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Original Article

Characterization of Specific IgE Antibody Related to Antigen 5 of *Echinococcus granulosus*

**AR Khabiri, F Bagheri, MR Siavashi, M Assmar*

Dept. of Parasitology, Pasteur Institute of Iran, Tehran, Iran

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Abstract

Background: Anaphylactic reactions, such as urticaria, edema, respiratory symptoms, and anaphylactic shock often complicate the course of Cystic Echinococcosis (CE). **Methods:** To investigate the role of the IgE immunoreactive antigen 5 (Ag 5) in the sero-positive patients with CE, we determined N-terminal of 57 kDa subunit of Ag5 responsible for IgE and C-terminal of this active antigen related to induction of IgG specifically. **Results:** Immunoblotting analysis showed that specific IgE to 57-kDa subunit related to inter-chain disulphide band of two 22 kDa and 38-kDa component of Ag5 and conformational epitope on this subunits. In addition, since the 57 kDa component arise from the removal of the C-terminal portion of 22 kDa subunit of Ag5, thus IgE specifically recognized N-terminal of 22 kDa subunit which remain bounds to the other component, whereas IgG reacted with C-terminal of 38 kDa component of Ag5. **Conclusion:** Recognition of the specific binding site on the 57 kDa subunit of Ag5 could leads to understanding the mechanism regulating IgE/IgG production in some immune circumstances that IgE tends to some dominate, whereas in other IgG predominates.

Keywords: *Cystic Echinococcosis, Allergy, IgE, Antigen 5*

Introduction

Cystic Echinococcosis (CE), caused by infection with larval *Echinococcus granulosus* has public health importance not only in areas of endemicity but also in countries or regions where migration of infected people and exchanges of livestock occurs (1). In human cystic hydatid cases, which are seropositive, IgG and IgE antibodies against cyst fluid antigens predominate. Differences in the antibody class switching response have been noted between asymptomatic patients and more advanced cases that had undergone surgery (2). Asymptomatic preferentially induce IgG and surgical cases IgE, which suggest a class switching as the disease progresses (3). In helminthic infections, IgE production may be responsible for the protective immune response to the parasite or immune-mediated pathology or both (4). In particular, in *Necator americanus*, *Ancylostoma caninum*, and *Schistosoma mansoni* infections, the level of IgE correlates with the protected status of the host. In contrast, in *Echinococcus granulosus* and some other helminthiases, as in *Strongyloides stercoralis* infection, the presence of IgE is associated with pathological allergic reactions (5, 6). The elevated IgE have tight links with allergy in patients with CE are key biological features of the disease. A variety of allergic symptoms has described in patients with CE that include urticaria that may be only mild or present as chronic and apparently idiopathic urticaria. Anaphylactic shock is the most impressive and severe clinical presentation of IgE-dependent allergic reactions in CE (7).

Echinococcus allergens, i.e., those specific antigens from *Echinococcus* that are able to induce IgE secretion, have especially studied for *E. granulosus*. They may be found among the main *E. granulosus* well-define antigens used for diagnosis such as antigen B (AgB) and Ag5 as well as among less-studied antigens elonga-

***Corresponding Author:** Tel/ Fax: +98 21 66968855, E-mail: Alireza_khabiri@yahoo.com

tion factor -1 (EgEF-1 β/δ) and Cyclophilin (EA21) (8, 9).

Elongation factor-1 is a conserved constitutive protein responsible for the binding of aminoacyl-tRNA to the ribosomes and located predominantly in the endoplasmic reticulum and probably released into the hydatid fluid only after degeneration of the protoscoleces due to aging, calcification or drug treatment. This protein have double pathological role, including allergic reaction and interfering on the susceptibility to human CE (10). Cyclophilin (EA21) as EgEF-1 β/δ is a conserved constitutive protein with enzymatic peptidyl-prolyl isomerase (PPI-ase) activity, which is essential to protein folding in vivo. Proteins of the cyclophilin family have been described as allergens in molds *Psilocybe cubensis* (*Psi c2*), *Aspergillus fumigatus* (*Asp f11*) and *Malassezia furfur* (Mal *f6*), and in *Birch pollen* (*Bet v7*) (11, 12). Association of IgE specific with Ag5 has received very little attention and could be fruit fully studied to confirm the occurrence of allergic reactions in patients with parasitic infection, in this study our aims was to seek and characterize allergic molecules from Ag5 that behave as molecular markers of allergic reaction during human CE. By western blotting, we assessed the 57-kDa subunit of Ag5 responsible for reactivity of specific IgE in patients with CE.

