Number: 013H4803) at a dilution of 1/800, and were developed with Diaminobenzamidine tetrahydrochloride (0.05%) and H_2O_2 (1/5,000). Bound IgE antibodies were detected with alkaline phosphatase-conjugated monoclonal antihuman IgE (Sigma, St. Louis, MO, Lot number: 064H4823) at a dilution of 1/800, and were developed with Fast Red/Naphthol (Sigma) prepared as described (13).

RESULTS

When subjected to SDS-electrophoresis and Western blotting, HF antigens were recognized by IgG from hydatidosis patients with a specificity similar to what we reported in a recent study (5) of unfractionated HF series of bands with MW ranging from 12–110 kDa was revealed after incubation with IgG, but only those bands with MW of 12–14 kDa, and of 34 kDa were specific for hydatid disease, and lacked cross-reactivity with cysticercosis disease. Also, the GP antigen fraction, obtained after Con A-chromatography of hydatid fluid, was recognized as a similar series of MW bands, However, only a 42 kDa band was specifically recognized by IgG from patients suffering hydatid disease. When assayed against Ag5/B as antigen, IgG specifically recognized bands with MW of 12–14, 20, 34, 39 and 42 kDa (Table 1).

The IgE subclass of immunoglobulins from sera from cysticercosis patients or from healthy volunteers did not yield any detectable bands in total hydatid fluids after SDS-PAGE and immunoblotting (Fig. 1). In contrast, when sera from hydatidosis patients was assayed, IgE revealed a number of low MW bands (12–14, 20, 34, and 37 kDa). These, together with a 40 kDa antigen were found when the Ag5/B fraction of HF was used as antigen source. In contrast, IgE revealed only a single 39 kDa band in the GP fraction of HF, but that was also observed after incubation with sera from healthy volunteers and from cysticercosis patients (Fig. 1, column C).

Immunoglobulin A recognized a series of medium to high MW bands in HF and the Ag5/B fraction of HF after immunoblotting. Of these, only a strongly developed 42 kDa band was specific for hydatid disease. When total HF was used as antigen source, a specific 110 kDa band was also faintly visible. Both the 42 and 110 kDa bands were strongly

TABLE 1. Specific Recognition of Antigen Subunits (kDa) by Sera From Patients With Hydatid Disease, After SDS-PAGE and Western Immunoblotting

	Immunoglobulin subclasses		
Source of antigen[a]	IgG	IgE	IgA
HF	12–14, 34	12–37	42, 110
GP	42	ND	42, 110
Ag 5/B	12–42	12–40	42

[a]HF, total hydatid fluid; GP, glycoproteins obtained after Con A chromatography; Ag 5/B, HF fraction enriched in antigens 5 and B.
ND = not detected.

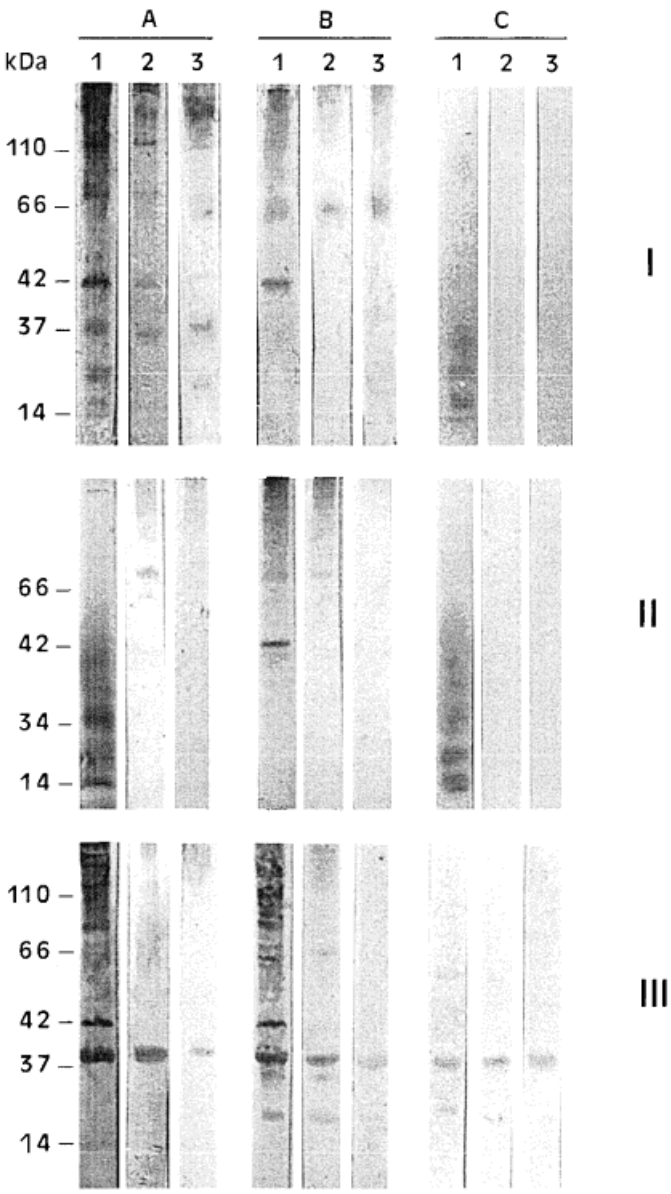

Fig. 1. Immunoblots of total hydatid fluid antigen (I), fraction Ag5/B (II), and GP antigen preparation (III). Blotted antigens were incubated with pools of sera from patients with hydatidosis (lane 1), cisticercosis (lane 2) and from healthy subjects (lane 3). Bound antibodies were detected with conjugate of antihuman immunoglobulin IgG (**A**), IgA (**B**), and IgE (**C**).

and specifically revealed when the GP fraction of HF was used as an antigen source (Fig. 1, column B).

