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Echinococcus granulosus human infection stimulates low avidity anticarbohydrate IgG2 and high avidity antipeptide IgG4 antibodies

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SUMMARY

Total IgG and IgG subclasses recognizing carbohydrate and peptidic epitopes from native and periodate treated partially purified hydatid cyst fluid (ppHCFA) and protoscolex somatic antigens (PSA) were tested by ELISA in hydatid patients sera. Binding of the major cross-reactive antiphosphorylcholine antibodies was inhibited with free reagent. A predominant anticarbohydrate antibody response against ppHCFA and PSA is shown. Although the main contributing IgG subclass to the antipeptide response against both antigens was IgG4, IgG1 also significantly contributed to the anti-PSA peptidic epitopes response. Western blot showed that IgG1 antibodies strongly recognized in ppHCFA a periodate susceptible 38 kDa antigen. The IgG4 antibodies mainly recognized the periodate-resistant 12, 16 and 24 kDa antigens. In addition, IgG2 antibodies recognized three strongly periodate-susceptible broad bands (116, 55 and 24 kDa antigens). PSA-specific IgG1 and IgG4 antibodies showed similar patterns of antigen recognition as well as no significant reduction of reactivity after periodate treatment while the IgG2 antibody recognition was strongly affected by this treatment. Furthermore, IgG2 showed significantly lower avidities than IgG1 and IgG4 antibodies recognizing both antigens. In conclusion, hydatid patients showed an enhanced production of low avidity anticarbohydrate IgG2 as well as high avidity antipeptide IgG4 antibodies.

Keywords E. granulosus, *IgG subclasses, carbohydrate epitopes, antibody avidity, phosphorylcholine, hydatid disease*

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INTRODUCTION

Unilocular hydatid disease, caused by the metacestode stage of *Echinococcus granulosus*, is a chronic parasitic disease resulting in the development of cysts in man, domestic and wild animals (McManus & Smith 1986).

In spite of the development of parasite-specific humoral and cellular immune responses in the intermediate host (reviewed by Heath 1986) viable hydatid cysts frequently persist for long periods (Gemmel *et al.* 1986). In addition, protoscoleces (PSC) of *E. granulosus*, released through accidental cyst rupture or spillage during surgery, are capable of generating secondary infections (Lightowlers *et al*. 1990). This is consistent with the fact that helminths are known to evade or modulate host immune response, so persisting over long periods in immune competent hosts (Maizels *et al*. 1993).

The exposure of the hapten phosphorylcholine has been proposed as a possible evasion strategy exploited by helminths and other parasites (Maizels & Selkirk 1988). Previously, Mitchell *et al*. (1977) suggested that phosphorylcholine may be produced by parasites to promote the production of low affinity antibodies. The presence of phosphorylcholine and antiphosphorylcholine antibodies in *E. granulosus* antigens and in sera from hydatid patients, respectively, has been reported (Shepherd & McManus 1987, Coltorti *et al*. 1990, Sterla *et al.* 1997).

Previous research also showed that carbohydrate epitopes play a relevant role in the host-parasite relationship in helminth infections. A suggested mechanism to evade host immunity is the production of 'blocking' (IgM and IgG2) antibodies that may block the antibody-dependent effector systems mediated by other isotypes (Butterworth *et al*. 1988). The relevance of carbohydrate epitopes in the immune response to *E. granulosus* has been reported both in natural (Sterla *et al*. 1997) and experimental infections (Ferragut & Nieto 1996) using hydatid fluid from fertile cysts as antigen. Further studies on *E. granulosus* PSC

antigens (PSA) showed that carbohydrate epitopes are highly immunogenic in mice generating a low avidity response as well as hypergammaglobulinaemia (Miguez *et al.* 1996). The predominance of low avidity anticarbohydrate antibodies of IgG3 and IgM isotypes in late experimental mouse infection has been described (Severi *et al.* 1997). It has been shown that carbohydrate antigens induce T-independent antibody responses strongly restricted to these isotypes in mice (Mond *et al.* 1995). Moreover, the patterns of anti-PSA antibody response were different in mice infected with viable PSC compared with those inoculated with dead PSC, thus suggesting that the live parasite may be modulating the host immune response (Severi *et al*. 1997).

