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Th_1 and Th_2 Inducing Cytokines in Cystic Echinococcosis

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SUMMARY: This study included 62 subjects who presented at the Turgut Özal Medical Centre in Malatya. Of these, 32 were infected with the larval stage of Echinococcus granulosus and the remaining 30 were healthy controls. The ratio of males to females was 0.45. Cystic echinococcosis (CE) occurred in all age groups; the youngest case was 12 years and the oldest case was 74 years old. The frequency of hydatid cysts located in liver was (78.1%) followed by lungs (15.7 %) and one in the brain. All patients recovered completely except for one woman (35 years old) in whom multiple cysts (21cysts) were detected in liver and omentum two years after the first operation. In 68.7 % of the cases, the cytokine that was most frequently increased was IL-4. The IL-2 cytokine increased to 46.8% followed by IL-10 (40.6 %). These results suggest that there are different immunoregulatory events and the cytokine response during CE may be in part related to the slight monocytosis observed in CE patients. In conclusion, concurrent Th1 and Th2 cytokine-type profiles were expressed in CE with the predominance of type Th2 cytokines.

Key Words: Cystic echinococcosis, IL-2, IL-4, IL-10

Kistik Ekinokokkozta Th1ve Th 2 yi İndükleyen Sitokinler

ÖZET: Bu çalışma Turgut Özal Tıp Merkezi/Malatya'da toplam 62 olgunun katılımıyla düzenlendi. Bunların 32'si larval dönem CE enfeksiyonu olan, 30'u ise sağlıklı bireylerdi. Erkek, kadın oranı 0.45 idi. CE, her yaş grubunda görüldü; en genç hasta 12 yıl yaşında, en yaşlısı ise 74 yıl yaşındaydı. Kist hidatik lokalizasyonu en sık karaciğerde (%78,1), daha sonra akciğerde (%15,7) ve bir vakada da beyindeydi. 35 yaşında, karaciğer ve amentumda multipl kistleri (12 kist) olan bayan hasta dışında bütün olgular tamamen iyileştiler. Olgularda en fazla yükselen sitokin %65,7 oranıyla IL-4 oldu. IL-2'dek artış %46,8, IL-10 artışı %40,6 olarak bulundu. Bu sonuçlar, CE'da farklı immunregulatör olayda ve sitokin cevabı'nın olduğunu ve bu hastalarda hafif monositozun olabileceğini gösterdi. Sonuç olarak, CE' da Th1 ve Th2 sitokinlerinin bir arada bulunduğu ve Th2 sitokinlerin daha baskın olduğu görüldü.

Anahtar Sözcükler: Kistik Ekinokokkoz, IL-2, IL-4, IL-10

INTRODUCTION

Cystic echinoccoccosis (CE) is an important cestode infection endemic in Turkey (1). It is a potentially dangerous disease and may involve vital organs such as brain. It is primarily disease of herbivorous animals and man is infected accidentally (5). Clinical features of the disease are highly variable and depend on organs involved, size of cysts and their sites within the affected organ, complications caused by rupture of cysts, and subsequent immunologic reactions (16). The roles of cytokines in host immunity seem to be quite complex and may differ with genera and species of helminth, its size and location within the host, its metabolic products, and species of the host (2, 16). Moreover, conflicting cytokine responses were reported in many studies and attributed to technical and methodological points, differences in dose and type of the used stimulus or antigen and the course of the infection (4, 6, 8, 11, 12).

CE induces two very distinct Th_1 and Th_2 cytokine secretion patterns. They were implicated in the inactive and active stages of hydatid disease (13). Th_2 cells express IL-4, IL-5, IL-6 and IL-10 and they are associated with susceptibility to the disease, whereas Th_1 cells produce IL-2, IFN-γ and they are to related to protective immunity (9, 19). T. Resting T cells normally do not synthesise or secrete IL-2 unless they are stimulated by antigens or after exposure to mitogens. IL-4 It is known as cell growth factor 'BCGF' (10). It stimulates growth and differentiation of B cells to IgG_1 and IgE isotypes. IL-10 is primarily secreted by Th_2 cells and has an anti inflammatory cytokine and suppresses Th_1 cytokines production (10).

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Most studies on CE cytokines are mainly based on *in vitro* experiments, i.e. determination of cytokine production following stimulation peripheral blood mononuclear cell or T helper cells of patients with crude and B antigens (3, 7, 12). These studies demonstrated an increase in the production of some cytokines such as gamma-interferon, IL-4, and IL-5. Others demonstrated concurrent intervention of the Th_1 or Th_2 cells (6, 9, 14). The clinical status, differences between primary and recurrent infections and severity of the clinical expression of the disease probably also affect human immunological responses to the parasite (7).

