

Molecular and immunological characterization of the C-terminal region of a new *Echinococcus granulosus* Heat Shock Protein 70

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SUMMARY

By screening an expression library of *Echinococcus granulosus* with IgE from sera of patients with cystic echinococcosis (CE) and allergic reactions, we isolated the C-terminal region of a new heat shock protein (HSP)70 of *E. granulosus*. The protein, named Eg2HSP70, has close homology with the C-terminal region of *Dermatophagoides farinae* and human HSP70. We investigated the humoral and cell-mediated immune responses to this antigen in patients with CE grouped according to the presence of allergic reactions. Immunoblotting detected total IgG, IgG4 and IgE specific to Eg2HSP70 (83% of sera contained IgG, 31% IgG4 and 57% IgE). No significant difference was found in immunoglobulin percentages according to the presence of allergic reactions. Immunoblotting inhibition showed that no IgG or IgE specific to Eg2HSP70 cross-reacted with *D. farinae* or human HSP70. Eg2HSP70-stimulated PBMC from 26 patients produced significantly greater amounts of TNF- α , IFN- γ and IL-10 than unstimulated cultures in all patients, irrespective of the presence of allergic reactions ($P < 0.05$). They also produced significantly greater amounts of IL-4 than unstimulated cultures exclusively in patients with allergic reactions ($P < 0.05$). These findings show that Eg2HSP70 is a new antigenic molecule inducing both B and T cell responses.

Keywords *Echinococcus granulosus*, HSP70, IgE, immune response, Th1/Th2 cytokines

INTRODUCTION

Cystic echinococcosis (CE), caused by *Echinococcus granulosus*, is one of the most severe helminthic infections in humans and is distributed world-wide (1). Acute infection with *E. granulosus* typically initiates a cascade of cellular and humoral events in the immunocompetent host (2). The distinguishing feature of the host–parasite relationship is that the chronic infection and detectable humoral and cellular responses against the parasite coexist. The main clinically observed immunological consequences of CE arise from allergic reactions caused by the Th2 activation associated with the production of high levels of specific IgE antibodies (3). In an earlier study we found that two *E. granulosus* allergens, elongation factor 1 β/δ (EgEF-1 β/δ) and cyclophilin (EA21), cause the allergic reactions manifested by patients with CE and play a critical role in the host–parasite relationship by inducing specific Th2 responses (4,5).

Continuing our programme of research into the immunology of hydatidosis, to characterize allergenic molecules that cause the IgE response in human *E. granulosus* infection we screened an *E. granulosus* cDNA library with IgE from sera of patients with CE and allergic reactions. We isolated a clone expressing the C-terminal region of a new heat shock protein 70 (HSP70), named Eg2HSP70. We investigated the humoral and cell-mediated immune responses to this antigen in patients with CE grouped according to the presence of allergic reactions.

PATIENTS AND METHODS

Blood samples

Blood samples were obtained from 79 patients with CE (61 with cysts in the liver, three with cysts in the lung, one with cysts in the kidney, one with cysts in the spleen, one with

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Table 1 Clinical features of the 79 patients with cystic echinococcosis

