

Increase of IgE Anti-*Encephalitozoon cuniculi* Antibody Levels in Septic Patients

Juan C. Andreu-Ballester^{1*}, Constantino Tormo-Calandín², Carlos García-Ballesteros³, Victoria Amigo³, Ana Peiró-Gómez⁴, Juan Ruiz del Castillo⁵, Carlos Peñarroja-Otero⁶, Ferran Ballester⁷, Soledad Fenoy⁸, Carmen del Aguila⁹ and Carmen Cuéllar⁹

¹Head of Research Department, Arnau de Vilanova Hospital, Valencia, Spain

²Professor of Catholic University, School of Medicine, Valencia, Spain

³Hematology Department, Arnau de Vilanova Hospital, Valencia, Spain

⁴Head of Emergency Department, Arnau de Vilanova Hospital, Valencia, Spain

⁵Professor in School of Medicine, University of Valencia, Valencia, Spain

⁶Head of Medicine Department, Casa de la Salud Hospital, Valencia, Spain

⁷Centre for Public Health Research (CSISP), Valencia, Spain

⁸Department of Parasitology, San Pablo CEU University, Madrid, Spain

⁹Department of Parasitology, Faculty of Pharmacy, Complutense University, Madrid, Spain

*Corresponding author: Juan C. Andreu-Ballester M.D. Ph.D., Research Department, Arnau de Vilanova Hospital, San Clemente 12, 46015, Valencia, Spain, Tel: +34639562863; E-mail: jcandreu@ono.com

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Abstract

Objective: We recently demonstrated that the biggest reduction of T cells in septic patients was produced in the $\gamma\delta$ T subset. This depletion was directly proportional to the severity of the septic process, and it was associated with mortality. We hypothesized that microsporidia can harness the deficit of $\gamma\delta$ T cells in septic patients to proliferate and contribute to the worsening of the sepsis.

Methods: In this retrospective study, we analyzed anti-*Encephalitozoon cuniculi* antibody levels in sera from 46 septic patients, and compared them with a similar control group of healthy subjects. As a secondary objective we aimed to relate anti-*E. cuniculi* antibody levels with $\alpha\beta$ and $\gamma\delta$ T cells in these patients.

Results: Forty-eight percent of septic patients were positive for IgE anti-*E. cuniculi* vs 13.0% of healthy subjects (OR: 3.67, CI95% 1.64-8.20, P=0.001). The frequency of $\alpha\beta$ and CD56⁺ $\gamma\delta$ T cell subsets decreased in septic patients with positive anti-*E. cuniculi* IgE antibodies. This decrease was more potent in the CD3⁺CD56⁺ $\gamma\delta$ T cell subset. The genitourinary focus (urinary tract infections and pyelonephritis) produced a significant higher percentage of anti-*E. cuniculi* positive cases (11/13 (84.6%), OR=11.0, CI 95% 2.1-58.5, P=0.003).

Conclusion: There was a greater level expression of IgE anti-*E. cuniculi* in septic patients, reaching almost 50% of positive. The presence of IgE anti-*E. cuniculi* in septic patients was related to a decrease of $\alpha\beta$ and $\gamma\delta$ T cells in peripheral blood. This decrease was more potent in the CD3⁺CD56⁺ $\gamma\delta$ T cell subset. Microsporidia may be heavily involved in the physio-pathological evolution of sepsis.

Keywords: Sepsis; Severe sepsis; Septic shock; Microsporidia; Anti-*Encephalitozoon cuniculi* antibodies; $\alpha\beta$ and $\gamma\delta$ T cells

Introduction

Sepsis remains a challenge for modern medicine, not only for the steady increase in incidence in all countries but also for its high mortality and health care costs [1-3]. One of the main problems of the septic disease is the etiological diagnosis. In 40-50% of patients the causative organism is undetectable, since cultures are negative [4,5]. This implies that the antibiotic treatment can sometimes be empirical, until culture results. T cells are fundamental in the immune response to infection. T cells are divided into two distinct populations characterized by the TCR receptor expressing on its surface. So, now we can distinguish T cells expressing the $\alpha\beta$ -TCR heterodimer ($\alpha\beta$ T cells), or the $\gamma\delta$ -TCR heterodimer ($\gamma\delta$ T cells) [6].

During sepsis, apoptosis has been regarded as an important cause of cell death with a decrease in the number of lymphocytes as well as, epithelial and parenchymal cells [7-9]. We recently demonstrated that a decrease in all lymphocyte subpopulations according to the expression of $\alpha\beta$ or $\gamma\delta$ TCR receptor is produced in this disease. The higher reduction observed in our study was produced in $\gamma\delta$ T cells, and the decrease of this subpopulation was related to mortality of septic patients [10]. This transient diminution of T cells could promote the proliferation of some opportunistic agent and contribute to the severity of the disease.

