

Immunological characterization of *Echinococcus granulosus* cyclophilin, an allergen reactive with IgE and IgG4 from patients with cystic echinococcosis

E. ORTONA, S. VACCARI, P. MARGUTTI, F. DELUNARDO, R. RIGANÒ, E. PROFUMO, B. BUTTARI, O. RASOOL*, A. TEGGI† & A. SIRACUSANO
Department of Immunology, Istituto Superiore di Sanità, Rome, Italy, *Department of Medicine, Unit of Clinical Allergy Research, Karolinska Institutet and Hospital, Stockholm, Sweden and †Department of Infectious Disease, 2nd Faculty of Medicine and Surgery, Rome, Italy.

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SUMMARY

By immunological screening of a cDNA library derived from protoscoleces of *Echinococcus granulosus* with IgE from patients with cystic echinococcosis (CE) and allergic manifestations, we isolated a protein identical to *E. granulosus* cyclophilin. The protein, named EA21, has close homology with *Malassezia furfur* cyclophilin allergen (Mal f 6) and with human cyclophilin. Using immunoblotting (IB) with a polyclonal antibody specific to EA21, we identified *E. granulosus* cyclophilin both in protoscoleces and in sheep hydatid fluid. Of the 58 sera from patients with CE, 29 (50%) were IgE positive to EA21, whereas, despite the high sequence homology, none were IgE positive to Mal f 6 or human cyclophilin. Only 26 of the 58 patients (45%) had IgG specific to EA21, whereas all patients (100%) had IgG specific to Mal f 6 and human cyclophilin. IB analysis showed that serum IgE-binding reactivity to EA21 differed significantly in patients with and without allergic reactions (20 of 25, 80% versus nine of 33, 27%; $P < 10^{-4}$). Conversely, five of the 25 patients who had CE-related allergic manifestations (20%) and 21 of the 33 who did not (63%) had specific IgG4 ($P = 10^{-3}$) and total IgG to EA21. EA21 induced a proliferative response in 15 of 19 (79%) patients' PBMC regardless of the allergic manifestations, but it induced no IL-4 production. Overall, these findings suggest that *E. granulosus* cyclophilin is a conserved, constitutive, parasite protein that does not cross-react with cyclophilins from other organisms and is involved in the allergic symptoms related to CE.

Keywords *E. granulosus* cyclophilin allergic reactions IgE IgG4

INTRODUCTION

Allergenic molecules probably enhance IgE responses by their enzymatic or other biological activity [1]. The cyclophilins, proteins that are remarkably conserved through evolution and found in all existing organisms, possess enzymatic peptidyl-prolyl isomerase (PPI-ase) activity, which is essential to protein folding *in vivo*. Proteins of the cyclophilin family have already been described as allergens in the moulds *Psilocybe cubensis* (Pci c 2), *Aspergillus fumigatus* (Asp f 11) and *Malassezia furfur* (Mal f 6), and in birch pollen (Bet v7) [2–6].

Cystic echinococcosis (CE) is an infection by cestode larvae of *Echinococcus granulosus* that form the hydatid cyst containing the protoscoleces. *Echinococcus granulosus* in humans triggers a variety of hypersensitivity reactions, ranging from

benign urticaria and short episodes of shaking chills or fever, or both events, to potentially fatal bronchial spasms, angioneurotic oedema and anaphylactic shock [7]. The search for *E. granulosus* allergenic molecules has highlighted the importance of specific antigens present both in fluid and in protoscoleces from the hydatid cyst [8,9].

Our primary aim in this study was to seek and characterize allergenic molecules that behave as molecular markers of allergic reactions during human cystic echinococcosis. By screening an *E. granulosus* cDNA library with IgE from patients with allergic manifestations related to CE, we isolated a protein identical to the known *E. granulosus* cyclophilin, EA21 [10]. To identify a possible cross-reaction between EA21 and two known homologous cyclophilins we assessed whether sera from patients with CE, from atopic subjects and from healthy donors reacted with EA21, with cyclophilin from the yeast *Malassezia furfur* and from human cyclophilin. By immunoblotting (IB) we assessed the IgE, total IgG and IgG4 antibody responses to EA21 in patients with CE, grouped according to the presence of allergic reactions. To determine EA21-induced cellular reactivity and IL-4 production we used a peripheral blood mononuclear cell (PBMC) assay.