Patients with allergic reactions					Patients without allergic reactions				
No.	Sex/Age	Cyst		Treatment ^b	No.	Sex/Age	Cyst		Treatment ^b
		Location	Type ^a				Location	Type ^a	
1	F/60	Multiple	CE1	P	1	F/65	Liver	CE1	P
2	M/73	Liver	CE1	S	2	F/65	Multiple	CE1	S
3	M/35	Liver	CE1	S	3	M/75	Liver	CE1	S
4	F/45	Multiple	CE1	S	4	M/21	Liver	CE1	P
5	M/72	Liver	CE2	S	5	F/62	Liver	CE1	P
6	M/45	Liver	CE2	P	6	M/67	Spleen	CE1	S
7	F/65	Liver	CE2	P	7	F/65	Liver	CE1	P
8	M/54	Liver	CE2	P	8	M/77	Muscle	CE1	S/P
9	M/35	Liver	CE2	Pair	9	M/53	Liver	CE1	P
10	M/55	Liver	CE3	P	10	F/77	Liver	CE1	S
11	F/48	Liver	CE4	S	11	M/55	Liver	CE1	P
12	F/29	Liver	CE4	S	12	M/54	Liver	CE2	S
13	F/64	Liver	CE4	P	13	F/45	Liver	CE2	S
14	F/56	Lung	CE4	S	14	F/36	Liver	CE2	P
15	F/29	Liver	CE4	P	15	F/23	Lung	CE2	P
16	F/20	Liver	CE4	S	16	F/28	Liver	CE2	S
17	F/60	Liver	CE4	P	17	M/65	Multiple	CE2	S
18	M/58	Liver	CE4	S	18	M/53	Liver	CE3	P
19	M/76	Liver	CE4	S/P	19	M/71	Liver	CE4	P
20	M/76	Liver	CE5	S	20	M/53	Kidney	CE4	P
21	F/37	Liver	CE5	S	21	M/50	Multiple	CE4	S
22	M/60	Liver	CE5	P	22	F/48	Liver	CE4	P
23	F/39	Liver	CE5	S	23	M/60	Liver	CE4	S
24	F/48	Multiple	CE5	P	24	M/72	Liver	CE4	S
25	F/45	Multiple	CE5	S	25	M/57	Multiple	CE4	S
26	F/64	Lung	CE5	S	26	M/42	Multiple	CE4	S
27	F/37	Liver	CE5	P	27	F/42	Liver	CE5	S
28	F/60	Liver	CE5	S/P	28	F/35	Multiple	CE5	P
29	M/60	Multiple	CE5	S	29	M/29	Lung	CE5	S
30	F/53	Liver	CE5	S	30	M/26	Liver	CE5	S
31	M/58	Liver	CE5	P	31	F/32	Liver	CE5	P
32	F/41	Liver	CE5	S	32	M/48	Liver	CE5	S
33	F/60	Liver	CE5	S	33	M/60	Liver	CE5	S
34	M/50	Liver	CE5	S	34	M/65	Liver	CE5	P
35	M/76	Liver	CE5	S	35	M/30	Liver	CE5	S
36	F/52	Liver	CE5	S	36	M/28	Multiple	CE5	S
					37	F/37	Liver	CE5	P
					38	M/55	Liver	CE5	S
					39	M/45	Liver	CE5	P
					40	M/45	Liver	CE5	S/P
					41	F/62	Liver	CE5	P
					42	F/48	Liver	CE5	P
					43	F/58	Liver	CE5	S

^aAccording to the ultrasonographic classification proposed by the WHO Informal Group on Echinococcosis. ^bP, pharmacological; S, surgical.

cysts in the muscle and 12 with cysts in multiple sites) (Table 1) and from 17 control subjects, nine with atopic disorders (eight with polyspecific allergic reactions and one with monospecificity to *Dermatophagoides farinae*) and eight

non-atopic healthy donors. CE was diagnosed clinically, serologically and by ultrasound. Patients with CE were grouped according to the presence of allergic reactions: 36 patients with allergic reactions characterized by skin

manifestations such as intense itching and urticaria at the time of serum sampling, who reported negative skin prick tests for the major allergens, and 43 patients without allergic reactions. All procedures were approved by the local Ethical Committee and all participants gave their informed consent to the study.

Production of recombinant Eg2HSP70

The cDNA library was prepared as previously described (6). The cDNA clones were screened with a pool of five sera from confirmed positive CE patients showing strong allergic manifestations such as itching and urticaria at the time of serum sampling and with high antibody reactivity in an *E. granulosus* specific IgE ELISA (7). The nucleotide sequence of the cloned cDNA insertion was obtained with an automated sequencer ABI prism 310 Collection (Applied Biosystems, Foster City, CA, USA) and translated protein sequences were analysed by the Genetics Computer Group Sequence Analysis Software Package (8). To search for sequence similarities we used the BlastP software with BLITZ post-processing in swissprot, swissall, swissnew, swall, and trembl databases (Human Genome Center, Baylor College of Medicine, Texas, USA). The selected cDNA clone was subcloned into the *Bam*HI/*Kpn*I restriction site of the QIA express vector, pQE31. The fusion protein was expressed in *Escherichia coli* SG130009 cells, purified by affinity of NI-NTA resin for the 6X histidine tag and eluted under denaturing conditions (urea) according to the supplier's instruction (Qiagen GmbH, Hilden, Germany). The purified protein was analysed by 12% SDS-PAGE under reducing conditions and the molecular size was confirmed by immunoblotting using monoclonal antibody anti-histidine tail (Qiagen).