In man, high levels of IgG4 were associated with high levels of IgE and eosinophilia in helminth infections (Maizels *et al*. 1993). As IgG4 is neither citophilic nor complement activator, it might block the antiparasite IgE activity (Hagan *et al*. 1991). However, the coexpression of IgE and IgG4 may be beneficial as IgG4 may block IgE-mediated allergic responses (Maizels *et al*. 1993). An enhanced specific IgG4 response to cyst fluid antigens has been described in hydatid patients (Hira *et al*. 1990, Aceti *et al*. 1993, Wen & Craig 1994, Ioppolo *et al*. 1996, Shambesh *et al*. 1997).

The aim of this study was to analyse the contribution of parasite-specific IgG subclasses, the nature of the recognized epitopes in the *E. granulosus* antigens and the avidity of the antibodies of different IgG subclasses; in order to asses the immunobiological relevance of the human antibody response to *E. granulosus* antigens during cystic hydatid infection.

MATERIALS AND METHODS

Parasite antigen preparations

Hydatid cyst fluid antigen (HCFA)
Hydatid fluid was aseptically aspirated from fertile cysts obtained from livers and lungs of Uruguayan cattle. Hydatid cyst fluid antigen (HCFA) was prepared according to Ferragut & Nieto (1996).

Partially purified hydatid cyst fluid antigen (ppHCFA)
A hydatid cyst fluid fraction, highly enriched in parasite components, was obtained according to Oriol *et al*. (1971) and applied to a Sephadex G-200 column (Pharmacia); the protein peak appearing with the void volume and containing antigens 5 and B was used as antigen (ppHCFA).

PSA

PSC were allowed to sediment and washed several times with sterile 0·05 M phosphate buffer saline pH 7·2 (PBS); then they were disrupted by sonication and cleared by centrifugation according to Miguez *et al*. (1996).

Carbohydrate and protein analysis

Carbohydrate analysis
The carbohydrate content of each antigenic preparation was determined by a modified orcinol-sulphuric assay (White & Kennedy 1986) according to Miguez *et al*. (1996).

Protein analysis

The protein content of each antigenic preparation was determined by bicynchoninic acid method (BCA Protein Assay Reagent, Pierce, IL, USA) using bovine serum albumin (BSA) as standard, according to the manufacturer's instructions.

Sera

The serum samples analysed were collected from 42 surgically confirmed hydatid patients and 15 healthy donors. The group of hydatid patients consisted of 30 with hepatic cysts, three with pulmonary cysts, eight with multiple and/or disseminated cysts and one with a renal cyst. A pool of serum samples from 50 hydatid patients was prepared as positive control. All serum samples were stored at – 20°C until used.

Alkaline phosphatase conjugate

Anti-sheep immunoglobulin serum was raised in rabbits. The immunoglobulin fraction was precipitated with ammonium sulphate according to Hudson & Hay (1989) and conjugated with alkaline phosphatase (Type VII-T, Sigma) according to Voller *et al.* (1976).

Enzyme-Linked immunosorbent Assay (ELISA)

Determination of parasite-specific IgG
Microtitre plates (Greiner Labortechnik) were coated with 0·1 ml/well of a solution 1 μg/ml (ppHCFA) or 5 μg/ml (PSA) in 0·05 M carbonate buffer pH 9·6, incubated overnight at 4°C, and blocked with 0·15 ml/well of PBS-1% BSA. After one h of incubation at room temperature (RT) the plates were washed four times with PBS-0·05% Tween 20 (PBS-T). Serum samples diluted 1/50 in 0·05 M Tris-HCl buffer saline pH 7·2/0·05% Tween 20/0·5% BSA/0·02 M phosphorylcholine chloride (calcium salt, Sigma, St Louis, MO, USA), were incubated in triplicate (0·1 ml/well) for one h at RT. The optimal concentration of phosphorylcholine was determined by titration. After washing, a