E. Ortona and S. Vaccari contributed equally to this paper.

Correspondence: Dr Alessandra Siracusano, Department of Immunology, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italy.

E-mail: siracusano@iss.it

PATIENTS AND METHODS

Blood samples

Blood samples were obtained from 58 patients (23 males and 35 females; mean age 46.1 years, range 14–78) with CE (44 with cysts in the liver, three with cysts in the lung, one with cysts in brain, one with cysts in muscle and nine with cysts in multiple sites), 15 subjects with atopic disorders as proven by the results of skin prick tests (12 with polyspecific allergic reactions, two with monospecificity to *Dermatophagoides farinae* and one with monospecificity to *Olea pratensis*), and 30 non-atopic healthy donors. Hydatid cysts were classified into five types: type I (simple cysts), type II (multiple cysts), type III (cysts with detachment of the wall), type IV (heterogeneous hyperechoic cysts) and type V (calcified cysts) [11]. Patients with CE were grouped according to the presence of allergic reactions: 25 patients with allergic reactions characterized by skin manifestations, such as intense itching and urticaria, at the time of serum sampling, reported negative skin prick tests for the major allergens; 33 patients without allergic reactions (Table 1). Twenty patients with allergic reactions had peripheral blood eosinophilia. CE was diagnosed on the basis of evidence from imaging techniques (ultrasonographic scanning or nuclear magnetic resonance or both), serological assays, surgery or adjuvant medical treatment. All procedures were approved by the local Ethical Committee and all subjects gave their informed consent to the study.

Production of recombinant EA21

The cDNA library was prepared as previously described [12]. The cDNA clones were screened with a pool of five sera from clinically-confirmed positive CE patients showing severe allergic manifestations, such as itching and urticaria, at the time of serum sampling, and with strong reactivity in an *E. granulosus*-specific IgE ELISA (O.D.₂₈₀ > 2) [13]. The nucleotide sequence of the cloned cDNA insertion was sequenced with an automated sequencer (ABI prism 310 Collection, PE, Applied Biosystems, Foster City, CA, USA) and translated protein sequences were analysed by the Genetics Computer Group (Madison, WI, USA) Sequence Analysis Software Package [14]. To search for sequence similarities we used the BlastP software with BLITZ postprocessing 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 site of the QIA express vector, pQE31. The 6X fusion protein was expressed in *Escherichia coli* SG130009 cells, purified by affinity of NI-NTA resin for the 6Xhistidine tag and eluted under denaturing conditions (urea) according to the supplier's (Qiagen, GmbH, Hilden, Germany) instructions. Before the protein was used to immunize mice, it was dialysed in phosphate-buffered saline (PBS) for 2 days at 4°C to remove urea. After dialysis the protein was divided into aliquots and kept at –80°C for subsequent use.

Production of recombinant Mal f 6

Recombinant *Malassezia furfur* cyclophilin (Mal f 6) was prepared from a clone previously isolated by Lindborg [5]. The protein was eluted in denaturing conditions as described above.

Antigens

Sheep hydatid fluid was collected in Sardinia from fertile cysts. Protoscoleces were removed by centrifugation for 1 h, 4°C at 10 000 g, and stored at –20°C until use. The supernatant fluid was

concentrated 10× by collodium bags (Sartorius AG, Göttingen, Germany) and used in SDS-PAGE at the concentration of 2 µg/lane. Human cyclophilin was purchased from Sigma Aldrich, Milan, Italy.

