INTERLEUKIN LEVELS IN CEREBROSPINAL FLUID FROM CHILDREN WITH NEUROCYSTICERCOSIS

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Abstract. No information about the levels of pro-inflammatory interleukins has been described in children with neurocysticercosis (NCC). The levels of tumor necrosis factor-α (TNF-α), interleukin (IL)-5, IL-6, and IL-12 in the cerebrospinal fluid from children with NCC were determined by enzyme-linked immunosorbent assay (ELISA). Twelve children with NCC, six with active and six with inactive disease, and six children without NCC were studied. TNF-α was undetectable in CSF from controls and five children with inactive NCC, whereas the levels were significantly higher (median 22.1 pg/ml; P = 0.008) in all children with active NCC. Levels of IL-6 were low in active and inactive NCC patients but two subjects with active subarachnoid disease had high levels. IL-5 and IL-12 were not detected. This study shows that high levels of TNF-α are present in CSF from children with active NCC. IL-6 levels are higher when infection occurs in the subarachnoid space.

INTRODUCTION

Cysticercosis is caused by the larval form of the tapeworm Taenia solium. The prevalence of this infection is high in developing countries of Latin America such as Mexico, Brazil, Colombia, Peru, and Chile.1,2 It is considered to be an emerging disease in the United States.3

Neurocysticercosis (NCC) is the most important clinical form of cysticercosis since it may produce serious neurologic disabilities or even permanent brain damage. These clinical manifestations include seizures, mental deterioration, intracranial hypertension, and hydrocephalus.4,5,7,9-12 The severity of the symptoms has been related to the number of cysticerci, their localization in the brain, the degree of acute inflammatory response, and the age of the patient.5,7,9,11-14 Although NCC has been studied less in children than adults, there are age-associated differences in the clinical presentation and inflammatory response in the central nervous system. For example, hydrocephalus is frequent in adults and uncommon in children, and encephalitis is more frequent in children.5,7,9-12

The most important aspect of the pathogenesis of NCC is the inflammatory response in the brain.9,11,13-15 Its intensity has been associated with the number of cysticerci and their localization in the brain. Thus, the reaction differs when cysticerci are found in the parenchyma, the subarachnoid space, and the ventricular system.3,6,9-16

Host-inflammatory mediators are important in the pathogenesis of severe parasitic diseases, such as malaria, leishmaniasis, and trypanosomiasis.17-24 In the case of NCC, we have demonstrated by immunohistochemical analysis of brain tissue from adults with NCC that at least four types of immune responses against NCC (antibodies, NK cells, macrophage and neutrophil infiltration, and a combination of macrophages and T cells) may occur. The intensity and type of immunity appeared to be associated with T-helper type 1 (TH1) and not T-helper type 2 (TH2) responses. In many lesions, there are pro-inflammatory cells and interleukins, including, IL-12, IL-2 and transforming growth factor-β (TGF-β). In particular, a T cell-rich infiltrate with high levels of TNF-α is induced by cysticerci in leptomeninges.16 On the other hand, in a 12-month follow-up study of 17 adults with active parenchymatous NCC, IL-1β levels were normal in serum and CSF and soluble IL-2 receptor (sIL-2R) levels were elevated in serum but not CSF. TNF-α was undetectable in both serum and CSF.25 In another study, TNF-α was elevated in CSF from 3 of 17 adults with active subarachnoid NCC, and IL-1β and IL-6 were elevated in 10 of these patients.26 More recently, high levels of IL-5 in serum and CSF have been found in adult patients with NCC but IL-8 levels remained low.27 This suggests that selected eosinophil mediators may also be involved in the pathogenesis of NCC.

The presence of pro-inflammatory interleukins in children with NCC has not been documented. Thus, we hypothesized that the levels of pro-inflammatory molecules might be associated with the activity and severity of NCC in children. Therefore, the aim of this work was to study the levels of TNF-α, IL-5, IL-6, and IL-12 in CSF from pediatric patients with NCC, and correlate the expression of these cytokines with activity and the brain localization of the infection.

PATIENTS AND METHODS

Patients. Twelve pediatric patients with NCC were included. The age range of the patients was 5–13 years (median 7 years). The diagnosis of NCC was based on neurologic symptoms6,9 and a characteristic image of the brain using cranial computed tomography (CT-scan) and magnetic resonance imaging (MRI). To confirm the infection by cysticercus, the presence of specific antibodies against the parasite was determined in CSF samples by Western Blot analysis performed as described by the manufacturer (Immunetics, Inc., Cambridge, MA). A strip with a positive control in the kit was used as reference.