Production of recombinant *Dermatophagoides farinae* and human cognate HSP70

Recombinant *D. farinae* HSP70 (Mag 29) and human cognate HSP70 (HSC70) were prepared from previously isolated clones (9,10). To amplify by PCR the C-terminal region of *D. farinae* HSP70 we used the following specific primers: 5'-GCATGGATCCAAAGACGATATTGAACGTA-3' (with the *Bam*HI restriction site); and 5'-GCATGAGCTCTTAATCTACTTCTTCAATGGTG-3' (with the *Sac*I restriction site). To amplify the C-terminal region of human HSC70 we used the following specific primers: 5'-GAC-TGGATCCAAGGAAGACATTGAA-3' (with the *Bam*HI restriction site); and 5'-GCATGGTACCTTATTCAACCTCTTCAATGTGGG-3' (with the *Kpn*I restriction site). The proteins were eluted in denaturing conditions as described above.

Immunoblotting

The humoral immune response was determined by immunoblotting (IB), after 12% SDS-PAGE in reducing conditions, as previously described (6). In brief, Eg2HSP70, *D. farinae* HSP70 and human HSC70 were used as antigen at the concentrations of 3 µg/lane and were revealed by human sera diluted 1 : 50 for IgG and IgG4 detection and 1 : 10 for IgE detection. A goat anti-human IgE peroxidase labelled serum (Cappel, Cochranville, PA, USA), a mouse monoclonal anti-human IgG4 antibody (BD Biosciences, Heidelberg, Germany) and a goat anti-human IgG peroxidase labelled serum (Bio-Rad, Richmond, CA, USA) were used as second antibodies. A goat anti-mouse IgG peroxidase labelled serum (Bio-Rad) was used as third antibody. Strips were developed with peroxidase substrate, 3'-3' diaminobenzidine (Sigma-Aldrich, St Louis, MO, USA).

Immunoblotting inhibition

To inhibit specific IgE and IgG, the human sera pool diluted 1 : 10 in PBS-Tween was incubated overnight at RT with 0.1, 1 and 10 µg/mL of the inhibitors *D. farinae* HSP70 and human HSC70, according to the method reported by Huang *et al.* (11). Unabsorbed sera and sera absorbed with 10 µg/mL of Eg2HSP70 were run in parallel as controls. Strips were incubated overnight at RT with the mixtures and then developed as described above. The results were analysed by laser densitometry GS 700 (Bio-Rad).

Proliferation assay

Proliferation was assayed by the established procedure (12). In brief, triplicate cultures of PBMC were prepared at the concentration of 10⁵ cells/well, with the addition of Eg2HSP70 at the concentration of 8 µg/mL. This antigen concentration was proved effective in preliminary experiments. The quantitative chromogenic *Limulus* ameobocyte lysate test (QLC-1000 BioWhittaker Inc, Walkersville, MD), conducted according to the manufacturer's instructions, detected no measurable endotoxins in the final preparation. After 8 days of culture at 37°C in a humidified atmosphere containing 5% CO₂ in air, the proliferative response was assessed after the addition of 0.5 µCi ³H-methyl-thymidine (specific activity 1 mCi/mmol) (Amersham Life Science, Buckinghamshire, UK) to each well. After a further 20 h at 37°C, cells were harvested on glass fibre filter paper (Wallac, EG & G Company, Turku, Finland), using an automatic cell harvester (Harvester 96, MACH III M, TOMTEC, Orange, CT, USA) and analysed in a β counter (1450 Microbeta Plus, Wallac EG & G Company, Turku, Finland). Net counts per minute (cpm) of triplicate cultures were determined and

