

Serum antibody & Th2 cytokine profiles in patients with cystic echinococcosis

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Background & objectives: Human cystic echinococcosis (CE), caused by *Echinococcus granulosus*, is one of the most important and widespread parasitic zoonoses. T helper cell-2 (Th2) dominated immunity in CE is associated with increased susceptibility to the disease, while T helper cell-1 (Th1) cell activation is assumed to induce protective immunity. Hence, in order to investigate *in vivo* Th2 cell activation and serum complement levels, the present study was aimed to detect serum levels of specific IgG, IgE, interleukin (IL)-4, IL-10, C3c and C4 in confirmed CE patients.

Methods: Specific IgG levels in serum was measured by enzyme linked immunosorbent assay using recombinant *E. granulosus* antigen-B/2 (RecEg-AgB/2) and serum IgE, IL-4, IL-10, C3c and C4 were quantified by nephelometry in 45 surgically confirmed patients with CE, and 10 healthy controls.

Results: Specific IgG ($P<0.0001$), IgE ($P<0.05$), IL-4 ($P=0.0197$) and IL-10 ($P<0.01$) levels were significantly elevated in CE cases compared to healthy controls. IL-4 could be detected in 34 patients (75.55%) and six controls (60%) in a low concentration. The IgE concentration was elevated (>120 U/ml) in 36 (80%) cases of CE and in one healthy control.

Interpretation & conclusion: Our results showed higher C3c and C4 levels in CE patients than healthy controls. No significant association was found between IgE concentrations and cytokine levels. The results of this study point to a cytokine profile suggestive of Th2 cell dominance *in vivo* in CE.

Key words Cystic echinococcosis - cytokines - host immune response - Th1/Th2 cells

Human cystic echinococcosis (CE), caused by *Echinococcus granulosus*, is one of the most important and widespread parasitic zoonoses¹. In general human CE is diagnosed and characterized in the late symptomatic stages when significant pathology has already occurred². Many (60-80%) confirmed CE patients only are seropositive for antibodies³, which may be due to

the antibodies forming immune complexes in some patients⁴. Several studies have shown an elevated IgG subclass (IgG1 and IgG4) antibody response in advanced human CE⁵. While parasite-specific antibodies appear not to exhibit a direct restricting role on the growth of metacestodes in humans, the immunological effector function may be attributed primarily to T cells⁶.

Both humoral and T-cell mediated responses, constituted of T helper cell-1 (Th1) and T helper cell-2 (Th2) type reactions, seem to play an important role against infections and are considered to be regulated by cytokines⁷. Th1 cells produce interferon- γ (IFN γ), interleukin, (IL-2), tumour necrosis factor- β (TNF- β), and are responsible for both humoral and cell-mediated immune responses⁸. Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 cytokines, which facilitate the production of IgE, IgG1-IgG4 isotype switching and IgA synthesis⁸. Th2 type responses are predominant in patients with chronic extracellular parasite infections⁹. IgG1 and IgG4 appear to be the most active antibody subclasses in both human cystic and alveolar echinococcosis⁵. Fauser and Kern¹⁰ have demonstrated that re-stimulation with crude *E. granulosus* antigen induced or enhanced Th2 cytokine mRNA expression (especially IL-5 and IL-10) by peripheral blood mononuclear cells from patients with CE. Another study showed a decrease in IL-1 and TNF cytokine levels in CE patients compared to controls¹¹.

Host complement system interact with the parasite, and damage it, in both pre-cystic as well as established cystic phases of infection¹². Even in the established cyst, because the laminated layer is permeable to host macromolecules¹³, the outer syncytial tegument of the germinal layer is permanently exposed to complement levels. Complement triggers inflammatory response by the infection followed by complement activation with the release of C4a, C3a and/or C5a. However, Diaz *et al*¹⁴ found no significant alteration of complement levels in plasma of patients with CE.

In India, *E. granulosus* infections have been identified in different intermediate hosts including buffaloes¹⁵, cattle^{15,16}, sheep¹⁶, goats¹⁶, pigs¹⁷ and camels¹⁸. Molecular evidence indicates that three different *E. granulosus* genotypes have been found to be prevalent in India¹⁹⁻²¹. In particular, the intraspecific variants or strains may play an important role with regard to infectivity, pathogenicity, epidemiology and control²².