Microsporidia are obligate intracellular parasites considered as emerging pathogens that have recently been classified as fungi [11,12]. They are ubiquitous and capable of infecting all groups of animals. The organisms are characterized by the production of small and environmental resistant spores which have a unique mode of entering

host cells via a polar tube. Three are the species belonging to *Encephalitozoon* genera isolated from human infections: *E. cuniculi*, *E. hellem* and *E. intestinalis*. They infect both animals and humans, causing opportunistic infections in immunocompromised individuals, such as Acquired Immune Deficiency Syndrome (AIDS), [13-15] although some evidence suggested that asymptomatic infections were common in immunologically stable patients [16,17]. Clinical features of microsporidiosis are broad and include a variety of manifestations such as intestinal, ocular, renal and pulmonary disorders, although the immune status of the host is important in the clinical course of the disease [18]. Clinically silent chronic infections usually developed in immunologically competent hosts, although clinical signs could develop after early infections [19,20]. In these cases, spore shedding has been described occasionally, but detectable amounts of microsporidial DNA may be excreted via urine that could indicate a capacity of microsporidia to disseminate in this population [21,22].

In immunocompromised hosts, microsporidial infections develop into serious diseases that may result in death. The most common clinical symptoms are chronic diarrhea and mal-absorption although, systemic diseases can develop. The first cases were recognized in AIDS patients with CD4⁺ T lymphocytes lower than 100 cells/ml. However, encephalitozoonosis has also been described in transplant recipients [23,24]. These parasites have been detected in numerous locations producing a great variety of manifestations due to their dissemination capacity. In the case of *E. cuniculi* infection often produce disseminated disease although diarrhea or interstitial nephritis and cholecystitis have been described.

We propose the hypothesis that microsporidia can harness the deficit of lymphocytes in patients with sepsis to proliferate and contribute to the worsening of the disease.

Our main goal was to quantify the levels of antibodies against *E. cuniculi* in sera from septic patients compared to a healthy control group. As a secondary objective we aimed to relate anti-*E. cuniculi* antibody levels with $\alpha\beta$ and $\gamma\delta$ T cells in these patients.

Material and Methods

Study population

In this retrospective study, sera from 46 patients who met the criteria for sepsis in our previous study were analysed [10]. All patients were admitted to Emergency and Intensive Care Unit of Arnau de Vilanova and Doctor Peset Alexandre Hospitals of Valencia, Spain. Sepsis was defined according to internationally established criteria [25], as well as their different stages, sepsis without organic failure and with organic failure (severe sepsis) [26,27]. In addition, patients had to meet the following requirements: not suffer immunodeficiency or autoimmune diseases, have not been vaccinated in the last six months, and not be subjected to immunosuppressive therapy. The control group (46 subjects) was recruited from relatives of patients admitted to hospital who were not relatives of septic patients. They should have the same characteristics of the patients in addition to not suffer acute infectious diseases. Both groups were matched by sex and age (\pm 5 years). The Research and Ethics Committee of both hospitals approved the study.

Blood sample analysis

Blood cell counts were performed using Coulter LH750 automated hematology analyzer (Beckman Coulter, Fullerton, CA). Monoclonal

antibodies used: CD45, CD4, CD8, CD3, for the peripheral blood subpopulations and CD4, CD8, CD56, CD2, CD3, TCR $\alpha\beta$ and TCR $\gamma\delta$ for the T $\gamma\delta$ lymphocyte study.

Fluorescence analysis was performed using a Beckman-Coulter multiparameter flow cytometry analyzer, Cytomics FC 500, Florida (USA) and later analyzed with CXP Software. Minimums of 50,000 events were measured. Absolute counts of circulating cell subsets were calculated using the percentages obtained by flow cytometry and the leukocyte count was obtained from the hematological analyzer using a dual-platform counting technology.

$\gamma\delta$ T lymphocyte populations were analyzed with Phycoerythrin-Cyanine 5.1 (PC5) conjugated anti-human TCR $\gamma\delta$; Beckman Coulter, Miami, USA (clone: IMMUNO 510). $\alpha\beta$ T lymphocytes were analyzed with Phycoerythrin-Cyanine 5.1 (PC5) conjugated anti-human TCR $\alpha\beta$; Beckman Coulter (Clone: IP26A).