A

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gggatggtga atgatggcga gaagttcaaa caagaggatg aaaggcagag 50
ggatgctgtg gcagccaaga atgggttga gagctatggc ttcaccatgc 100
cgtcgacagt ggaagatgag aaggtgaagg ataagataag cgagtcggat 150
cgaagaaga taacggagag tgtgaagaga cgatcagttg gttggatggg 200
aaccaacagg cggataagga ggagtacgca cagacagaag gagttggaga 250
gtgtgtgcgc aatcccatca tcacgagtg accaggagcg aggtggtgct 300
ggtggcatgc ctggaggtat gccaggagcg atgcctggtg gtggtggcat 350
gggtggtgcg agttctggtg gcgctggtcc cactattgag gaggttgact 400
aatgcgtttc tgctctgcc ctcgatgatt tccagacacg ttgtatgctt 450
ctccccctt tctttggtgt aatgtatttg tattttaagt aaagacttac 500
tgtcga 506

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B

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Eg2Hsp70  -----GMVNDAEKFKQEDERQDRVAAKNGLSEYAFTMPSTVED-EKVKDKISES  49

EgHsp70    ---IER*****K*****S*K*****E**G**  567
HuHsc70    KEDIER**QE***Y*A***K***K*SS**S*****N*KA*****LQG**NDE  565
Mag29      KDDIER**KE**SY**E**DK***I***S**G***Q*KA*LDE-*AI*S*V**E  54

Eg2Hsp70  DRKKITESVKRRSVGWMGTNRRIRRST--HRQKELESVCAIPSSRVYQERGGA--  100

EgHsp70    **RR*M*KCEE-T*K*LDG*QQAEKEEYE*****NPIIAKM***A**VGG  621
HuHsc70    *KQ**LDKCNE-IIN*LDK*QTAEKEEFE*Q*****K**NPIITKL**SA**MP-  618
Mag29      *****LDK*DE-VLK*LDA*ALAEKDEFE*QR*****NPIITKL**QA***GA  108

Eg2Hsp70  -----GGMPGGMPGGMPGGGGMGGAS----SGGRGPTIEVD  133

EgHsp70    IPGGIPG*****T***GIPA*MA**M*GDPS*****  665
HuHsc70    -----*****F***GAPPS-----GASS*T*****  646
Mag29      -----*****P***P**TD*S**GAAGGDC*KS*****  145

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Figure 1 (a) Nucleotide sequence of the cDNA clone encoding Eg2HSP70 and (b) comparison of the new recombinant Eg2HSP70 amino acid sequence with the C-terminal amino acid sequences of *Echinococcus granulosus* HSP70 (EgHSP70), human HSC70 (HuHSC70) and *Dermatophagoides farinae* HSP70 (Mag29). *Identical residues.

the proliferative response was expressed as stimulation indices (ratio between the mean cpm in stimulated and unstimulated cultures). A stimulation index of 2 was taken as the threshold level for a positive reaction.

Cytokine assay

To test cytokine production, 8 µg/mL of Eg2HSP70 was added as stimulus to PBMC cultured as previously described (13). Five days later, supernatants were collected and analysed by ELISA kits for quantitative determination of IL-4, IL-10, TNF-α and IFN-γ (Quantikine High Sensitivity kit ELISA human IL-4, Quantikine kits ELISA human TNF-α, IFN-γ and IL-10, R & D Systems Inc, Minneapolis, MN, USA). The ELISA kit yielded 0.13 pg/mL sensitivity for IL-4, 3.9 pg/mL for IL-10, 4.4 pg/mL for TNF-α and 3.0 pg/mL for IFN-γ.

Statistical analysis

Differences between percentages were evaluated by Fisher's exact test. Wilcoxon and Mann-Whitney sum-rank test were used to compare differences between geometric means. Differences with a confidence interval of 95% or higher were considered statistically significant ($P \leq 0.05$).

Nucleotide sequence accession number

The GenBank accession number for the cDNA sequence used in this study is AF450087.