Although the Th1 - Th2 cell concept with respect to susceptibility and resistance to infection with *E. granulosus* is widely accepted, data related to cytokine production *in vivo* among the Indian populations are lacking. Hence in the present study an attempt was made to detect serum levels of *E. granulosus* specific IgG, IgE, IL-4 and IL-10 to assess Th2 cell activation *in vivo*. Also serum C3c and C4 levels were quantified in CE cases to assess the inflammatory activity of the disease.

Material & Methods

The present study was conducted in Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, after obtaining the Institute Research Committee approval of the protocol. Patients seen during a period of two years (January, 2004 to December, 2006) were all inpatients and serum sample was collected from them prior to surgery. A written consent was obtained from all patients and controls. Approximately 5 ml of serum sample was collected before surgery from 45 surgically confirmed patients with CE (14 male and 31 female), and 10 healthy controls (6 male and 4 female). The age of the patients with CE ranged from 22 to 65 yr, with a mean age of 44.93 yr (median = 45 yr) and healthy controls were 26 and 54 yr old with a mean age of 37.8 yr (median = 37.5 yr). The CE patients (n=23) were identified to be infected by sheep strain (G1), five by buffalo strain (G3) and 17 by cattle strain (G5). These strains were identified by polymerase chain reaction based restriction fragment length polymorphism (PCR-RFLP) analysis of the internal transcribed spacer-1 gene using *Cfo* I and *Alu* I restriction endonucleases²⁰.

Patients & controls: The patients group included 45 cases of surgically confirmed cases of CE. The cysts removed during surgery were confirmed to be of hydatid cyst aetiology by histopathological evidence of germinal layer and by demonstration of scolices and hooklets in the aspirated HCF. The control group included healthy blood donors and students who have not suffered from CE or any other disease in the recent past.

Detection of specific IgG antibodies by enzyme linked immunosorbent assay (ELISA): All the reagents used in this study were obtained from Genei, Bangalore, India, unless specified. The recombinant *E. granulosus* antigen-B/2 (RecEg-AgB8/2) was produced²³ in the laboratory. Polyvinyl high binding microtiter ELISA plates (NUNC, DK-4000 Roskilde, Denmark) were coated with 100 μ l/well (0.5 μ g/well) of 1:100 dilution of the RecEg-AgB8/2 in carbonate bicarbonate buffer pH 9.6 and incubated overnight at 4°C undisturbed.

Quantification of IgE and cytokines in serum: Serum concentrations of IgE, cytokines such as IL-4, IL-10 and complement proteins such as C3c and C4 were quantified by nephelometric analysis using Nephelometer (BN ProSpec, Dade Behring, Marburg, Germany). The tests were performed according to the

manufacturer's instructions. C3c and C4 antibodies were obtained from Dade Behring Diagnostics India Private Ltd., New Delhi, India. IgE, IL-4 and IL-10 antibodies were obtained from Invitrogen, USA.

Statistical analysis: The statistical analysis for comparing the subgroups was done using Chi square test. $P < 0.05$ was considered as significant.

Results

IgG antibody levels were elevated significantly ($P < 0.0001$) in CE patients compared to healthy individuals. IgE concentration was elevated (>120 U/ml) in 36 (80%) CE cases and in one (10%) healthy individual ($P < 0.05$). The mean concentrations of cytokine and complement protein levels in healthy individuals plus one standard deviation (SD) was taken as cut-off. IL-4 and IL-10 were detected in 34 (75.55%), 43 (95.55%) cases of CE patients and 6 (60%), 9 (90%) healthy controls respectively (Table). IL-4 levels were elevated (>1.35 pg/ml) in 25 (55.55%) CE cases and in 1 (10%) healthy individual and IL-10 levels were elevated (>2.28 pg/ml) in 32 CE cases (71.11%) and in 1 (10%) healthy individual. C3c levels were increased (>1.26 g/l) in 30 CE cases (66.66%) and 1 (10%) healthy individual and C4 levels were raised (>0.72 g/l) in 18 CE cases (40%) and 1 (10%) healthy individual. When compared to healthy individual, serum IgE ($P < 0.05$), IL-4 ($P < 0.01$), IL-10 ($P < 0.01$), C3c ($P < 0.05$) and C4 ($P < 0.05$) levels were significantly raised in the patients with CE, irrespective of the strain of *E. granulosus*.