Materials and Methods

Antigen preparation Hydatid preparations enriched for Ag5 were obtained from the cyst hydatid fluid (CHF) based on the method of Orial et al. (13). Briefly, The HF was dialyzed against acetate buffer (pH 5.0) and after centrifuged at 52000g for 30 min, the precipitate dissolved in phosphate buffer (pH 8.0), and centrifuged. The supernatant collected and passed through a sepharose-4B proteinG column (Pharmacia LKB, Uppsala, Sweden). Finally, dialysis performed against phosphate saline buffer (PBS, 0.15 M. pH 7.4).

Sera The sera samples were collected from 45 surgically confirmed hydatid patients and cut-off values were calculated based on 53 sera from healthy adult individuals.

Screening of IgE positive sera IgG and IgE specific Ag5 were measured by ELISA test according to Short et al. (14) with some modifications. Briefly, microtitration plates (Maxisorp, NUnc, Roskilde, Denmark) were coated with 5 µg/ml of Ag5 diluted in 0.1 M carbonate/ bicarbonate buffer (pH 9.6). Sera were diluted 1:100 and 1:20 for IgG and IgE, respectively in PBS containing 2.5% bovine serum albumin. Goat anti-human IgG or IgE conjugated to horseradish peroxidase (Sigma, USA), diluted 1:3000 and 1500, respectively used as the second antibody. TMB and H_2O_2 applied to visualize the antigen-antibody reaction. Optical density (OD) was registered at 450 (OD_{450}) after the addition of stop solution (H_2SO_4, 2.5 N). Mean OD± 3 standard deviation (SD) of the OD values obtained for the healthy sera were used to establish a cut-off value. Values greater than the Cut-off value was considered positive for anti-hydatid antibodies.

SDS-PAGE and Immunoblotting Purified Ag5 was subjected to SDS-PAGE in 10% homogeneous polyacrylamide gels as described by Laemli (15). Antigens were separated under reducing and/or non- reducing conditions (with or without 5% 2-mercaptoethanol) and were transferred from unstained gels to nitrocellulose membranes by means of the Fast transfer (Pharmacia LKB) following standardized procedures**.** Efficiency of transfer was verified by staining with Ponceau S solution (0.3% W/V in 3% V/V trichloroacetic acid). Membranes were blocked for 1 h at room temperature (RT) in Tris buffer (0.1 M with 1% Tween20) and bovine serum albumin (1% W/V). The blocked membrane cut into strips and incubated overnight at RT with pool positive and negative sera (diluted 1:500 and 1:100 for IgG and IgE). After washing, membranes further incubated with peroxides labeled goat anti- human-IgE and IgG (Sigma) 1:1500 and 1:5000 dilutions, respectively for 2 h at RT. Finally membranes

washed and visualized by adding Diaminobenzamidine tretrahy-drochloride (DAB) (0.06% W/V) solution con-taining H_2O_2 (0.1% V/V).

Results

Screening of patient sera Qualitative analysis of IgE response in patient sera with CE allowed us to identify two distinct groups of CE sero-pasitive, IgG plus IgE and/or IgE positive relative to negative controls. In CE patients 100% positive reaction were recorded for IgG antibody, whereas IgE could be detected in only 64.4% of tested sera. Data analysis also showed that all of the IgE positive had IgG specific to Ag5, whereas 16 of the 45 patients (35.6%) were negative for specific IgE to Ag5.

SDS-PAGE and Western blotting Fig. 1 shows the different components of the Ag5 purified on SDS-PAGE under non-reducing. In this condition the Ag5 show 67- and 57 kDa subunit in gel staining with comassie blue. The relative amount of some smaller component may arise from the process of two main bands or other hydatid cyst fluid during Ag5 purification. Western blotting performed under this condition showed that sera from CE patients with IgG and IgE reacted to the 57 kDa subunit but western blotting performed under reducing conditions showed that IgG is related to the 38 kDa subunit of Ag5 and IgE do not recognize this subunit of Ag5 (Fig.2).

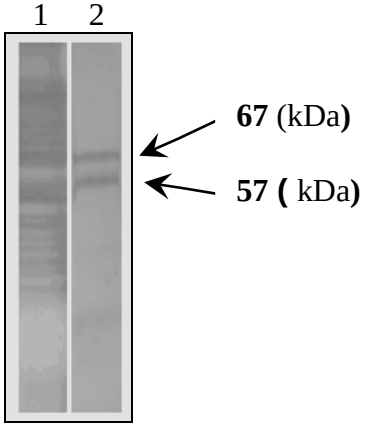

Fig. 1: Antigen 5 from *Echinococcus granulosus* hydatid cyst fluid before (Line 1) and after (Line 2) purification. Under non-reducing condition the Ag5 show 67- and 57 kDa subunit in gel staining with comassie blue. The relative amount of some smaller component may arise from the process of two main bands or other hydatid cyst fluid during Ag5 purification.