Different methods are used to determine cytokines levels. Specific cell culture is common and optimal one. By this method, peripheral blood mononuclear or spleen T cells were *in vitro* incubated at ambient CO_2 and stimulated with specific antigens or mitogens and mRNA for each cytokines was determined using double sandwich ELISA or molecular methods such as reverse-transcriptase polymerase reaction (2, 3, 11, 13). Unfortunately, such methods are rarely performed in our country. Instead, cytokines determination was attempted in serum. However, serum levels can be affected by many factors such as autoimmune diseases, type of consumed food. The number of clinical studies on cytokines in human CE is limited. The present study was focused on determining the effect of infection with the larval stage of *E. granulosus* on serum levels of IL-2, IL-4 and IL-10.

MATERIALS AND METHODS

A total of 32 patients with CE (10 males and 22 females), and 30 healthy controls (12 males and 18 females) were recruited for this study. The ratio of males to females among CE patients was 0.45. Complete blood counts (CBC) was performed on all subjects. CE was diagnosed by microscopic examination of cyst fluid for scolices or hooklets and results of radiographic examination, histopathological evaluation, and compatible clinical findings. A commercial kit, indirect haemagglutination test (Cellognost Echinococcosis, Behring Diagnostics Gmbh, Marburg, Germany), was used for detecting CE antibodies. Stool examination was made on all studied individuals to exclude patients with intestinal parasites.

The age range of the patients with CE was 12-74 years with a mean 46.5±19. 6 years (median=42 years). Healthy control subjects were between 16 and 47 years old with a mean age of 27.7±9.7 years (median=24.5 years). Twenty five cysts were located in liver mostly in right lobe (Table 1). In one woman (74 years old), there were cysts in both liver and lung. Cysts located in both brain and lungs were observed in an old man (68 years old). All cysts were found to be surrounded by fibrous layer except that of brain. Few cases of sterile kidney cysts were excluded from this study since they were identified as simple epithelial cysts on the basis of histopathological examination and negative indirect haemagglutination test.

Decision was made whether control subjects were healthy according to results of blood examination (routine parasitological, biochemical and CBC tests) in addition to apparent healthy status with no history of current illness and pathological condition. Venous fasting blood samples were collected before initiating antiparasitic therapy and surgical operation and on the day of the surgery and three months after that. Blood samples were centrifuged at 2000×g for 10 minutes to obtain the serum. The lipaemic or haemolysed sera were discarded. The other sera were divided into 3 tubes for each subject and stored immediately at -20 °C until analysis. For each determination, a new aliquot was used since freezing and thawing of a sample more than once may partially destroy its biological reactivity. Permission for all procedures was taken from the local Ethical Committee and all subjects gave their informed consent to the study.

Cytokines: Serum levels of IL-2, Il-4 and IL-10 were measured by ELISA following the manufacturer's instructions. IL-2 had 5 pg/ml detection limit (Cytoscreen Immunoassay human IL-2 kit, Biosource International Inc., USA). IL-4 and IL-10 had 0.4 pg/ml and 5 pg /ml detection limits respectively (The Pelikine human IL-4 ELISA kit, Central Laboratory of the Netherlands Red Cross Blood Transfusian Service, The Netherlands).

Statistical analysis: Mann-Whitney U, Chi-square and Fisher's tests were used for evaluation of data. Pearson's linear correlation test was used for assessment of correlation between parameters. The minimum level of significance was defined at $p<0.05$. All above mentioned analysis were performed using the Standard Package for Social Sciences (SPSS) version 9.0 for Windows (SPSS).

RESULTS

The study covered 62 subjects attending Turgut Özal Medical Centre/Malatya. They were 32 with larval stage *E. granulosus,* and 30 healthy controls. CE occurred in all age groups; the youngest case was 12 years and the oldest case was 74 years old.

Serum antibodies titres were found to be either equal or higher than 1/512 by indirect haemagglutination test. The CBC results showed normal total white blood cells count at range of 4.1- 8.7 x1000 /ml) but there was slight increase in monocytes percentages in majority of patients (30/32, 93.7%) at range of (11.3- 19.1%, normal range 0.9-6%). The frequency of hydatid cysts localized in the liver was (78.1%) followed by lungs (15.7%) and one both lung and brain case (Table 1). All patients were recovered completely after surgical intervention and antiparasitic therapy with albendazole except one woman (35 years old) in whom as many as 21 cysts were detected in liver and omentum two years after first operation. The normal serum concentration of IL-2 and IL-10 were less than 5 pg /ml and the mean level of IL-4 was <0.04pg/ml according to kit

values. The cytokine values of individuals having CE infections higher than those of controls were considered as increased. Table 2 shows the mean serum levels of studied cytokines. They were significantly increased in CE group at 46.8 %, 68.7 %, 40.6 % respectively (P <0.001) (Table 3) but they were significantly decreased after successful treatment. No statistically significant correlation was detected between any of the studied cytokine levels.