horseradish peroxidase (HRP) conjugated monoclonal anti-human IgG (Fab) (The Binding Site Ltd, Liverpool, UK) diluted 1/2000 in PBS-T-0·5% BSA (PBS-T-BSA) according to the results of checker-board titration was added. After one h of incubation at RT and washing, substrate solution (2·2 mM o-phenylene-diamine dihydrochloride in 0·1 M phosphate/citrate buffer pH 5·0, with 0·01 ml of H2O2/ 50 ml of solution) was added (0·1 ml/well), and stopped with H2SO4 1N (0·05 ml/well) after ten min of incubation. Optical density (OD) was read at 492 nm using a vertical pathway spectrophotometer (Labsystems Multiskan, MCC/ 340, Helsinki, Finland).

Determination of parasite-specific IgG subclasses

ELISA was performed as previously described with the following modifications: after incubation of serum samples affinity-purified sheep IgG fractions recognizing each of human IgGi (i = 1, 2, 3, 4) subclasses (The Binding Site Ltd) were added diluted 1/2000 in PBS-T-BSA (0·1 ml/well). After one h of incubation at RT the plates were washed and a HRP conjugated rabbit IgG fraction antisheep IgG F(ab)2 (Cappel Teknika Co., Durham, NC, USA) (0·1 ml/well) was added. After one h of incubation at RT the assay was continued as described above.

Avidity index determination

Avidity indexes of anti-ppHCFA and anti-PSA IgG subclasses (IgG1, IgG2, IgG4) were determined according to Pullen *et al.* (1986). ELISA was performed as described above with some modifications. Serum samples were appropriately diluted, so as to give an OD492 approximately equal to 1·0, and were dispensed (0·1 ml/well) in seven wells. Supernatants were discarded after one h of incubation at RT and potassium thiocyanate was added to each of five wells of every tested sample at KSCN concentrations of 3·0, 2·0, 1·5, 1·0 and 0·5 M in PBS-T (0·1 ml/well) and just PBS-T to other two wells. Plates were incubated 15 min at RT under shaking; after washing ELISA was performed as described above. Data were rejected as unrealiable if the maximum OD492 was below 0·5.

SDS-PAGE and immunoblot

SDS-PAGE was performed according to Laemmli (1970) using a 12·5% acrylamide gel and 1% SDS. HCFA and PSA were separated under reducing conditions (2%, 2-mercapto-ethanol). Western blot was done according to Towbin *et al.* (1979). After 30 min incubation with blocking solution (PBS-1% Tween 20) the nitro-cellulose strips were washed three times for five min with PBS-0·1% Tween 20. Then the strips were incubated one h in sera diluted 1/100 in buffer Tris-HCl pH 7·3/0·1% Tween 20/0·02 M phosphoryl-choline hydrochloride (calcium salt). After washing, the strips were incubated one h with the sheep IgG fraction antihuman IgGi subclass (i = 1, 2, 4, diluted 1/1000 1/500 and 1/1000, respectively, in PBS-0,1% Tween 20). After washing, the strips were incubated one h in a solution of alkaline phosphatase conjugated rabbit antisheep immunoglobulins diluted 1/750 in PBS-0·1% Tween 20/1% rabbit normal serum. All incubations were done under rocking. The blots were developed according to Farr & Nakane (1981). The substrate solution containing BCIP (5-bromo-4-chloro-3-indoyl phosphate p-toluidine salt) and NBT (p-nitro-blue-tetrazolium chloride) was added according to the manufacturer's instructions (Bio-Rad).