The level of C-reactive protein (CRP) was measured in serum of patients with a heterogeneous enzymatic sandwich immunoassay with an end point immunofluorescence reading method (Vitros Chemistry Products[®]).

Erythrocyte sedimentation rate (ESR) was measured in the TEST 1 (Alifax, Padova, Italy) using a quantitative capillary photometry-based technology.

Encephalitozoon cuniculi antigen and determination of specific antibodies

The *E. cuniculi* antigen was obtained from *E. cuniculi* (USP-A1) spores cultured on E6 monolayer following Aguila et al. protocol [28]. Briefly, spores were disrupted using glass beads, 2.5% SDS and 2% mercaptoethanol. Soluble antigens were obtained from the supernatant after centrifugation. Protein content was determined by the Bradford method and adjusted to 0.8 $\mu\text{g/ml}$ to coat ELISA plates. Duplicate dilutions of human sera at 1/200 in PBS-Tween, containing 0.1% BSA were added and incubated. Horse radish peroxidase (HRP) conjugate goat anti-human IgG (Biosource International, Camarillo, CA) was used. For IgE determination, test sera were added in duplicate at a 1/2 dilution. A murine monoclonal antibody against ϵ human IgE chain (IgG1 κ , E21A11, INGENASA, Madrid, Spain) was added and incubated, followed by a goat anti-mouse IgG1 (γ) HRP conjugate (CALTAG Laboratories, Burlingame, CA) [29,30]. Values higher than the mean of the Optical Densities (O.D.) of the 46 serum samples of control subjects plus once their standard deviation were considered as *E. cuniculi* positive.

Statistical analysis

Characteristics of the patients, variables of immunoglobulin levels and lymphocytes subsets in each study group were numerically and graphically described. Normality of distributions was tested by the Kolmogorov-Smirnov test. When normality was accepted, Student t test for paired samples was used to compare the means of quantitative variables. When normality was not accepted, the non-parametric Wilcoxon test was used instead. Differences between cases and controls according to the sepsis categories were always analysed using the Wilcoxon test. Categorical variables were compared by means of Pearson's Chi-Square test and Spearman's correlation coefficients. The level of significance was taken as a *P* value of less than 0.05 (bilateral contrast). Data were analyzed using the statistical software SPSS, version 18.

Results

Characteristics of patients with sepsis

Ninety-two subjects were included in the study, 46 patients with sepsis (29 male and 17 female), and 46 healthy controls with a similar age and sex distribution. The mean age of the patients with sepsis was 61.9 ± 25.4 , and the mean age of the controls was 65.6 ± 20.3 , $P=0.44$. Table 1 shows the characteristics of the patients with sepsis.

	Mean \pm SD		No. (%) of patients
Age (yrs)	61.9 \pm 25.4	Stages of sepsis	
APACHE II score	10.5 \pm 4.8	- Sepsis	28 (60.9)
SOFA score	2.0 \pm 1.2	- Severe Sepsis	18 (39.1)
ESR	77.6 \pm 25.1	Organic Failure	
CRP	313.3 \pm 113.1	Acute Renal Failure	7 (15.2)
	No. (%) of patients	Neurologic	7 (15.2)
Sex		Acute Respiratory Failure	5 (10.9)
- Male	29 (63.0)	Shock	4 (8.7)
- Female	17 (37.0)	Acute Hepatic Failure	3 (6.5)
Diagnosis		Hematologic	3 (6.5)
- Pneumonia	13 (28.3)	Metabolic	1 (2.2)
- Urinary Tract Infections	12 (26.1)	No. Organs with failure	
- Acute Appendicitis	6 (13.0)	0	28 (60.9)
- Acute Cholecystitis	5 (10.9)	1	12 (26.1)
- Acute Cholangitis	3 (6.5)	2	3 (6.5)
- Enterocolitis	2 (4.3)	3	1 (2.2)
- Acute Diverticulitis	1 (2.2)	4	1 (2.2)
- Abscess	1 (2.2)	5	1 (2.2)
- Pelvic inflammatory disease	1 (2.2)	Positive Cultures	19 (41.3)
- Undetermined	2 (4.3)	In-hospital death	4 (8.7)

Table 1: Characteristics of Patients with Sepsis (N=46).

Continuous variables are expressed as means \pm Standard Deviation (SD). C-reactive protein (CRP). Erythrocyte sedimentation rate (ESR). Positive cultures included blood (n=12), urine (n=6), sputum (n=2), exudate (n=1), stool (n=1). Severe Sepsis included Septic Shock (N=4). Undetermined sepsis represents the patient with fever and well-defined criteria of systemic inflammatory response without a well-defined septic focus. In our study, 2 patients with undetermined sepsis had positive blood cultures for *Escherichia coli*.