Table 1. Location sites of hydatid cysts .

Sites	Number	Percentage
Liver only	25	78.1
Lung only	5	15.7
Both liver & lung	1	3.1
Both lung & brain	1	3.1
Total	32	100

Table 2. The cytokine levels in patients with CE and healthy controls (mean ±SD).

Cytokines (pg/ml)	Healthy controls (n=30)	Patients with CE (n=32)		P value*
		Pre-treatment	Post-treatment	
IL-2	1.2 ± 1.1	6.5 ± 5.7	2.8 ± 1.8	<0.001
IL-4	0.19 ± 0.11	3.2 ± 2.7	0.61 ± 0.98	<0.001
IL-10	2.1 ± 1.1	4.1 ± 2.7	2.6 ± 1.5	<0.001

*Mann-Whitney U test

Table 3. The numbers and percentages of persons infected with CE showing increased cytokines.

Cytokines (pg/ml)	Increased[a]		Normal[b]		Total	P-value[c]
	n	%	n	%		
IL-2	15	46.9	17	53.1	32	<0.001
L-4	22	68.7	10	31.3	32	<0.001
IL-10	13	40.6	19	59.4	86	<0.001

a: CE patients with increased cytokine levels; **b:** CE patients with normal cytokine levels; **c:** Chi- Square test

DISCUSSION

CE is in fact a chronic disease and presence of cysts for several months or years may have a major role in modulating cytokine production via prolonged stimulation by cyst fluid antigen. Humoral and cellular response characterized by elevation in some serum antibodies and cytokines (2,6,13). At the cyst area, there is cellular infiltration consisting of neutrophils, macrophages, eosinophils, and fibroblasts (14). There is no severe inflammatory process and cysts are surrounded by a fibrous layer which separates the laminated layer from the host tissues. The exception is brain where no fibrous layer surrounding the cyst as observed in one brain case during this study. Till now, it is unknown whether such cellular infiltration is due to innate host response and parasite releasing chemotactic substances or due to cytokine's effects of Th0 / Th1 subsets (14).

The importance of cytokine responses in the pathogenesis of CE was previously studied in animal models (2,6,8). The experimental studies in mouse-model echinococcosis and human echinococcosis demonstrated that cytokine profile varies with the age of cyst. Generally speaking, there is Th0 or Th1 response in early infection and Th2 response in late and chronic infections (14). In another study, it was reported that IFN-γ and IL-10 were elevated in the first three months of infection (6).

In assessing the results of this study, IL-4 was significantly increased in CE group 68.7 % (P <0.001). This clearly indicated the importance of IL-4 in immune response to CE. IL-2 was elevated in nearly half of patients (Table 2). It is also important to note that Th1 cell activity may co-exist with Th2 cell activity in some cases (14). The percentage of increased IL-10 was 40.63 % and this could increase hydatid cyst survival and growth since IL-10 inhibits effector cellular immune response via its potent anti-inflammatory action (18). Another important factor in CE that may affect the cytokine profile is the site of location of cyst within liver. Torcal et al (16) showed significant increase in values of TNF -α, IL-1, IL-2, IL-4, and IgE in patients having cysts located in the central area of liver than peripheral location. However, our results indicated a bias towards Th2 response (IL-4, IL-10). Further investigations are necessary in larger patient groups to support our present findings and assess the role of other unstudied cytokines in disease progress and treatment.

On comparing cytokine profiles of the present study with those of alveolar echinococcosis (15, 17), Different cytokine profiles were found in cystic versus alveolar echinococcosis, the parasitic agents being very similar but the clinical course was very different.

Albendazole is routinely used in postoperative therapy in patients with CE. Nevertheless, in the present study many secondary cysts recurred in the abdomen of one female patient at interval of 2 years after the first operation. This is most probably the result of complication of the first surgical intervention. The patient had significantly increased serum IL-2, IL-4 and IL-10 levels. This finding could mean an enhanced immunological response when cysts are multiple and secondary due to probably increased cyst antigen stimulation and in turn more activation of immune cells.

In conclusion, the results point to the presence of both Th1 and Th2 cytokine profile in CE with Th2 predominance. CE also causes activation of monocytes that may be involved in CE pathophysiology.

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