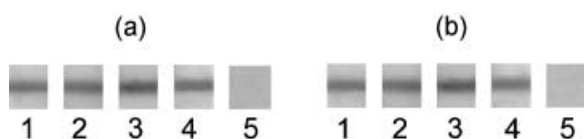
RESULTS

Eg2HSP70 characterization

Immunoscreening with IgE from a pool of sera from patients with CE and allergic reactions identified a strongly reactive clone. The amino acid sequence, predicted from the 505 base-pair (bp) open reading frame of this clone, is 133 residues long, the predicted molecular mass is 14 kDa, and the isoelectric point is 9.3. The amino acid sequence is highly homologous with HSP70 of other parasites and in particular has 63.5% identity with the C-terminus of a previously isolated HSP70 from *E. granulosus*, 51.4% identity with the C-terminus of human HSC70 and 49.3% identity with the C-terminus of *D. farinae* HSP70 (9,10,14) (Figure 1). The C-terminal amino acid motif EEVD was consistent with previously characterized cytoplasmic HSP70 genes and the four amino acid motif (GGMP) characteristic of *Plasmodium falciparum*, *Toxoplasma gondii* and of other

Table 2 Pattern of total IgG, IgG4 and IgE reactivity to Eg2HSP70 in 79 patients with cystic echinococcosis, grouped according to the presence of allergic reactions, and in 17 control subjects

	No. of serum samples	Total IgG (%)	IgG4 (%)	IgE (%)
Patients with cystic echinococcosis				
With allergic reactions	36	29 (81)	14 (40)	21 (59)
Without allergic reactions	43	37 (87)	11 (26)	24 (56)
Total patients	79	66 (83)	25 (31)	45 (57)
Control subjects				
With atopy	9	5 (57)	1 (11)	3 (33)
Without atopy	8	3 (37)	1 (12)	1 (12)
Total subjects	17	8 (47)	2 (12)	4 (24)
Diagnostic sensitivity		83	31	57
Diagnostic specificity		53	88	76

**Figure 2** Competitive inhibition immunoblotting of human serum pool binding to Eg2HSP70 inhibited with (a) human HSC70 and (b) *Dermatophagoides farinae* HSP70. Lane 1, unabsorbed serum; lane 2, serum absorbed with 0.1 µg/mL inhibitor; lane 3, serum absorbed with 1 µg/mL inhibitor; lane 4, serum absorbed with 10 µg/mL inhibitor; lane 5, serum absorbed with 10 µg/µL Eg2HSP70. Panel (a): immunoblots revealed with peroxidase-labelled goat anti-human IgE. Panel (b): immunoblots revealed with peroxidase-labelled goat anti-human IgG.

parasites, compared with three copies starting at position 101 (15,16).

Humoral immune response

In patients with CE, IB analysis detected total IgG, IgG4 and IgE specific to Eg2HSP70 (83% of patients' sera contained IgG, 31% IgG4 and 57% IgE). No significant difference was found in immunoglobulin percentages according to the presence of allergic reactions. In the control group, sera from subjects with and without diagnosed atopy also reacted to Eg2HSP70 (47% had IgG specific to Eg2HSP70, 12% had IgG4 and 24% had IgE) (Table 2).

Evaluation of cross-reactivity of Eg2HSP70 with human HSC70 and *D. farinae* HSP70

IB analysis showed that 12 (27%) of the 45 patients (10 patients without allergic reactions and two patients with allergic reactions) IgE positive to Eg2HSP70 were also IgE positive to *D. farinae* HSP70, whereas none of them was IgE

positive to human HSC70. Of the 66 patients IgG positive to Eg2HSP70, 35 (53%) were also IgG positive to *D. farinae* HSP70 and human HSC70. IB inhibition analysis showed that no IgG or IgE specific to Eg2HSP70 cross-reacted with HSP70 of *D. farinae* or human HSC70. Pre-absorption of the sera pool with Eg2HSP70 completely blocked the IgE and IgG reactivity to Eg2HSP70 itself (Figure 2).

Cellular immune response

Eg2HSP70 induced a proliferative response in 11/39 (28%) patients' PBMC; stimulation indices ranged from 0.4 to 16.6. No significant difference was found in mean stimulation indices according to the presence of allergic reactions (data not shown). Eg2HSP70-stimulated PBMC from total undivided patients and from patients with and without allergic manifestations produced significantly higher amounts of TNF-α, IFN-γ, and IL-10 than unstimulated cultures. Eg2HSP70-stimulated PBMC from total patients and from patients with allergic disease produced significantly greater amounts of IL-4 than unstimulated cultures (Figure 3).