Anti-*Encephalitozoon cuniculi* antibodies

IgE anti-*E. cuniculi* levels of septic patients were almost double those of healthy control subjects (Figure 1A). However, no differences were observed when patients with sepsis and severe sepsis were compared (Figure 1B). Moreover, not significant differences were observed between the control group and the two groups of sepsis related to serum levels of IgG anti-*E. cuniculi*.

IgG and IgE anti-*E. cuniculi* levels were analyzed according to the time of evolution of sepsis. The highest levels of specific IgG were obtained after three days from the onset of symptoms (fever) compared to the group of patients with a lower time of evolution (1-2 days) ($P=0.034$). No differences were seen in the case of anti-*E. cuniculi* IgE levels.

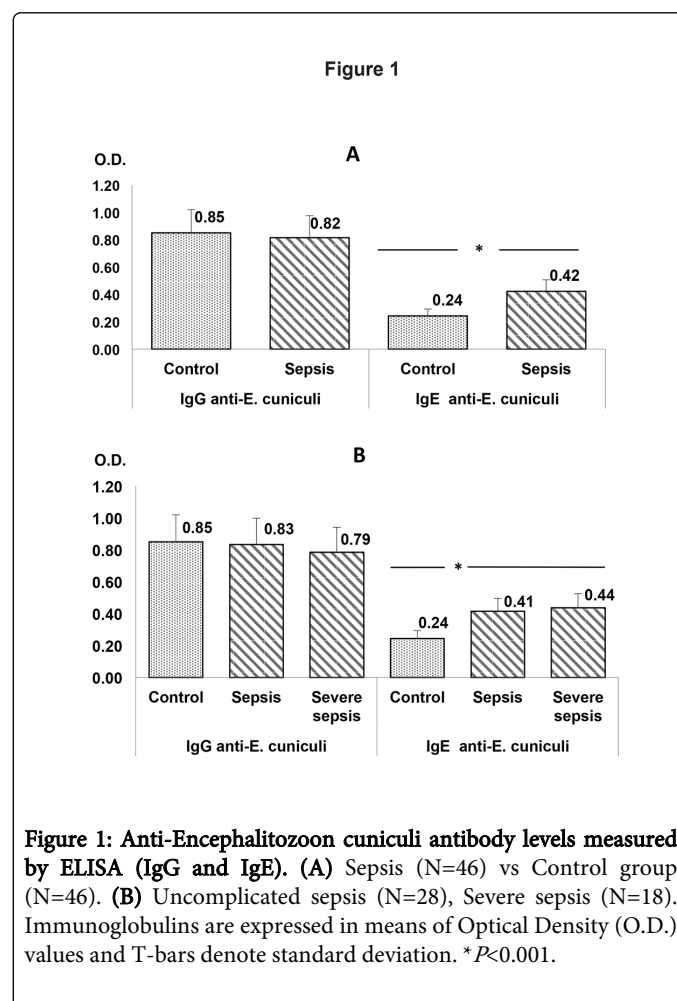


Figure 1: Anti-*Encephalitozoon cuniculi* antibody levels measured by ELISA (IgG and IgE). (A) Sepsis (N=46) vs Control group (N=46). (B) Uncomplicated sepsis (N=28), Severe sepsis (N=18). Immunoglobulins are expressed in means of Optical Density (O.D.) values and T-bars denote standard deviation. * $P<0.001$.

T cell subsets in septic and control healthy subjects

The frequency of $\alpha\beta$ (Figure 2A) and $\gamma\delta$ (Figure 2B) T cell subsets was greatly diminished in septic patients vs control healthy subjects. The frequency of $\alpha\beta$ T cells in patients with severe sepsis was decreased more significantly than in patients with non-severe sepsis and this decrease was only significant in $CD3^+$ and $CD3^+CD4^+$ $\alpha\beta$ T cells. However, all $\gamma\delta$ T cell subsets significantly decreased in severe sepsis vs organic uncomplicated sepsis.