Proliferation assay

Proliferation was assayed by the established procedure [15]. In brief, triplicate cultures of PBMC were prepared at the concentration of 10⁵ cells/well by the addition of 180 µl cell suspension and 20 µl sterile EA21 (8 µg/ml). The quantitative chromogenic *Limulus* amebocyte lysate test (QLC-1000 BioWhittaker, Inc, Walkersville, MD, USA), conducted according to the manufacturer's instructions, detected no measurable endotoxins in the final preparation. In all experiments, cultures with phytohaemagglutinin (2 µg/ml) and cultures without antigen were set up as positive and negative controls. After 8 days of culture at 37°C in a humidified atmosphere containing 5% CO₂ in air, the proliferative response was assessed by the addition of 20 µl containing 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). ³H-methyl-thymidine uptake into cell DNA was measured by reading samples in a β counter (1450 Microbeta Plus, Wallac EG & G Company). Net counts per minute (cpm) of triplicate cultures were measured and the proliferative response was expressed as Δ cpm (cpm in stimulated culture minus cpm in unstimulated cultures). A mean Δ cpm value of healthy blood donors, plus 2 standard deviations, was taken as the cut-off for a positive reaction.

Cytokine assay

To assess IL-4 secretion by PBMC from patients with CE, 8 µg/ml EA21 was added as a stimulus to PBMC cultured as previously described [16]. Five days later, supernatant fluids were collected and analysed by Quantikine High Sensitivity kit ELISA for quantitative determination of IL-4 (R & D Systems Inc., Minneapolis, USA). The ELISA kit yielded ranges of 0.25–16 pg/ml.

Serological tests

IB, after 12% SDS-PAGE in reducing conditions, was performed as previously described [9]. In brief, EA21, Mal f 6 and human cyclophilin were used as antigens at 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, Hercules, CA, USA) were used as second antibodies. A goat anti-mouse IgG peroxidase-labelled serum (Bio-Rad) was used as third antibody. For the immunocalization, protoscoleces and hydatid fluid were used as antigens. Mouse polyclonal antiserum to EA21 was obtained with standard procedures. To ascertain isotype specificity of the goat anti-human IgE peroxidase-labelled serum we tested it in a sandwich ELISA with human IgG purified by Protein A affinity chromatography.

Statistical analysis

All values are means ± s.d. Differences between percentages were evaluated by Fisher's exact test. Differences between arithmetic means were evaluated by two-tailed Student's *t*-test. Differences

Table 1. Clinical features of the 58 patients with cystic echinococcosis

Patient no.	Sex/Age	Eosinophilia (%)	Cyst type	Cyst site	Pharmacological treatment
Patients with allergic manifestations					
1	M/45	>5	V	Liver	NO
2	F/45	>5	II	Liver	NO
3	F/30	<5	IV	Liver	NO
4	M/60	<5	V	Liver	NO
5	F/65	>5	II	Liver	YES
6	F/65	<5	V	Liver	NO
7	F/35	>5	IV	Liver	NO
8	F/40	>5	V	Multiple	NO
9	F/50	>5	III	Liver	NO
10	M/40	>5	IV	Liver	NO
11	F/25	>5	II	Lung	YES
12	F/50	>5	II	Liver	NO
13	F/30	>5	IV	Liver	NO
14	M/50	>5	II	Liver	NO
15	M/40	>5	II	Liver	NO
16	F/30	>5	V	Liver	NO
17	F/30	<5	II	Liver	NO
18	F/30	>5	III	Liver	NO
19	M/40	>5	V	Liver	NO
20	M/40	>5	II	Liver	NO
21	F/60	>5	V	Liver	YES
22	M/30	<5	II	Liver	NO
23	M/40	>5	III	Multiple	NO
24	F/70	>5	V	Liver	NO
25	M/35	>5	II	Lung	NO
Patients without allergic manifestations					
1	M/45	<5	V	Liver	NO
2	F/50	<5	II	Liver	NO
3	M/60	<5	II	Muscle	NO
4	F/60	<5	V	Multiple	NO
5	M/30	<5	II	Liver	YES
6	M/60	<5	V	Liver	NO
7	M/30	<5	I	Multiple	YES
8	M/50	<5	I	Multiple	YES
9	F/45	<5	V	Liver	NO
10	F/65	<5	II	Liver	YES
11	F/50	<5	V	Multiple	NO
12	F/30	<5	V	Liver	NO
13	F/40	<5	IV	Liver	NO
14	M/14	<5	IV	Liver	NO
15	F/30	<5	V	Liver	NO
16	F/40	<5	I	Brain	NO
17	F/60	<5	I	Liver	YES
18	F/30	<5	III	Liver	NO
19	M/40	<5	V	Liver	NO
20	M/40	<5	II	Liver	YES
21	F/65	<5	IV	Liver	NO
22	M/78	<5	II	Multiple	YES
23	F/50	<5	V	Liver	NO
24	M/55	<5	V	Liver	NO
25	M/60	<5	V	Multiple	NO
26	M/65	<5	V	Liver	NO
27	F/50	<5	IV	Liver	NO
28	F/30	<5	V	Liver	NO
29	F/60	<5	II	Liver	YES
30	F/35	<5	I	Multiple	YES
31	F/75	<5	IV	Liver	NO
32	F/55	<5	V	Liver	NO
33	F/52	<5	II	Lung	YES