DISCUSSION

Heat shock proteins originally came to notice on account of their increased levels in organisms exposed to conditions of stress. Current knowledge shows that as well as generating specific immune responses against infectious agents and cancer, HSPs possess immunostimulatory properties (17–20). Like other members of the HSP70 family, Eg2HSP70, the new recombinant protein we isolated and characterized by screening an *E. granulosus* cDNA library, is an antigenic molecule inducing both B and T cell responses.

To study the immunological reactivity of HSP70 of *E. granulosus*, we investigated the C-terminal variable region

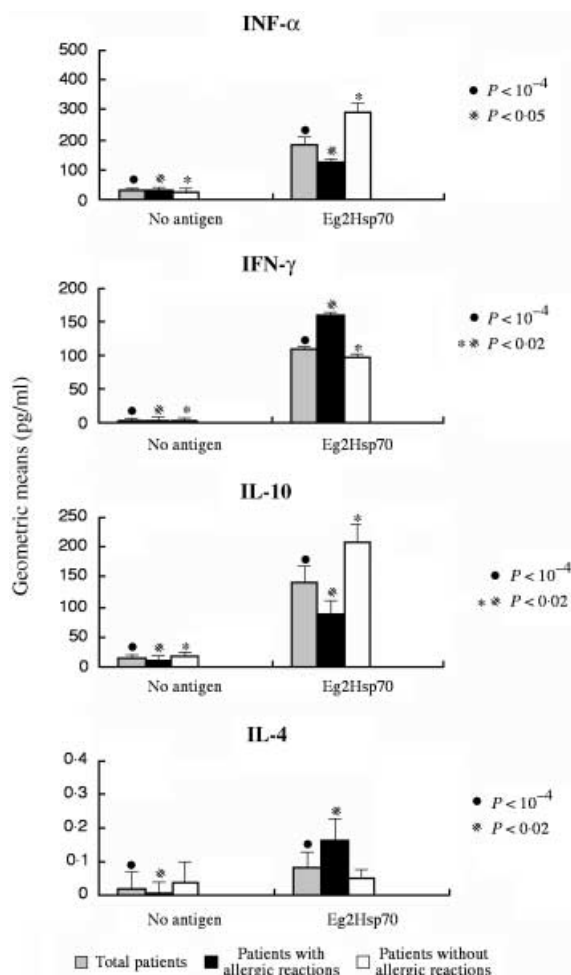


Figure 3 Eg2HSP70-stimulated Th1 and Th2 cytokine production in 26 patients grouped according to the presence of allergic reactions (11 with allergic reactions and 15 without). All data are expressed as geometric means $\pm$ SE. Wilcoxon and Mann–Whitney sum-rank tests were used to compare differences between means. Differences with a confidence interval of 95% or higher were considered statistically significant ($P \leq 0.05$).

of *E. granulosus* HSP70, the dominant antigenic region of the molecule (21). The HSP70 molecules, in particular their C-terminal region, are major allergens of fungi such as *Aspergillus fumigatus*, *Penicillium citrinum*, *Alternaria alternata*, and *Cladosporium herbarum*, and of mites such as *D. farinae*, and *D. pteronissus* (9,22–25). Our finding that 57% of patients with CE were IgE positive to Eg2HSP70 clearly implies that the C-terminal region of *E. granulosus* HSP70 is also an allergen.

Unlike our previous results showing an association between allergic reactions and IgE specific to EgEF-1 β / δ and EA21 in patients with CE, in this study using the

new antigen, Eg2HSP70, we found no association (3,4). A possible reason is that Eg2HSP70 could lack the multiple epitopes capable of evoking allergic reactions. In parasitic disease, the ratio of IgE to IgG4 is critical in determining the expression of allergic symptoms (26). We exclude the possibility that our patients' sera contained 'blocking' IgG4, because we found serum IgG4 specific to Eg2HSP70 in only a few patients, regardless of whether they had allergic reactions.

Recent studies have disclosed that the IgE-binding epitopes of parasite proteins resemble the IgE-binding epitopes of known allergens. Hence they extend to proteins of distinct species the definition of 'pan allergen' (27). The conserved IgE-binding peptides might be the common denominator responsible for dual immune responses, against parasites and allergens. Surprisingly, despite the high sequence homology of Eg2HSP70 with *D. farinae* HSP70, and the IgE positive reactions to *D. farinae* HSP70, in patients with CE with and without allergic reactions, our IB inhibition analysis disclosed no cross-reactive linear epitopes between Eg2HSP70 and the C-terminal region of HSP70 from *D. farinae*. Why patients with CE who had negative skin prick tests for the major allergens, including *D. farinae*, had serum IgE reactive for *D. farinae* HSP70 remains unclear.