Classification was made as suggested by Sotelo and others28 with some modifications suggested by Aguilar-Rebolledo29 for pediatric patients using MRI as described in Table 1. Thus, NCC was first classified as active or inactive, and the active form was further distinguished as simple active or complicated. The simple active forms include nodular, cystic, or granulomatous lesions with or without focal edema. The complicated active forms include diffuse edema or en-
Measurements of TNF-α/H9251

Active complicated forms
Nodular or cystic lesions with rim enhancement and focal edema
Mixed lesions (a combination of previous lesions)
Single or multiple lesions, nodular or cystic without hydrocephalus
Single or multiple lesions, without infarction, arachnoiditis, or intracranial hypertension

Active simple forms
Single or multiple lesions with rim enhancement
Nodular lesion with rim enhancement and focal edema
Cystic lesion with rim enhancement
Mixed lesions (a combination of previous lesions)

Inactive forms
Nodular or cystic lesions with rim enhancement
Encephalitis with multifocal or diffuse edema
Miliary distribution of lesions
Lesions with infarction and edema
Lesions with arachnoiditis
Intraventricular forms
Lesions with hydrocephalus

CSF from six pediatric patients with neurological disorders of non-inflammatory processes were obtained as negative controls. The diagnoses of the six controls were hemispheric stroke, radicular syndrome, trigeminal neuralgia, cerebral tumor, and Arnold-Chiari malformation (two patients). A CSF sample from an adult patient with NCC was also used as positive control.

ELISAs for TNF-α, IL-5, IL-6, and IL-12. CSF aliquots of 100 μL were analyzed in duplicate using ELISA kits with specific anti-human TNF-α, IL-5, and IL-12 antibodies (Endogen Inc., Boston, MA), or with specific anti-human IL-6 antibodies (Boehringer-Mannheim, Germany) according to the instructions of the suppliers. A linear regression standard curve for every interleukin was constructed with known concentrations of either TNF-α, IL-5, IL-6, or IL-12. Results were expressed in pg/ml.

Statistical analysis. Differences in the levels of all interleukins determined in CSF from patients with inactive and active forms of NCC or between NCC and non-NCC children were analyzed using the Mann Whitney U test.

Ethical approval. This study was approved by the ethical committee of the Hospital de Pediatría, CMN Siglo XXI, Instituto Mexicano del Seguro Social (IMSS). Informed consent from parents or legal guardians of children was obtained.

RESULTS

Patients. Of the twelve patients studied, six were asymptomatic and had inactive NCC with a single cysticercus and parenchymatous localization. The other six patients were symptomatic and had active NCC (Table 2). One (patient 7) had a single, uncomplicated parenchymatous cysticercus (Figure 1a). The other five had complicated forms of the disease. Two patients (8 and 12) had a single lesion with diffuse edema around the lesion (Figure 1b), one patient (10) had multiple parenchymatous and intraventricular lesions (Figure 1c), and the other two (9 and 11) had a miliary form with subarachnoid and parenchymatous localization (Figure 1d).

The cell number and protein concentration in CSF were increased only in the two patients with active, complicated and subarachnoid NCC (patients 11 and 12). The anti-cyto-

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Type of NCC*</th>
<th>Age in years</th>
<th>Number of lesions</th>
<th>Localization</th>
<th>TNF-α, pg/mL</th>
<th>IL-6, pg/mL</th>
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<td>1</td>
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<td>11</td>
<td>Active complicated</td>
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<td>12</td>
<td>Active complicated</td>
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<td>single</td>
<td>subarachnoid</td>
<td>49.4</td>
<td>753.8</td>
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</table>

* All inactive forms of NCC were asymptomatic whereas all active forms were symptomatic.
† P = 0.005 comparing patients with inactive NCC versus patients with active NCC, using the Mann Whitney U test.
‡ P = 0.005 comparing patients with inactive NCC versus patients with active NCC, using the Mann Whitney U test.
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**Figure 1.** T1 Gad MRI of a patient with simple active form of NCC and another three with complicated active form. 

- **a)** Coronal view of a patient with simple active form; showing rim enhancement in the head of left caudate nucleus (arrow).
- **b)** Coronal view of a patient with complicated single active form of NCC; a strong rim enhancement in the left parietal lobe with an intense vasogenic edema, typical of a strong inflammatory response (arrow).
- **c)** Sagittal view showing strong enhancement in the left parietal lobe. Another cyst with scolex and capsule is observed in the occipital horn of the lateral ventricle (arrows).
- **d)** Axial view showing a multiple (miliary distribution) rim enhancement in both parenchyma and subarachnoid space (arrows). In panels c and d, some of these parasites in hyaloid phase that represent degenerating cysts are shown.
Immunetics, Inc., Cambridge, MA). Lane separately to every strip according to instructions by the supplier were combined with 1 ml of sample dilution buffer and applied to the Western Blot assay was positive in all the CSF samples from patients with NCC. A more intense response was observed in those patients with active NCC compared with patients with inactive NCC. All six controls were negative (Figure 2).

Interleukin levels in CSF. TNF-α was undetectable in the CSF from the six control individuals without NCC. TNF-α was detected at a concentration of 5.7 pg/ml in one out of six with inactive and asymptomatic NCC (Table 2). In contrast, all six patients with symptomatic and active NCC had detectable levels of TNF-α (median 22.1 pg/ml). The highest levels were in complicated cases, particularly when the cysticerci were in the subarachnoid space (patients 11 and 12). The difference in the levels of TNF-α between the two groups was statistically significant \( (P = 0.008) \).