In an attempt to depict the possible correlation of serum immunoglobulin and cytokine levels with that of the strain of *E. granulosus*, significant difference was observed in serum specific IgG antibody levels between sheep (G1) and buffalo strain (G3) [$P < 0.01$; 95% confidence intervals (CI): 0.092 to 0.550], but not between sheep-cattle and cattle-buffalo strains. Besides, only the C3c levels were significantly raised in case of sheep strain compared to buffalo strain ($P < 0.05$; 95% CI: 0.060 to 1.644). There were no significant differences in serum IgE, IL-4 and IL-10 levels detected with respect to different strains of *E. granulosus*.

Discussion

The production of IgG antibodies in CE may depend on the number, size, location and condition of hydatid cysts.³ IgG4 antibodies result from a Th2 lymphocyte response²⁴. Specific IgG and IgE antibody levels were significantly elevated in clinically expressed CE cases compared with the control group. IgG1/IgG4 subgroup antibody levels were not measured in this study. The specific IgG antibody levels were elevated in all surgically confirmed patients with CE while the IgE levels were elevated in 80 per cent of cases of CE and in only one healthy control. However, antibodies alone do not appear to have a significant role in effective host immune responses, which can control or kill the hydatid cysts²⁵. Host age, sex and strain also influence susceptibility/resistance to hydatid infection²⁶. Th2 dominated immunity is associated with increased susceptibility to the disease, while Th1 cell activation is assumed to induce protective immunity⁷⁻⁹. As compared to controls, concentrations of the Th2 cytokines IL-4 and IL-10 were significantly raised in CE patients.

Since significant association between serum IgE levels and disease status has been shown earlier²⁷, the relation between serum cytokine levels (IL-4 and IL-10) and IgE concentration was investigated in this study. There was a prominent increase in IL-10 levels among all patients with CE in comparison to the healthy controls, whereas IL-4 was detected only in 75.55 per cent cases, which may be because IL-10 suppresses not only Th1-type but also Th2-type cytokines by inhibition of antigen presenting cells²⁸. This study did not find any association between IgE and either of the cytokines as shown by Wellinghausen *et al*²⁹ in patients with alveolar echinococcosis. Though IL-4 was not detectable in all CE patients and controls, nephelometric analysis to study the Th2 cell activation among the patients with CE seems to be more authenticated than previous studies *in vitro*¹¹ as well as *in vivo*^{30,31}.

Elevated levels of serum complement proteins, C3c and C4, were detected in patients with CE compared to healthy controls. However, these complement levels in CE cases were not very high compared to healthy controls, unlike in patients with other cestode or helminthic infections, as shown earlier^{12,32}.

Table. Immunoglobulin and cytokine levels in patients with CE and healthy controls

	Specific IgG	Total IgE (U/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)	C3c (g/l)	C4 (g/l)
Patients (n=45)	0.59±0.2	333.91±460.84	1.81±1.54	4.38±3.64	1.47±0.81	0.64±0.37
Controls (n=10)	0.10±0.007	39.1±32.1	0.60±0.74	1.37±0.91	0.90±0.36	0.36±0.27

Several intra-specific variants or strains have been described within *E. granulosus*, showing differences in their pathogenicity in humans. In most parts of Europe the sheep strain plays an important role for humans, in Asia and Africa considerable importance is ascribed to the camel strain³³. In India, cattle and buffalo strains seem to be prevalent apart from sheep strain^{19,21,34}. Even though, there was significant difference in serum specific IgG levels between sheep and buffalo strains, this study showed similar patterns of IgE and cytokine levels in CE patients infected with all the three strains (G1, G3, G5). However, experimental studies are necessary to confirm whether these cytokine up-regulation is due to specific hydatid infection and to demonstrate the pathogenicity of different strains in India.

In conclusion, our data point to a cytokine profile suggestive of Th2 cell dominance *in vivo*. We did not however check the IgG1-IgG4 antibody class switch and suppression of Th1 type immune responses in patients with CE. Further studies are necessary not only to investigate regulation of specific immune response in CE but also to determine the role of serum cytokine measurements in evaluation of pathogenicity of different strains prevalent in India.

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