Heat shock proteins of the 70 kDa family appear to be phylogenetically highly conserved across human, parasitic and bacterial species, facilitating immunological cross-reactions between pathogens and self-HSP70 (21). In theory, the unmistakable resemblance of parasitic HSPs to host homologous HSPs might render them identifiable to the immune system as self, thus obviating a response and providing a good example of 'antigen mimicry'. Our results indicate that in *E. granulosus* infection this tolerance does not occur and that despite its 51.4% identity with human HSC70, Eg2HSP70 is an immunogenic peptide like several parasite HSP70s (28). The lack of IgG cross-reactive epitopes between Eg2HSP70 and human HSC70 supports the hypothesis that Eg2HSP70 cross-reacting antibodies in healthy subjects are not natural self-antibodies, but are primed by the continuous contact with HSPs from commensals or microbes of low virulence (28). Consistent with our study, Colebrook & Lightowlers reported that EgHSP70 does not induce autoimmune reactivity with human HSP70 in hydatid patients (14).

In our patients, IgG-immunoblotting yielded low diagnostic specificity for CE (53%), probably owing to the conserved sequence of Eg2HSP70. We therefore conclude that Eg2HSP70 is of no use in the routine immunodiagnosis of CE. Accordingly, others have already reported that recombinant parasitic HSPs lack serological specificity (14,28). In this study we evaluated by IB only the qualitative humoral response specific to Eg2HSP70, without considering the

outcome of the disease. Further studies should investigate whether the quantitative determination by ELISA of antibodies specific to Eg2HSP70 might be useful in clinical follow-up to predict the outcome of CE.

The new antigen that we studied not only induced a humoral immune response but also induced a cell-mediated response. In PBMC from patients with CE, Eg2HSP70 stimulated moderate T-cell proliferation and elevated Th1 (IFN- γ and TNF- α) and Th2 (IL-4 and IL-10) cytokine production. In particular, it induced elevated IL-4 production exclusively in PBMC from patients with allergic reactions. Control of IgE synthesis in response to an allergen depends essentially on Th2 cells that switch on B cell IgE synthesis (29). In previous *in vitro* and *in vivo* studies we showed that *E. granulosus* antigens influence the Th1/Th2 balance by eliciting IL-4, IL-10 and IL-13 (13,30–32). In this highly Th2-polarized microenvironment, in patients with CE and allergic reactions, Eg2HSP70 could elicit IL-4 production not through its intrinsic ability but by strengthening the generalized Th2 polarization previously established. The antigen that we isolated seems not to act in the setting of a genuine Th1 to Th2 cytokine switch; it probably concurrently stimulates Th1 (or Th0) cells to secrete TNF- α and IFN- γ and Th2 cells to secrete IL-10 and IL-4.

The repeat unit (GGMP), which is repeated three times within the 3' gene of Eg2HSP70, and only once in the 3' gene of EgHSP70, is characteristic of HSP70 of other parasites, but is not observed in most other organisms (33). Since these highly conserved repeats are localized in the less conserved C-terminal region of the gene, it is possible that these motifs have a functional significance. In *Toxoplasma gondii* virulent strains consistently had four copies of this repeat unit, compared with five copies in all avirulent strains; it is possible that the deletion of one repeat unit has some significant impact on the synthesis or stability of the protein. Interestingly, the GGMP tetrapeptide repeat region has been shown to be an immunodominant epitope that results in the immune response of the host focusing on this region instead of more critical epitopes of the parasites (16). Further studies of this cytoplasmic HSP70 in *E. granulosus* may provide new insights into the functions of repetitive sequences in HSP70.

In conclusion, our study again emphasizes the complex mechanisms underlying anti-*E. granulosus* immune responses. It also highlights the difficulty in understanding the host–parasite relationship, mainly owing to the array of antigenic patterns that can elicit protective, suppressive or pathological immune responses.

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