Sodium metaperiodate treatment of native antigens

To destroy carbohydrate epitopes the ELISA plates or the nitrocellulose strips coated with either of two antigens (ppHCFA and PSA or HCFA and PSA, respectively) were treated with 0·02 M sodium m-periodate in 0·05 M sodium acetate buffer, pH 4·5 according to Woodward *et al.* (1985). After this treatment the ELISA and immunoblot were performed as previously described.

Data analysis

To compare the results obtained from different ELISA plates, data were expressed as the number of standard deviations (SD) with respect to the mean ($\bar{x}$;) optical density value observed in normal serum samples. Thus the results were expressed as:

$$\mathrm{Ni} = \frac{\mathrm{OD} - \bar{x}}{\mathrm{SD}}$$

N applies for IgG and Ni applies for IgGi (i = 1, 2, 3, 4). In addition, in each plate the reference pool of 50 serum samples from surgically confirmed hydatid patients as well as four individual serum samples from healthy donors were included. Thus the mean OD value observed in normal serum samples was calculated including the values obtained from these four serum samples in all plates. The hydatid patients pool served as an additional control. To obtain comparable values among the assays of different subclasses (Severi *et al.* 1997) Ni were introduced as independent variables in the following multiple regression function:

$$\mathrm{N} = \mathrm{b0} + \mathrm{b1\ N1} + \mathrm{b2\ N2} + \mathrm{b3\ N3} + \mathrm{b4\ N4}$$

The bi coefficients of multiple regression were used to define new values for IgG (N′) and for each IgG subclass (N′i):

$$\mathrm{N'} = \mathrm{N'1} + \mathrm{N'2} + \mathrm{N'3} + \mathrm{N'4}$$ (F distribution; $P < 0{\cdot}001$).

The evaluation of the contribution of each IgG subclass, expressed as relative percentage of total IgG was performed as following:

$$\%\mathrm{IgGi} = \frac{\mathrm{N'i}}{\sum_{i=1}^{i=4} \mathrm{N'i}} \times 100$$

The Z-test for pairs of data was used to determine the significance of differences between arithmetic means of percentages ($P < 0{\cdot}05$).

The Student's *t*-test for pairs of data was used to determine the significance of differences between arithmetic means ($P < 0{\cdot}05$).

The antibody concentration determined in the absence of thiocyanate was assumed to represent the total binding of specific antibodies, and those measured in the presence of decreasing concentrations of thiocyanate were converted to percentages of the total. Linear regression analysis of the log (% binding) *vs* molar concentration of potassium thiocyanate was carried out and the avidity index, representing the molar concentration of thiocyanate required to reduce the initial OD to 50%, was calculated. Data were rejected as unreliable if the initial absorbance was below 0·5.

Relative molecular weights of antigens recognized by hydatid patients sera were determined using a logarithmic plot of migration of a set of molecular weight standards included in every assay.

RESULTS

Chemical composition of antigen preparations

Protein and carbohydrate concentrations were determined in HCFA, ppHCFA and PSA antigens and the ratios of protein to carbohydrate concentrations were calculated. The results are shown in Table 1.

Table 1 Chemical composition of antigens

Ag	P/CH
HCFA	2·10
ppHCFA	2·96
PSA	0·73

P/CH: ratio of milligrams of protein to milligrams of carbohydrate.

ELISA

Specific total IgG and IgG subclasses

Table 2 shows the mean value of each parasite-specific IgG subclass expressed as the relative percentage of total parasite- specific IgG, calculated as described under data analysis, using both antigens with and without periodate treatment. Significantly higher IgG2%s were obtained using both native antigens, ppHCFA and PSA, as compared with percentages shown by both periodate treated antigens. In addition, the IgG2% using PSA was significantly higher than that using ppHCFA. No significant differences were found in IgG1%s before and after periodate treatment with both antigens but, significantly higher values resulted from treated and nontreated PSA as compared with treated and native ppHCFA. A higher IgG4% value was obtained with native ppHCFA as compared with native PSA. Although both periodate treated antigens showed increased IgG4%s, the IgG4% observed with ppHCFA remained significantly higher than with PSA. With both treated and nontreated antigens the contribution of IgG3 subclass was low. Besides, native ppHCFA-specific antibodies belong mainly to IgG2 and IgG4 subclasses without significant differences between them. Periodate treatment increased IgG4 contribution, becoming significantly higher than that of any of the other three subclasses. The antinative PSA IgG2 antibodies were the main contributors among the specific IgG antibodies; however, periodate treatment greatly diminished their contribution, IgG1 and IgG4 antibodies becoming the more important ones without significant differences between them.