with a confidence interval of 95% or higher were considered statistically significant ($P \leq 0.05$).

RESULTS

Characterization of *E. granulosus cyclophilin*

The immunological screening with IgE from a pool of sera from patients with CE and allergic reactions allowed us to identify a strongly reactive clone. The amino acid sequence predicted from the 480 bp long open reading frame of this clone is 160 residues long and has 100% identity with a cyclophilin of *E. granulosus*, named EA21, 71% identity with a *Malassezia furfur* cyclophilin allergen, Mal f 6, and 72% identity with human cyclophilin (Fig. 1). To investigate the localization of cyclophilin and to determine the molecular mass of the native antigen, a mouse polyclonal antiserum specific to EA21 was used in IB with protoscoleces and hydatid fluid. The antiserum recognized a band of approximately 17 kD, both in protoscoleces and in hydatid fluid, in reducing conditions (Fig. 2).

Humoral immune response

IB analysis showed that 29 of the sera from patients with CE (50%) were IgE positive to EA21, whereas none were IgE positive to Mal f 6 or human cyclophilin (Table 2). Only 26 of the 58 patients (45%) had IgG specific to EA21, whereas all patients (100%) had IgG specific to Mal f 6 and human cyclophilin. In two of the 15 sera from atopic subjects (13%), IB detected IgE and IgG specific to EA21; one of these sera (7%) also contained IgE specific to Mal f 6 and all sera had IgG specific to Mal f 6 and human cyclophilin. No healthy subject was IgE positive to the three antigens, whereas all of them were IgG positive to Mal f 6 and human cyclophilin, and nine of the 30 (30%) were IgG positive to EA21.

IB analysis showed that serum IgE binding reactivity to EA21 differed significantly in patients with and without CE-related allergic reactions (20 of 25, 80% versus nine of 33, 27%; $P < 10^{-4}$) (Table 3). Conversely, five of the 25 patients with allergic manifestations (20%) and 21 of the 33 without (63%) had specific IgG4 and total IgG to EA21 ($P = 10^{-3}$).

Cellular immune response

EA21 induced a proliferative response in 15 of 19 (79%) patients, and the mean differences in Δ cpm values did not differ significantly ($P = 0.1$) in patients with and without allergic manifesta-

tions (Fig. 3). PBMC produced similar amounts of IL-4 (medium value: $0.48 \text{ pg/ml} \pm 0.60$; $0.48 \text{ pg/ml} \pm 0.48$).

DISCUSSION

We recently reported that a conserved *E. granulosus* protein is useful in clarifying the origin of the allergic reactions during echinococcosis infection [9]. In this study, we provide further evidence for the involvement of *E. granulosus* conserved antigens in the allergic manifestations during CE. We also extend previous knowledge by showing that *E. granulosus* cyclophilin is an allergen that behaves as a molecular marker of allergic reactions during human echinococcosis, and has a determinant role in the host-parasite relationship.

In this study, we obtained the reactive clone identical to EA21 by screening a cDNA library with IgE from patients with CE who had severe allergic skin manifestations. We found that EA21 is an allergenic molecule that is specifically recognized by IgE from patients with CE.