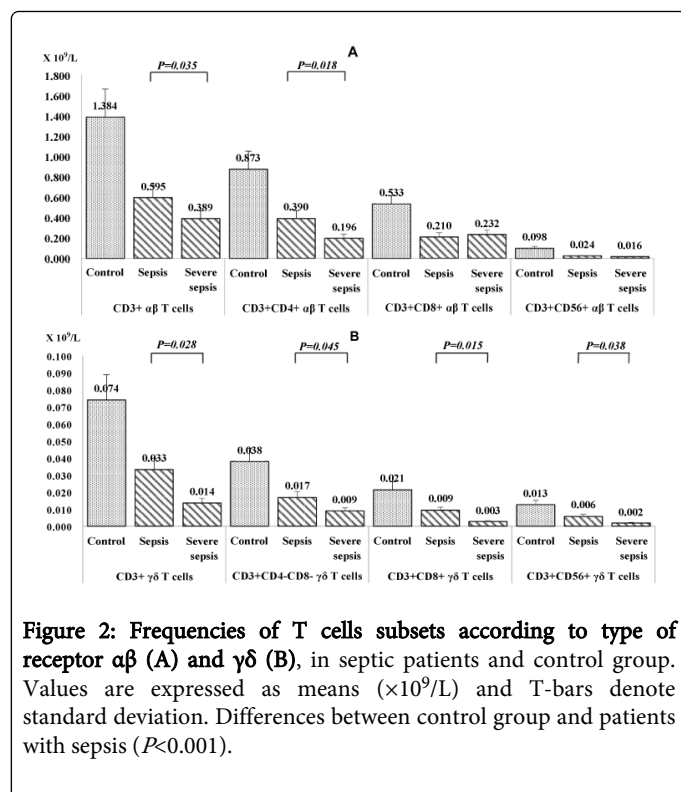


Figure 2: Frequencies of T cells subsets according to type of receptor $\alpha\beta$ (A) and $\gamma\delta$ (B), in septic patients and control group. Values are expressed as means ($\times 10^9/L$) and T-bars denote standard deviation. Differences between control group and patients with sepsis ($P < 0.001$).

IgE-IgG anti-*Encephalitozoon cuniculi* antibodies and $\alpha\beta$ - $\gamma\delta$ T cell subsets

Twenty-two of the 46 patients with sepsis (47.8%) were IgE anti-*E. cuniculi* positive vs 6/46 (13.0%) in the control healthy group (OR: 3.67, CI 95% 1.64-8.20, $P=0.001$). When the mean frequency of $\alpha\beta$ - $\gamma\delta$ T cell subsets was analyzed in the septic group of patients according to IgE anti-*E. cuniculi* positive-negative diagnostic, we observed a significant reduction in the case of all $\alpha\beta$ T cell subsets in the group of patients with IgE anti-*E. cuniculi* positive levels (Figure 3A), as well as, in the case of CD3+ and CD3+CD56+ $\gamma\delta$ T cells (Figure 3B). However, only 8/46 (17.4%) septic patients were IgG anti-*E. cuniculi* positive vs 11/46 (23.9%) observed in the control healthy group (OR: 0.83, CI 95% 0.53-1.30, $P=0.61$). Although the $\alpha\beta$ T cell population was always reduced in the group of septic patients that were IgG anti-*E. cuniculi* positive, we only observed significant differences in the case of CD3+CD56+ $\alpha\beta$ T cells.

Correlations among anti-*Encephalitozoon cuniculi* IgG and IgE levels with T cell subsets

In the healthy group, anti-*E. cuniculi* antibodies were negatively correlated with CD3+CD56+ $\alpha\beta$ T cell values, in both IgE (Spearman rho: -0.594, $P=0.001$) and IgG isotypes (Spearman rho: -0.468, $P=0.001$). Likewise, CD3+CD56+ $\gamma\delta$ T cells were negatively correlated with IgE anti-*E. cuniculi* (Spearman rho: -0.447, $P=0.002$). This means that the greater the number of CD3+CD56+ T cells the smaller the amount of anti-*E. cuniculi* antibodies (Figure 4A-4C).

However, in the group of patients with severe sepsis we observed a positive correlation between IgG anti-*E. cuniculi* antibodies and CD3+ $\gamma\delta$ T cells (Spearman rho: +0.607, $P=0.010$), CD3+CD4-CD8- $\gamma\delta$ T cells (Spearman rho: +0.607, $P=0.010$) and CD3+CD56+ $\gamma\delta$ T cells (Spearman rho: +0.627, $P=0.007$). This means that the greater the number of CD3+ $\gamma\delta$ T cells the greater the amount of IgG anti-*E. cuniculi* antibodies (Figure 4D-4F).