Table 2 Percentages of IgG subclasses

	% IgG subclasses			
	IgG1	IgG2	IgG3	IgG4
ppHCFA				
A	24 (± 11)	39*(± 20)	—	32*(± 18)
B	23 (± 9)	15 (± 9)	18 (± 12)	43*(± 17)
PSA				
A	30 (± 16)	47*(± 16)	11 (± 4)	9 (± 3)
B	32*(± 12)	13 (± 11)	19 (± 12)	36*(± 14)

*: highly significant difference among the values of the row (A or B for each antigen) (*Z*-test, $P < 0{\cdot}05$).
Mean value (± SD) of each parasite-specific IgG subclass, expressed as relative percentage of total parasite-specific IgG using both antigens, ppHCFA and PSA. A, without periodate treatment. B, with periodate treatment.

Avidity index

Table 3 shows the avidity indexes of antippHCFA and anti-PSA IgG1, IgG2 and IgG4, determined as previously described, using 17 serum samples of hydatid patients with cysts in liver ($n = 11$), lungs ($n = 2$) as well as disseminated and/or multiple cysts ($n = 4$). As serum samples appropriately diluted should give an OD492 approximately

Antigen	ppHCFA			PSA		
	Avidity index					
Sample	IgG1	IgG2	IgG4	IgG1	IgG2	IgG4
1*	1·9	1·8	1·9			
2*	2·4	1·5	1·5			
3*	1·6	1·6	1·7	1·9	1·0	2·8
4*	2·1	1·9	1·9	1·7	1·2	2·7
5	1·7	1·6	1·8	2·0	1·6	1·9
6	2·3	1·3	4·4	3·0	2·0	2·2
7	3·7	1·8	1·8	2·4	1·8	2·4
8	1·9	1·3	1·6	2·8	2·1	1·9
9	2·0	1·8	2·4	2·7	1·6	2·4
10	1·7	1·2	3·2	2·1	1·9	2·1
11				3·1	1·8	2·5
12	2·3	1·9	2·1	2·3	1·6	2·5
13				2·2	1·1	3·3
14	2·0	1·7	2·5	2·4	2·1	2·1
15	3·3	1·7	3·2	1·9	1·9	2·1
16				3·6	2·8	3·4
17				3·0	2·2	1·7
Mean	2·2	1·6	2·3	2·5	1·8	2·4
± SD	(± 0·6)	(± 0·2)	(± 0·8)	(± 0·5)	(± 0·4)	(± 0·5)

Table 3 Avidity indexes of parasite-specific IgG1, IgG2 and IgG4 antippHCFA (left) and anti-PSA (right) determined using serum samples from hydatid patients with liver cysts (1–11), pulmonary cyst (12 and 13) and disseminated and/or multiple cysts (14–17)

* Patients with hydatid disease diagnosed by ultrasound in a field study performed with asymptomatic population and later surgery confirmed.

equal to 1·0 and the OD obtained from serum samples in ELISA performed with ppHCFA or PSA were different, in some cases we had to choose different serum samples.

Anti-ppHCFA and anti-PSA IgG2 antibodies showed significantly lower avidities as compared with corresponding IgG1 and IgG4 antibodies ($P < 0{\cdot}05$).

Immunoblot

Serum samples from ten different individuals were tested for each IgG subclass using HCFA and PSA. Figure 1 show the results from serum samples of two hydatid patients with hepatic cysts (I and II) and a healthy donor.