In accordance with our previous data on the localization of conserved and constitutive protein elongation factor 1 β/δ , we also detected *E. granulosus* cyclophilin in the protoscoleces and in the surrounding hydatid fluid [12]. Whether cyclophilin is released only in deteriorating cysts during ageing, calcification or drug treatment, or whether it is an active process of secretion, remains unclear.

The cyclophilins are a new family of pan allergens because the mould cyclophilins Mal f 6 and Asp f 11 can be cross-reactive, and human cyclophilin binds IgE from sera of patients sensitized to *A. fumigatus* cyclophilin [6]. Recent studies have disclosed that the IgE-binding epitopes of a *Schistosoma japonicum* protein exhibit similarities to the IgE-binding epitopes of known allergens [17]. The conserved IgE-binding peptides might be the common denominator responsible for dual immune responses against parasites and allergens. Despite the high sequence homology of EA21 with Mal f 6 and human cyclophilin, no patient with CE was IgE positive to these proteins. Our finding that IgE from patients with CE exclusively recognize *E. granulosus* cyclophilin demonstrates that the parasite itself induces their production. Moreover, because patients' IgE recognized EA21 whereas healthy subjects' IgE did not, EA21 may be useful in monitoring allergic manifestations during infection. The IgE-positive reactions to EA21 in two atopic subjects who had polyspecific allergic reactions, including skin-prick test reactions to birch pollen

EA 21	-GVKCFDDISIGGKPAGRIVFALFDD-VPKTVENFRALCTGEKGF
Hum. cycl.	VNPTV****AVD*E*L**VS*E**AK*****A*****S*****
Mal f6	-MSNV****TKN*A*L*T*K*K*****V*****AA*****
EA 21	GYKGSKFHRIIPGFMCGGDFTAGNGTGGKSIYGSKFEDENFNHK
Hum. cycl.	*****C*****R*****E*****I*L*
Mal f6	**A**H***V**D**L*****A**A****QL*
EA 21	HSKPMMLSMANAGKNTNGSQFFITTAVTSWLDGKHVVFGVESGE
Hum. cycl.	*TG*GI*****P*****C**K*E*****K*KE*M
Mal f6	N**GL*****P*****V*****IDGM
EA 21	DVVKDMEAVGSSSGKTSQEVLIITDCGQ--
Hum. cycl.	NI*EA**RF**RN****KKIT*A****LE
Mal f6	N***AI**E**G***PRSRIE*AK**VC-

Fig. 1. Comparison of the amino acid sequence of EA21, *Malassezia furfur* cyclophilin (Mal f 6) and human cyclophilin.

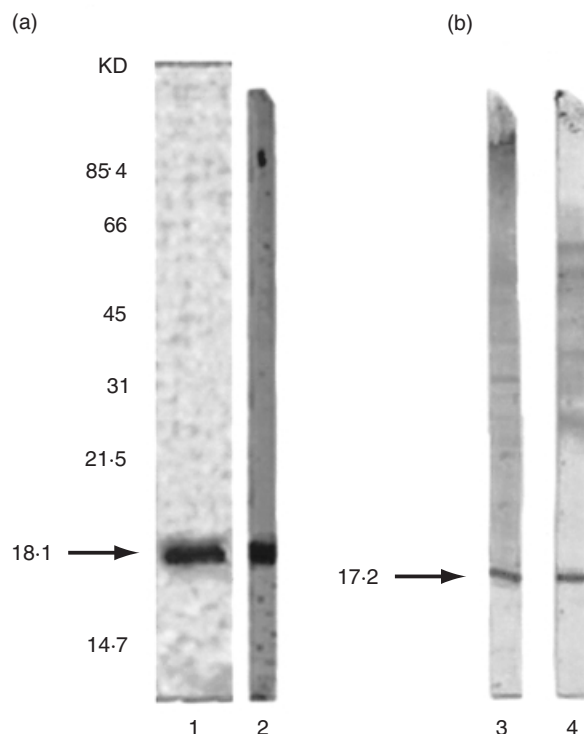


Fig. 2. (a) SDS-PAGE and immunoblotting of EA21. The protein was subjected to 12% SDS-PAGE and stained with Coomassie brilliant blue (lane 1), or transferred to nitrocellulose and revealed with the monoclonal antibody specific to the 6X histidine (lane 2). (b) Immunolocalization of *Echinococcus granulosus* cyclophilin. Immunoblotting after SDS-PAGE of protoscoleces (lane 3) and hydatid fluid (lane 4) revealed with the polyclonal antiserum specific to EA21. Molecular weights are indicated on the left.