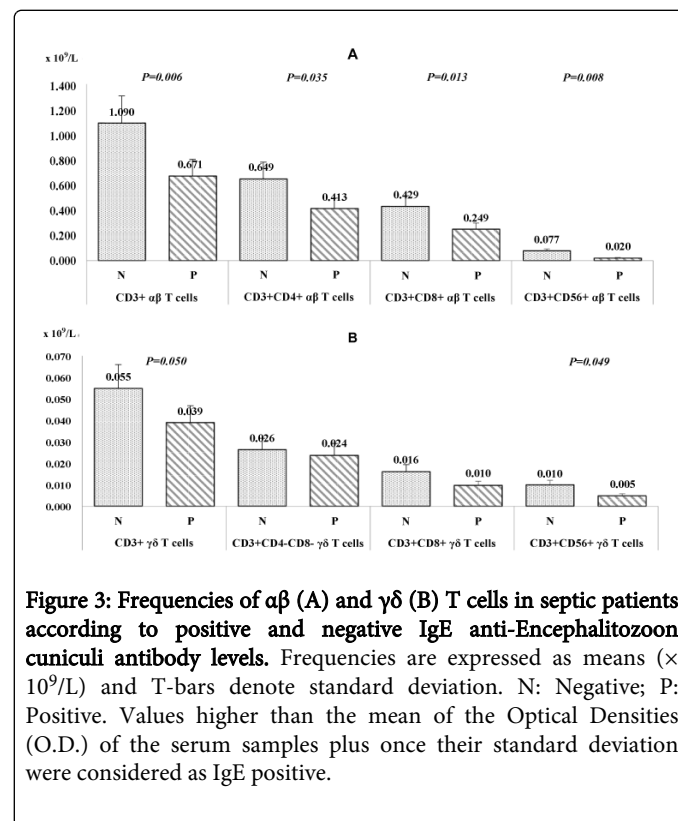


Figure 3: Frequencies of $\alpha\beta$ (A) and $\gamma\delta$ (B) T cells in septic patients according to positive and negative IgE anti-*Encephalitozoon cuniculi* antibody levels. Frequencies are expressed as means ($\times 10^9/L$) and T-bars denote standard deviation. N: Negative; P: Positive. Values higher than the mean of the Optical Densities (O.D.) of the serum samples plus once their standard deviation were considered as IgE positive.

Septic focus and IgG and IgE anti-*Encephalitozoon cuniculi* antibodies

When the association of septic focus with the percentage of patients with detectable levels of IgE anti-*E. cuniculi* was studied, the genitourinary focus (urinary tract infections and pyelonephritis) produced a significant higher percentage of positive cases (11/13 (84.6%), OR=11.0, CI 95% 2.1-58.5, $P=0.003$). On the other hand, abdominal and respiratory foci showed 29.4% and 23.1% of positive results, respectively.

The analysis of the organisms according to Gram stains with respect to IgE anti-*E. cuniculi* specific antibodies showed no relation between Gram positive organisms and presence of specific IgE. Instead, 69.2% (9/13) of the patients who were positive for Gram negative organism were IgE anti-*E. cuniculi* positive (OR=3.5, CI 95% 0.9-13.6, $P=0.067$). Table 2 shows the organisms isolated from cultures of different sample types from septic patients.