IL-6 levels in patients with inactive NCC ranged from 0.0–2.1 pg/ml with a median of 0.2 pg/ml. In the group with active NCC, IL-6 ranged from 0.3–753.8 with a median of 11.3 pg/ml. However, the difference between both groups of patients was not statistically significant \( (P = 0.06) \). The highest IL-6 values were observed in the two patients with active, complicated and subarachnoid NCC (patients 11 and 12). IL-5 and IL-12 were not detected in any of the samples.

**DISCUSSION**

Most knowledge of the pathogenesis of NCC is based on studies of adults. Although NCC has been less well-studied in children, clinical experience suggests that the clinical and inflammatory response may differ from that of adults. To our knowledge, this study is the first published description of pro-inflammatory interleukin levels in CSF from children with different forms of NCC. The results show an association between the presence of TNF-α in CSF and active, symptomatic, and complicated forms of NCC.

TNF-α might participate in the inflammatory process induced by antigens from cysticerci during active infection of the brain with NCC. As is the case for other parasitic infections, TNF-α may contribute to local tissue damage. This is supported by the fact that the highest TNF-α levels were observed in the most severe cases with active, complicated, and subarachnoid NCC. These results also suggest that the ability of cysticerci antigens to induce a variable TNF-α release depends on the site of infection.

The pro-inflammatory activity of TNF-α may be beneficial or deleterious to the host. Thus, picomolar concentrations of TNF-α can activate macrophages and enhance leukocyte-mediated killing of *Leishmania* and fungi. On the other hand, high concentrations of TNF-α could have a deleterious effect, as in cerebral malaria. In adults with NCC, an inflammatory response in brain tissue is also observed. It is characterized by the presence of TNF-α as well as IL-12, and IFN-γ in the vicinity of T cells.

Our results suggest that the number of cysticerci may not influence the levels of TNF-α, because one active and complicated case with multiple lesions had lower TNF-α levels than two active cases with single lesions (> 35 pg/mL). In contrast, the viability of the parasite may be important since all cases with active NCC had increased levels of TNF-α. The host-parasite interaction at different brain sites may play a role in the immune response against cysticerci, as suggested by the fact that the two cases with subarachnoid localization had the highest levels of TNF-α. This observation could be explained in terms of the higher influx of cells into the subarachnoid region relative to the parenchyma. Alternatively, the subarachnoid region may favor the release of antigens from the cysticercus, causing activation of cells that produce TNF-α. Thus living cysticercus in the subarachnoid region but not parenchymatous localization may express antigens that stimulate the release of TNF-α from pro-inflammatory cells. This is in agreement with the results of Rolfs and others who did not detect TNF-α in CSF from 17 patients with active parenchymatous NCC. However, Ostrosky and others reported elevated TNF-α levels in CSF in only 3 of 17 adults with subarachnoid NCC. These results contrast our findings in children and strongly suggest a difference in the inflammatory response against cysticercus in children versus adults.

TNF-α was detected in only one of six pediatric patients with inactive NCC. This may be due to a persistent release of antigens by the cysticercus in this patient and not in the other five patients. Additional studies are needed to define the mechanisms of induction of TNF-α release by cysticercus antigens.

Levels of IL-6 did not differ significantly between patients with inactive and active forms of NCC. However, high levels of IL-6 were found in the two children with active, complicated, and subarachnoid NCC. These results agree with those of Ostrosky and others who reported increased levels of IL-6 in the majority of adult patients with subarachnoid NCC. This is also supported by our previous immunochemical analysis that showed an increase in inflammatory cells in the subarachnoid space of adults with NCC. Thus, the...
subarachnoid region may contain more IL-6 and TNF-α producing cells that other areas of the NCC-infected brain. High IL-12 levels and a strong NK infiltrate have been observed in the brain tissue of adults with NCC.\(^{15}\) In contrast, IL-12 was not detected in any of the CSF samples from children with NCC. This suggests that the development of NK cells promoted by IL-12 in children may be less active than in adults. Accordingly, participation of NK cells may be less in children than in adults with NCC.

Our study also showed IL-5 was not present in the CSF from children with NCC. It is well known that the expression of IL-5 is associated with the development of eosinophils. Thus, the lack of IL-5 may explain our previous observations in the brain tissue of adult patients with NCC\(^ {16}\) in which eosinophils were scarce or undetectable.

As expected from previous studies of adults, pediatric patients with NCC produce antibodies against the glycoproteins of cysticerci. This also confirms the results of immunohistochemical examination of specimens of brain tissue from adults with NCC in which specific antibodies against cysticerci were observed.\(^ {16}\) Patients with the higher levels of TNF-α and IL-6 also showed a strong antibody response to the glycoproteins of the cysticerci. The clinical and physiologic significance of these phenomena remain unknown.

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