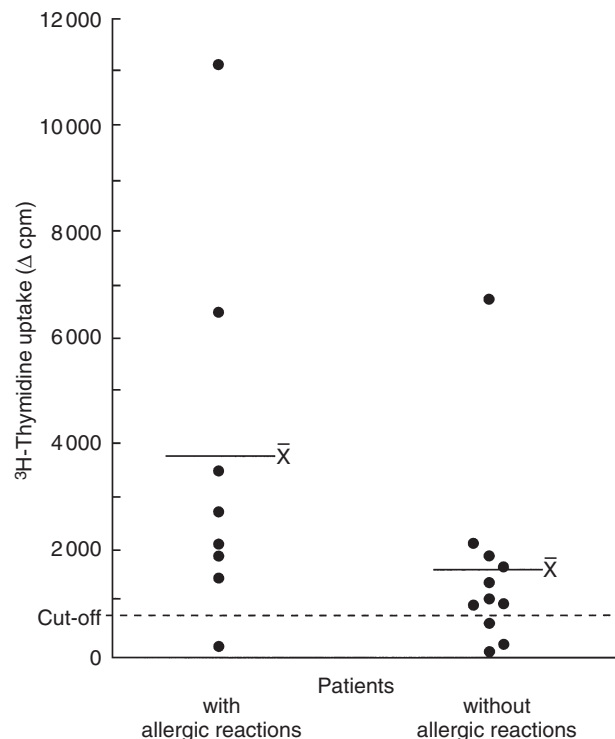


Fig. 3. Distribution of proliferation of peripheral blood mononuclear cells from 19 patients with cystic echinococcosis divided into two clinical groups according to the presence of allergic reactions in response to EA21. The mean Δ cpm value of uninfected human controls plus 2 standard deviations was taken as the cut-off level for positivity. $\bar{X}$ = mean Δ cpm values from patients with CE.

Table 2. Immunoblotting reactivity to EA21, Mal f 6 and human cyclophilin in sera from patients with cystic echinococcosis, atopic subjects and healthy donors

Serum samples	No. sera	No. of positive samples (%)					
		EA21		Mal f 6		Human cyclophilin	
		IgE	IgG	IgE	IgG	IgE	IgG
Patients with cystic echinococcosis	58	29 (50)	26 (45)	0	58 (100)	0	58 (100)
Atopic subjects	15	2 (13)	2 (13)	1 (7)	15 (100)	0	15 (100)
Healthy donors	30	0	9 (30)	0	30 (100)	0	30 (100)

Table 3. Pattern of IgE, total IgG and IgG4 reactivity to EA21 of 58 patients with cystic echinococcosis divided according to the presence of allergic reactions

Serum samples	No. of serum samples	IgE No. testing positive (%)	Total IgG No. testing positive (%)	IgG4 No. testing positive (%)
Patients with cystic echinococcosis				
with allergic reactions	25	20 (80)*	5 (20)†	5 (20)†
without allergic reactions	33	9 (27)*	21 (63)†	21 (63)†

Statistically significant differences by Fisher's exact test: * $P < 10^{-4}$; † $P = 10^{-3}$.

(*Betula verrucosa*), suggest the presence of cross-reactive epitopes between *E. granulosus* cyclophilin and birch cyclophilin (Bet v 7). This possible cross-reaction needs to be confirmed in future research with a larger panel of polyspecific or monospecific sera from atopic subjects. A possible limitation of our study is that to assess serological responses qualitatively, we used, in SDS-PAGE and IB, a recombinant protein purified in denaturing conditions. Although these experimental conditions disclosed linear epitopes alone, we cannot exclude the presence of conformational cross-reactive epitopes.