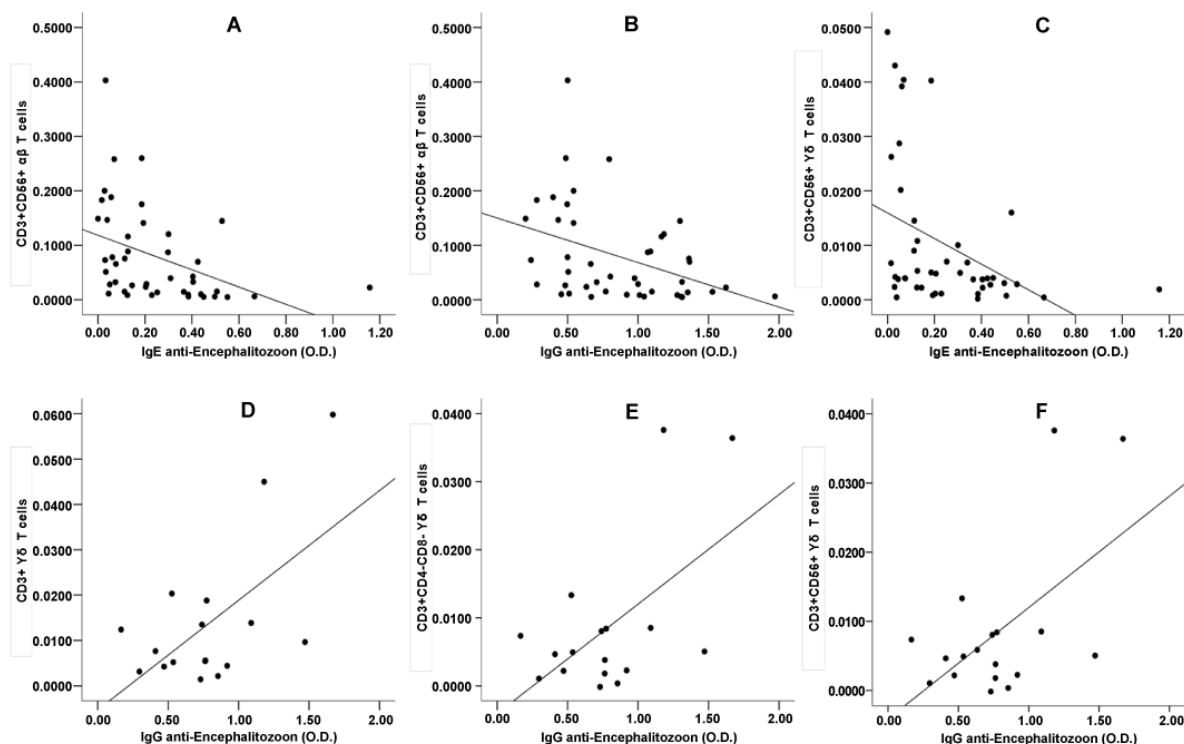


Figure 4: Correlations between IgG and IgE anti-Encephalitozoon cuniculi levels and T cells in healthy group. (A) Spearman rho (-0.594, $P=0.001$); **(B)** Spearman rho (-0.468, $P=0.001$); **(C)** Spearman rho (-0.447, $P=0.002$). Immunoglobulines are expressed as means of Optical Density (O.D.). T cell values are expressed as means ($\times 10^9/L$). **Correlations between IgG anti-E. cuniculi levels and T cells in severe sepsis. (D)** Spearman rho (+0.607, $P=0.010$); **E:** Spearman rho (+0.567, $P=0.018$); **F:** Spearman rho (+0.627, $P=0.007$). Immunoglobulines are expressed as means of Optical Density (O.D.). T cell values are expressed as means ($\times 10^9/L$).

	N (%)	Blood	Urine	Sputum	Exudate	Feces
Gram-negative						
- <i>Escherichia coli</i>	11	6	5			
- <i>Pseudomonas</i>	1	1				
- <i>Proteus mirabilis</i>	1		1			
- <i>Bacteroides fragilis</i>	1				1	
- <i>Klebsiella pneumoniae</i>	1	1				
- <i>Salmonella</i>	1					1
- <i>Haemofilus influenzae</i>	1			1		
- <i>Proteus vulgaris</i>	1		1			
Total Cultures N (%)	18 (78.3)	8 (34.8)	7 (30.4)	1 (4.3)	1 (4.3)	1 (4.3)
Gram-positive						
- <i>Streptococcus pneumoniae</i>	2	2				
- <i>Enterococcus faecalis</i>	1	1				

- <i>Staphylococcus epidermidis</i>	1	1				
Total Cultures N (%)	4 (17.4)	4 (17.4)				
Fungus						
- <i>Candida albicans</i>	1 (4.3)			1		
Total Cultures N (%)	23 (100.0)	12 (52.2)	7 (30.4)	2 (8.6)	1 (4.3)	1 (4.3)

Table 2: Organisms isolated from cultures of different sample types from septic patients.

Discussion

This is the first study that evaluates IgE and IgG anti-*E. cuniculi* antibody levels in septic patients, demonstrating that these patients had anti-*E. cuniculi* specific IgE levels higher than healthy subjects.

In an experimental study in mice, Furuya et al. [31] obtained three monoclonal antibodies against tube polar protein 1 (PTP1) of *E. cuniculi* belonging to the IgE isotype. These antibodies also recognized intracellular antigens. The high levels of IgE anti-*E. cuniculi* antibodies produced by septic patients may be indicative of the presence of microsporidia in the host's organs causing a primary immune response. This fact could be also indicating an increase in proliferation of these opportunistic agents provoking an acute infection.

Interestingly, IgG anti-*E. cuniculi* levels in septic patients were similar to those observed in sera from healthy subjects. This could indicate that the parasite did not induce an adequate and permanent adaptive immune response in these patients. This could be caused by an immune deficiency in these patients that did not provide proper immune responses. In this regard, we recently reported that a deficit of $\gamma\delta$ T cells [32] and interleukin-7 [33] in Crohn's disease patients, was related to a higher production of IgE anti-*E. cuniculi* antibodies. Likewise, 30% of Crohn's disease patients were positive by real time PCR for microsporidia while all subjects of the control group were negative, suggesting that Crohn's disease patients are a group at risk of microsporidiosis. Moreover microsporidia may be involved as a possible etiologic factor of Crohn's disease [34].