DISCUSSION

In 1912, Casoni (14) initiated the immunodiagnosis of human hydatid disease, showing that the intradermal injection of HF antigen caused an immediate hypersensitivity reaction. During later decades, and in view of the poor specificity of this historical test, other techniques were applied such as the complement fixation test, indirect haemagglutination, and latex agglutination tests, and finally ELISA. As immunological techniques advanced, a better knowledge of hydatid antigens became crucial. More than 10 parasitic antigens are present in the HF (15). Two of these antigens have been extensively studied, the thermolabile antigen 5 (16), and the thermostable antigen B (9). Ag 5 is a lipoprotein of varying high MW (~ 400 kDa) comprising subunits of 60–70 kDa, which themselves dissociate on disulphide bond reduction to two subunits of ~ 20 and 40 kDa. Ag B is a lipoprotein of ~150 kDa, comprising three subunits of 12, 16, and 20 kDa which are unaffected by reduction. In particular, the use of Western blotting techniques has confirmed the importance of these antigens in the immunodiagnosis of human hydatidosis (4). Recently, we showed that differences in purification protocols may change the ability of antigens to be recognized by specific IgG antibodies and therefore strongly determine the diagnostic value of the antigens (5). However, the detection of specific antibodies of classes other than IgG has been less studied, despite the documented presence in serum of patients suffering hydatidosis of specific IgM, IgA, and IgE. Immunoglobulin E is produced against potent allergies within the cyst, and the patients show cutaneous immediate hypersensitivity to these components (17), and has generally been measured in serum samples using the radioallergosorbent test (18).

Measuring specific IgE antibodies is effective only to monitor postoperative follow-up in a limited number of cases (19), and in the active disease, this technique lacks overall sensitivity (20). The experiments from the present work show that IgE from patients recognizes a series of bands with MW comprised between ~ 12 and 40 kDa in total HF as well as in the Ag 5/B fraction of HF. The same series of antigens were recognized by IgG from hydatidosis patients and not from healthy subjects or from those suffering cysticercosis. Sera from the latter patients were used to estimate the parasite-specific bands, since the cross-reactions between *Echinococcus sp* and *Taenia solium* present the most difficult problems because both parasite species can stimulate antibodies to Ag 5 (21). IgG managed to identify clearly a 42 kDa band in the GP fraction of HF, which, as we found earlier (5), renders the test highly specific (100%) and sensitive (95%) for hydatid disease. This is an absolute prerequisite for the immunodiagnosis, especially when used as an epidemiological tool with a low incidence disease such as unilocular hydatidosis. In contrast, the IgE failed to stain this antigen well enough, and this may explain the limited use of IgE-RAST in epidemiological research as well as in most pre- and postoperative follow-up procedures.

Infections by Echinococcus or other taeniids larvae also stimulate the production of specific IgA antibody (22). Although serum IgA has been shown to have antibody activity, it is probably less important biologically than the IgA found in secretions such as parotid saliva and bronchial and intestinal secretions. Secretory IgA plays a role in the creation of an

immune barrier against microorganisms at exposed surfaces (23), and clear evidence has been presented for a major role for these antibodies in protection following tapeworm infection by maternal colostrum transfer studies. Intestinal and colostral IgA antibodies produce protection, perhaps by preventing oncosphere invasion through the intestine (24). But although serum IgA may not be effective against established metacestodes, the possibilities of using specific IgA as a immunodiagnostic tool has barely been studied. Serum IgA in the present study was found to recognize a large series of Mw bands after Western blotting of HF antigens (Fig. 1, column B), including the 42 kDa band, also specifically recognized as IgG, was strongly and specifically developed, when total HF, the GP fraction, as well as when the Ag5/B fraction of HF was used as antigen. In the GP fraction also a 110 kDa band was specifically detected by serum IgA.

The resolutive power of Western immunoblotting, in contrast to most other immunological techniques, enabled the effective use of serum IgA to detect a reaction that was specific for hydatid disease. Since other immunodiaganostic techniques could have suffered too many false positive or negative results, together with the connotation of IgA as a predominant immunoglobulin in seromucous secretions, this immunoglobulin class may have been studied less in the immunodiagnosis of hydatid disease. Western blotting revealed the diagnostic 42 kDa band in partly purified HF fractions as well as in total HF. This suggests that the epitopes recognized by specific IgA are different from those recognized by specific IgG. The latter are employed most in the immunodiagnosis of hydatid disease and have been shown to be dependent on purification protocols (4,5,25,26). Therefore, searching specific secretory IgA in saliva, for instance, may have useful applications for the immunodiagnosis of hydatid disease, either through Western immunoblotting, or by simpler immunotechniques using purified 42 kDa antigen.

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