HCFA

Western blot analysis of anti-HCFA IgG1, IgG2 and IgG4 antibody responses in sera of hydatid patients showed different patterns of antigen recognition. Whereas IgG2 antibodies mainly recognized three antigens of 116, 55 and 24 KDa as broad bands (Figure 1a, lanes 7–9) IgG1 and IgG4 antibodies showed complex patterns of antigen recognition (Figure 1a, lanes 1–6). The IgG1 antibodies of most of hydatid serum samples tested (9/10) recognized the 38 kDa, probably a subunit of Ag5 (Figure 1a, lanes 1 and 2). In addition, some of them (2/10) recognized 24, 16 and 12 KDa antigens, probably subunits of Ag B (data not shown). In contrast, low molecular weight antigens were strongly recognized by IgG4 antibodies of most of the tested hydatid sera (9/10) (Figure 1a, lanes 4 and 5) but only one of them recognized the 38 KDa antigen (Figure 1a, lane 4). After periodate treatment of HCFA, a reduction in the reactivity was observed in all antigens recognized by IgG2 antibodies (Figure 1a, lanes 7 and 8) However, the patterns of recognition of IgG1 and IgG4 antibodies were less sensitive to periodate treatment, the 38 kDa band being one of the most affected (Figure 1a lanes 1, 2 and 4). In addition, no significant reduction of reactivity against the low molecular weights antigens was observed (Figure 1a, lanes 4 and 5).

PSA

Western blot analysis of PSA-specific IgG1, IgG2 and IgG4 antibody responses in sera of hydatid patients indicated that IgG2 recognized less antigenic bands than IgG1 and IgG4 antibodies (Figure 1b, lanes 7–9 and lanes 1–6, respectively). Most antigens recognized by IgG1 were also recognized by IgG4 antibodies (109, 96, 86, 47, 44 and 29 kDa) (Figure 1b, lanes 1–6). In addition, after periodate treatment of PSA an important reduction of IgG2 recognition of all bands (Figure 1b, lanes 7 and 8) as well as no significant reduction of IgG1 and IgG4 reactivity were observed, only few bands being affected (42, 44 and 47 kDa) in the later case (Figure 1b lanes 1, 2, 4, and 5).