As expected, $\alpha\beta$ and $\gamma\delta$ T cell levels in septic patients were lower than the observed in the control group. $CD3^+$ and $CD3^+ CD4^+ \alpha\beta$ T cells significantly diminished in patients with severe sepsis with respect to the organic uncomplicated sepsis. All the $\gamma\delta$ T cell subsets were significant lower in the severe sepsis patient group. It is interesting to note that almost 50% of septic patients showed IgE anti-*E. cuniculi* positive levels and decreases in all $\alpha\beta$ T cell subsets were significant in IgE positive patients. However, in the $\gamma\delta$ T cell population significant decreases were only observed for $CD3^+$ and $CD3^+ CD56^+$ phenotypes. This fact is important to bear in mind, taking into account that the mortality in patients with sepsis was associated with a reduction in $\gamma\delta$ T cells, specifically the $CD3^+ CD56^+ \gamma\delta$ T subset [10].

Natural killer T cells (NKT cells) belong to the innate immune response because they act in minutes or hours after antigen contact. NKT cells are an ongoing source of confusion in the literature. Flow cytometry revealed that up to 55% of hepatic, but <6% of peripheral $CD3^+$ lymphocytes expressed CD56, CD161; besides, they expressed $\alpha\beta$ or $\gamma\delta$ TCR [35].

Godfrey et al. [36,37] defined three types of NKT cells: Type I cells (classical NKT-cells), type II cells (non-classical NKT-cells) both CD1d-restricted and NKT-like cells (CD1d-independent NK1.1⁺T cells). Several subsets of $\gamma\delta$ T cells also express NK-cell markers such as NK1.1 [38], and these are usually not CD1d specific, so they have been included under the category of NKT-like cells. $CD3^+CD56^+ \alpha\beta$ and $\gamma\delta$ T cells analyzed in our patients can be included into the NKT-like cell category. Experimental studies in mice have shown the role of NKT cells in the pathophysiology of sepsis. However, the role of NKT cells expressing the $\gamma\delta$ receptor on sepsis is unknown [39]. Moreover, how NKT cells respond to fungi is unknown. In our study, the average levels of $\alpha\beta$ and $\gamma\delta$ NKT cells were below 50% in subjects with positive anti-*E. cuniculi* IgE (Figure 3). This decrease was more intense than the observed with the other T cell subsets.

Both $CD3^+CD56^+ \alpha\beta$ and $\gamma\delta$ T cell subsets were closely related to anti-*E. cuniculi* immune responses in healthy subjects. We have not found previous studies that report this subset in immunity against this pathogen in humans. There is only a study that showed an enhanced NK activity in a murine experimental infection by *E. cuniculi* [40]. Thus, in healthy control subjects there was an inverse relationship between anti-*E. cuniculi* antibody levels and $CD3^+CD56^+$ cells. This suggested that this T cell phenotype could have a direct effect on these microsporidia and their diminution can cause a lower stimulation of the specific antibody production. By contrast, in patients with severe sepsis, the correlation between $CD3^+CD56^+$ cells and specific IgG was positive, but no correlation with specific IgE was observed although specific IgE antibodies are involved in the experimental responses against this opportunistic agent [31]. Works are in progress in order to clear different immunological responses in health and septic subjects against microsporidia.

According to our results, microsporidia could be involved in the evolution of sepsis. They could be the root cause of the infections that trigger misdiagnosed sepsis. Nowadays, it is known that microsporidia can only be propagated in cell culture systems [41]. However, the use of cell cultures in routine clinical diagnosis is time consuming and laborious and only specialized laboratories maintain cell cultures with microsporidia. The best alternative is the use of serological tests with whole organisms or recombinant polar tube protein or spore wall protein as antigens [42].

It is known that $\gamma\delta$ T cells can be selectively activated by natural or synthetic phosphoantigens [43] and act by means of antibody-dependent cell-mediated cytotoxicity in the absence of an allogenic response [44]. For this reason, they could be potential candidates for therapy in patients with sepsis. In addition, some antibiotics have shown partial efficacy in the therapy of microsporidia [45].

Increasing evidence supports a central role for immunosuppression in sepsis. The initial immune response in sepsis is hyperinflammatory

(cytokine storm), but the response rapidly progresses to a hypoinflammatory response (immunosuppression) [46]. This immune dysfunction may lead to increase the host susceptibility to secondary opportunistic infections [47]. Our findings demonstrated that microsporidia may be heavily involved in the evolution of sepsis and justify the need for new investigations on antimicrobial agents against these opportunistic agents.

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