All the serum samples tested were IgG positive to Mal f 6 and human cyclophilin. One reason for this could be the ubiquitous presence of the saprophytic yeast, *M. furfur*, an organism that colonizes human skin and could have conserved epitopes that cross-react with human cyclophilin. Our data show that the presence of IgG specific to human cyclophilin is independent of *E. granulosus* infection. The lower IgG reactivity to EA21 observed in our serum samples suggests the presence of various immunodominant epitopes in the parasite cyclophilin. The high percentage of healthy donors who had positive IgG reactions to *E. granulosus* cyclophilin (30%) nevertheless precludes the use of this antigen in the immunodiagnosis of CE.

Despite the high percentage of patients with CE who had IgE specific to hydatid antigens, only 20% of these patients had allergic manifestations [9]. The IgE/IgG4 ratio is critical in determining the expression of allergic symptoms, and in other parasitoses, inhibition of allergic reactivity has been reported due to the presence of IgG4 'blocking antibodies' in the serum of infected individuals [18,19]. In an earlier study investigating the immune response to *E. granulosus* infection, we found a significant correlation between the production of IgE and IgG4 specific to hydatid fluid antigens. In line with others, we also reported the presence of IgG4 specific to antigen B in the active stages of the disease [20–22]. In a recent study on elongation factor 1 β/δ , we reported that 52% of CE patients' sera contained IgE whereas only 18% contained IgG4 specific to the recombinant protein, regardless of the presence of allergic reactions. We now show a high percentage of sera from patients with CE who had IgG4 antibodies specific to EA21. These findings underline two contrasting immunoglobulin associations, between IgG4 and protection against allergic reactions, and between IgE specific to EA21 and allergic manifestations. Hence, in CE as in other parasitoses, IgG4 apparently act to block pathogenic processes. As EA21 induces an IgE-specific response in patients with allergic manifestations along with an IgG4-specific response in those without, this molecule apparently acts as an allergenic protein. During clonal expansion, human B cells switch in successive steps from IgM to IgG4 and IgE. IgE and IgG4 isotypes may have other regulatory mechanisms, for example, the control exerted by HLA haplotype and HLA-independent genetic alterations leading to increased IgE production [23]. Further studies on the mapping of IgE and IgG4 epitopes are needed to clarify the various regulators of isotype switching.

In cellular and humoral immune responses to infections, cytokines have a role in regulating antibody isotype production. The cytokine IL-4 regulates synthesis of IgE and IgG4 [24]. Our cellular studies indicate that this antigen induces T-cell proliferation, but it is unable to induce IL-4 production in PBMC from patients with or without allergic manifestations. In previous *in vitro* and *in vivo* studies, we showed that *E. granulosus* antigens can influence the Th1/Th2 balance by eliciting IL-4, IL-10 and IL-

13 [16,20,25]. In this 'scenario', the ability of *E. granulosus* cyclophilin to elicit IgE production might depend not on an intrinsic ability to induce IL-4 production but on a generalized Th2 polarization.

Ample debate surrounds the mechanisms regulating IgE and IgG4 production. Even though both responses depend on Th2 polarization, in some immune circumstances (for example, allergy), IgE tends to dominate, whereas in others (including helminth infections), IgG4 predominates. Isotype switching leads to the production of two classes of immunoglobulins with antagonistic functions. The balance between the two could conceivably determine the clinical features. Finding out how the balance is controlled is a key question relevant to advances in the control of allergy by immunotherapy [26]. The research reported here takes our understanding of the mechanisms responsible for the allergenic manifestations in helminthic infections one step further.

We conclude that *E. granulosus* cyclophilin is a conserved, constitutive, parasite protein that is not cross-reactive with cyclophilins from other organisms, and that is involved in the allergic reactions manifested by patients with CE. The precise origin of these events could be investigated by constructing a test panel of parasitic allergens. Our data suggest that we should identify antigens and immunodominant epitopes inducing different Ig classes and subclasses able to endanger or protect the host.

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