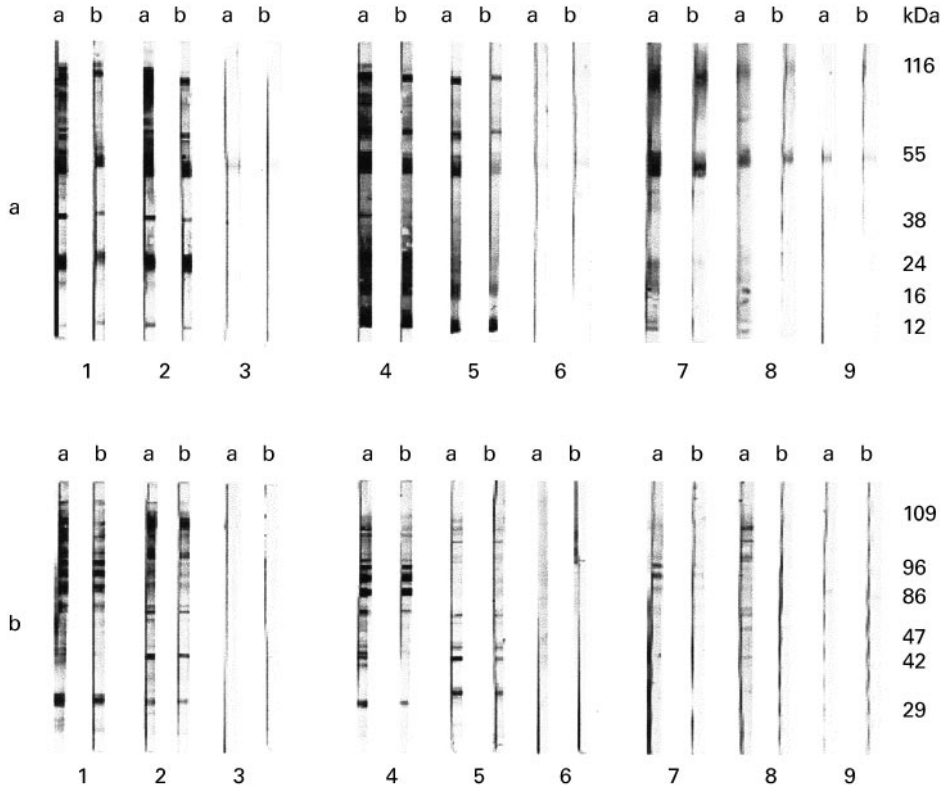

Figure 1 Antigen recognition patterns of HCFA (a) and PSA (b) by IgG1 (lanes 1–3), IgG4 (lanes 4–6) and IgG2 antibodies (lanes 7–9). Immunoblot analysis of native (a) and periodate treated antigens (b) probed with sera corresponding to: Lanes 1, 4 and 7, hydatid patient I; Lanes 2, 5 and 8, hydatid patient II; Lanes 3, 6 and 9, a healthy donor.

DISCUSSION

Selective expression of individual IgG subclasses in response to different antigens (proteins, carbohydrates, membranes) (Perlmutter *et al*. 1978) and clinical situations in which the minor IgG subclasses are enhanced, have been described in humans and animals (Oxelius 1984, Schur 1987, Hussain *et al.* 1994). Different patterns of antibody response involving specific IgG subclasses have been reported in parasitic infections (Dunne 1990, Maizels *et al*. 1995). Previous studies with hydatid patients showed contrasting results possibly due to the different methodologies used. Hira *et al*. (1990) showed that the responses in all IgG subclasses are enhanced in hydatid patients, IgG3 being a possible diagnostic marker. Aceti *et al*. (1993) showed a slightly higher titre of IgG1, IgG2 and IgG3 antibodies among sera from hydatid patients as compared with normal control sera. However, a relevant increase of IgG4 subclass antibodies was found in hydatid serum samples. Wen & Craig (1994) showed that specific antibodies of all IgG subclasses are present in hydatid patients, while the ELISA to determine specific IgG1 and IgG4 antibodies were more sensitive than the assays for specific IgG2 and IgG3.

Shambesh *et al*. 1997 suggest that a switch from predominant IgG1 response to IgG4 might occur in cystic echinococcosis patients as the disease progress.

Our results show the predominance of IgG2 and IgG4 antibodies in the parasite-specific immune response against native ppHCFA. The differences with the reports mentioned above may be due to the different methodologies and data analysis performed. In addition, in our case the major cross-reactive antiphosphorylcholine antibodies present in hydatid sera (Shepherd & McManus 1987, Sterla *et al*. 1997) were eliminated by competition (see Materials and Methods) in order to increase the influence of parasite-specific antibodies.

One approach to obtain reliable ELISA results is to express them in terms of the number of standard deviations with respect to the mean OD value of normal serum samples allowing a better comparison of results obtained from different plates (Rote *et al.* 1990, Font *et al*. 1991). As is well known ELISA results depend on both avidity and

concentration of antibodies being tested, as well as on the reagents used in each assay. To account for the complex relationship between ELISA reading (OD) and actual antibody concentration of different subclasses, in this paper we used the multiple regression analysis proposed by Severi *et al*. (1997). The experimentally determined regression coefficients were used to recalculate the titres of specific IgG subclasses expressed as described before. The multiple regression was statistically validated by a F distribution test.