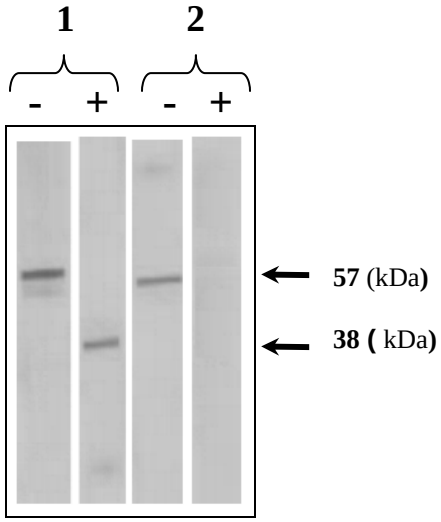

Fig. 2: Immunoblots of the antigen 5 from *Echinococcus granulosus* hydatid cyst fluid by anti-human IgG (Line1) and Anti-human IgE (Line 2) under reducing (+) or non-reducing (-) conditions: IgG react with both 57 kDa and 38 kDa subunits of Ag5 under non-reduce or reducing condition (arrow) but IgE recognized only 57 kDa component under non-reduce condition (arrow).

Discussion

Hydatid cyst fluids, specially, Ag5 has been a useful diagnostic tool for *E. granulosus* in human for many years. Despite this, there are major practical problems with the Ag5 such as, lack of accuracy that is associated with false-positive reactions, as it exists at present (16). Ag5 is a very-high-molecular-weight lipoprotein complex composed of 57-, and 67- kDa components that under reducing condition dissociated into 38-, and 22- to 24- kDa subunits. It is synthesized as a single polypeptide chain that is afterwards processed into two subunits; the 22- kDa subunit is generated by processing of the N-terminal region of the original polypeptide chain, whereas the 38- kDa component corresponds to the C-terminal portion (17). Part of cross-reactivity is related to the presence of phosphorylcholine in the Ag5.

In a previous study, we showed that IgG1 from patients infected with other parasites and from healthy controls was responsible for cross-reactivity with the 38 kDa subunit of Ag5 (18). The amino acid sequence of the 38- kDa subunit shows high similarity to that of serine proteases of the trypsin family, specifically to the major neutral proteases of mast cells (17). In this study, it was shown that the high percentage of IgE specific recognized 57 kDa subunit of Ag5 under non-reducing condition. It was shown that the 57-kDa component arises from the main 67- kDa forms of Ag5 in a process that involves the removal of the C-terminal portion of the 22- kDa subunit. We considered the possibility that same activation needed in the case of Ag5 for convert the 67 kDa inactive form into a 57 kDa form by proteolytic cleavage and removal of the C-terminal portion of 22 kDa subunit. Since the IgE reacted specifically to 57 kDa subunit of Ag5 under non-reducing condition, therefore the IgE/IgG ratio is critical in determining the expression of allergic symptoms of inhibition of allergic reactivity that has reported due to the presence of IgG in the serum of infected individuals.

Western blotting performed under reducing condition show in spit of the IgG recognized the 38 kDa submit of the Ag5. One speculation for non-reactivity of IgE to reduced 57 kDa could relate to inter-chain disulphide band that 2-mercaptoethanol dissociate disulphide band and impaired conformational antibody binding sites. Base on the alignment of amino acid sequence there is eight and seven cysteine residues on 38-, and 22- kDa subunit of Ag5 and the 38- kDa subunit contain at least one free thiol group which involved in inter- chain disulphide formation with 22 kDa subunit(19). In addition, reactivity of specific IgE to non-reduced Ag5 could also relate to N-terminal of 22 kDa subunit which reminds bounds to the bigger subunit. In this manner, the 57- kDa component arise from the removal of the C-terminal portion of 22- kDa subunit and this cleavage induce to disappear the 22- kDa subunit in reducing condition. Another study supports our idea which showed IgE epitope on EgEF-1 β/δ localized exclusively in the variable N-terminal regions that homology with a *S. stercoralis*, recombinant antigen recognized by IgE from patients with *Strongyloidiaius* (20). The IgE and IgG response directed to the N-terminal and C-terminal regions of the Ag5 subunit suggests that *E. granulosus* could manipulate the host immune response by addressing IgE and IgG antibodies to functional epitopes present in this region. Understanding the mechanism regulating IgE/ IgG production could be a key question relevant to advances in the control of Allergy and parasites.

In conclusion, our findings suggest that N-terminal and C-terminal of the Ag5 have distinct function in an IgE/ IgG induction molecule. Investigation on epitopes which recognized by specific IgE and/ or IgG on 57- kDa component and the mechanism of this subunit on allergic patients is currently understudy.

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