Periodate-sensitive epitopes are likely to be mainly carbohydrate epitopes, although some peptide epitopes may also be destroyed by this treatment (Woodward *et al*. 1984). After periodate treatment a sharp decrease in IgG2 antibody titres was observed while IgG4 became the most relevant antibodies recognizing peptidic (periodate-resistant) epitopes. These results are in agreement with previous reports showing IgG2 antibodies as the predominant human isotype in response to many carbohydrate antigens (Schur 1987, Dunne 1990). Butterworth *et al*. (1988) presented evidence in human infection by *Schistosoma mansoni* suggesting that antibodies of IgG2 and IgM isotypes reacting with egg carbohydrate antigens would block the effect of protective antibodies mediating ADCC reactions against the schistosomula. In addition, the relevance of HCFA carbohydrate epitopes in the immune response to *E. granulosus* has been shown both in natural (Sterla *et al*. 1997) and experimental infection (Ferragut & Nieto 1996). Previous research in mouse experimental infection showed different patterns of anticarbohydrate antibodies among PSA and HCFA-specific IgG (Ferragut *et al*. 1996, Severi *et al*. 1997) Therefore, we also performed the studies using PSA to compare with data from experimental infection. Consistent with results observed in mice (Severi *et al*. 1997) the PSA-specific antibody pattern showed significantly higher IgG2 levels as compared with the other three IgG subclasses; also strongly reduced by periodate treatment. In addition, IgG1 and IgG4 antibodies were the most important antibodies recognizing peptide (periodate-resistant) epitopes. Furthermore, IgG2% using PSA was significantly higher than using the ppHCFA, these results are in accordance with the chemical composition of antigenic preparations.

In accordance with the results of Wen & Craig (1994) using native hydatid cyst fluid antigens, our Western blotting results indicate that IgG1 and IgG4 antibodies show different binding patterns. The IgG1 antibodies strongly recognized the 38 kDa antigen, probably a subunit of Ag 5, while IgG4 is the predominant isotype that binds the low molecular weight antigens, 12, 16 and 24 kDa, probably subunits of antigen B. These findings are also in accordance with the report of Ioppolo *et al*. (1996) who found different percentages of positive reactions in the recognition of Ag B between the IgG subclasses, IgG4 being the predominant isotype. In contrast to Wen & Craig (1994), probably due to differences in experimental conditions, IgG2 antibodies mainly recognized three antigens as broad bands (116, 55 and 24 kDa) strongly affected by periodate treatment according to our ELISA results. As was expected the IgG1 and IgG4 antibody recognition patterns were weakly sensitive to periodate treatment, the 38 kDa band being one of the most affected while no significant reduction of reactivity against the low molecular weight antigens (12, 16 and 24 kDa) was observed, in accordance with previous reports (March *et al*. 1991, Sterla *et al*. 1997). Using PSA, IgG1 and IgG4 antibodies also showed similar patterns of recognition. After periodate treatment both isotypes showed no significant reduction of reactivity. In contrast, recognition by IgG2 antibodies was strongly affected by periodate treatment. Miguez & Nieto (1996) showed that PSA surface carbohydrate epitopes are highly immunogenic and generate a low avidity response in mice. Severi *et al*. (1997) found that the anticarbohydrate low avidity antibodies belong mainly to IgG3 and IgM isotypes in infected mice. In accordance with these studies our results show significantly lower avidities of IgG2 antibodies, the predominant isotype in the immune response to carbohydrate epitopes with both antigens (pp-HCFA and PSA), as compared with IgG1 and IgG4 antibodies.

In conclusion, our results show an important anticarbohydrate immune response in hydatid patients against both antigens (pp-HCFA and PSA), IgG2 antibodies being the predominant isotype. The enhanced production of low avidity anticarbohydrate antibodies in natural and experimental *E. granulosus* infection suggest that the parasite may be modulating the host immune response to evade effector mechanisms. This hypothesis is consistent with previous studies with other helminths showing that IgG2 would act as ''blocking'' antibodies (Butterworth *et al*. 1988). Furthermore, the antipeptide immune response in hydatid patients showed an important contribution of IgG4 antibodies which may also block the antiparasite IgE activity.

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