ANALYSIS OF THE PERIPHERAL IMMUNE RESPONSE IN PATIENTS WITH NEUROCYSTICERCOSIS: EVIDENCE FOR T CELL REACTIVITY TO PARASITE GLYCOPROTEIN AND VESICULAR FLUID ANTIGENS

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Abstract. In neurocysticercosis (NCC), it is thought that the long-term survival of the parasite within the human brain is due in part to the ability of the cestode to suppress the local immune response. When the parasite dies, the immunosuppression is apparently lost and a strong local inflammatory response then develops. In contrast, little is known about the immunologic response that may occur in the peripheral immune system of these patients. In this study, the status of the peripheral (extracerebral) cellular and humoral response was evaluated in patients with a history of NCC. The in vitro proliferation of peripheral blood mononuclear cells to mitogens and foreign antigens was similar in patients and controls. Importantly, a substantive response was elicited by two Taenia solium metacestode antigens. In addition, 8 of 10 patients had a detectable humoral response to the antigentic glycoproteins of the cestode. Considering both the cellular and humoral response, all of the patients with NCC presented an active peripheral immunity.

INTRODUCTION

The metacestode stage of the parasite Taenia solium causes the disease neurocysticercosis (NCC), which is a major public health problem in many developing countries. This infection results from the migration of T. solium oncosphere larvae to the brain where it transforms into a metacestode or cysticercus that can live for years. As the parasite dies, it deteriorates towards a final calcified stage. The clinical manifestations vary depending upon the viability of the parasite and/or its location within the brain. For example, seizures are a common symptom of single parenchymal cysts that are already calcified. In contrast, symptoms associated with increased intracerebral pressure (ICP) are generally a consequence of viable or dying parasites that are obstructing turnover of the cerebrospinal fluid or inducing local inflammation and edema.

Despite the transformation of a 30 micron-long oncosphere into a cysticercus that is 1 cm in diameter within the brain, there is usually no or a minimal immune response associated with viable parasites. In contrast, dying cysticerci usually induce intense infiltrates that are associated with pathology. These data are supported by radiologic and histopathologic studies suggesting that viable cysticerci, typical of other helminthic parasites, have evolved strategies to prevent the development of an effective immune response that may eliminate them. In the case of T. solium cysts, several strategies to avoid or manipulate localized immunity have been hypothesized. The first is immune evasion by masking the vesicular membrane of the parasite with host-derived proteins such as antibodies and complement components. A second strategy is molecular mimicry whereby the parasite synthesizes proteins that resemble host factors. Finally, the parasite may secrete products that can control the host’s local response such as the “metacestode factor” that inhibits cellular and humoral immune responses when it is inoculated into mice.

Only a few studies have directly addressed the effect of the brain infection by T. solium metacestodes on the peripheral (extracerebral) immunity of the host and have suggested peripheral immunosuppression. Moreover, the molecular basis underlying the lack of detectable anti-cysticercus antibodies in a proportion of biopsy-confirmed NCC cases has not been determined. The status of the cellular immune response in the periphery has been evaluated only in one study. The results indicated that peripheral cellular immunity in patients with NCC was diminished compared with control subjects when analyzed by in vitro proliferation of peripheral T lymphocytes in response to various lectin mitogens. Although the study suggested that patients with NCC have a generalized suppression of the cellular immune response, a detailed analysis of factors that could have biased these results was not reported. These parameters include the clinical status of the patient, viability of the infecting cyst, and most importantly, treatment with corticosteroids and antihelmintics. Therefore, it is necessary to determine the generality of the previous findings.

In the present study, the cellular and humoral immunity of patients with a history of NCC was assessed. In vitro proliferation of mononuclear cells towards mitogens and two foreign antigens (tetanus toxoid and Candida albicans extract) resulted in values comparable to those of controls. In addition, a significant T cell proliferative response to parasite antigens was found in patients with NCC but not in controls. Similarly, substantive antibody responses to parasite antigens were observed in the patients with NCC compared with the controls. Thus, the group of patients with NCC displayed an active peripheral immune response.

PATIENTS, MATERIALS, AND METHODS

Patient population. Patients diagnosed with NCC at the University of Texas Health Science Center between 1994 and 1996 were considered for this study. NCC was diagnosed conclusively in cases with a histologically confirmed cyst following craniotomy. A diagnosis of NCC was presumed when patients had suggestive clinical symptoms, positive radiologic scans, and in some cases serology compatible with NCC and/or a history of residence in regions endemic for NCC. Extra-cerebral cysticerci were not noted. The control population consisted of healthy North American
and Latin American individuals living in San Antonio, Texas at the time of the study. The ethical review process was studied and approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. All patients signed an approved informed consent form.

**Isolation of peripheral blood mononuclear cells.** Fresh blood specimens were collected from patients and control subjects. Mononuclear cells were separated by density centrifugation using lymphocyte separation medium (Organon Teknika Corporation, Durham, NC). The mononuclear interface was resuspended in RPMI 1640 medium (BioWhittaker, Walkerville, MD) with 2 mM L-glutamine (GIBCO-BRL, Rockville, MD), 100 IU/ml of penicillin G sodium, 100 μg/ml of streptomycin sulfate, 0.25 μg/ml of amphotericin B as antibiotic-antimycotic solution (GIBCO-BRL, Rockville, MD), 1 x minimal essential medium-vitamin solution (JRH Biosciences, Lenexa, KS), 23 mM HEPES buffer (JRH Biosciences), and 20% AB human serum (AB sera; NABI, Miami, FL). Fresh mononuclear cells were then used for the lymphocyte proliferation assay.

**Preparation or source of antigens.** Antigens for the lymphocyte proliferation assay included the T cell mitogens concanavalin A (Con A) and phytohemagglutinin (PHA; Sigma, St. Louis, MO), tetanus toxoid (TT; Connaught Laboratories, Swiftwater, PA), C. albicans extract (Ca; Greer, Lenoir, NC), and T. solium cyst antigens, including a glycoprotein (GP) extract and cyst vesicular fluid (VF). Antigens were obtained from cysticerci isolated from a naturally infected pig in Colombia. The fluid was collected after the cyst’s vesicular membrane was disrupted by repeated freezing-thawing, and the protein concentration of the VF was then determined by the Bradford method (BioRad, Hercules, CA). For the Western blot analysis, a glycoprotein extract of the parasite was prepared by passing a crude extract through a Lens culinaris affinity column, essentially as described previously.11

**Lymphocyte proliferation assay.** Fresh mononuclear cells were seeded in 96-well, round bottom plates at a concentration of 5 x 10⁶ cells/ml. Cells were then cultured in the presence of culture medium alone, or with 25 μg/ml of Con A, 10 mg/ml of PHA, 2.5 μg/ml of TT, 20 μg/ml of Ca extract, 1 μg/ml of cyst GP, or 200 μg/ml of cyst VF. The optimal concentration of each mitogen and antigen was chosen empirically by titration of each of the reagents. Eighteen hours before harvesting the cells, 1 μCi/ml of ³H-thymidine was added. The cells were harvested at day 4 for the mitogens, and at day 6 for antigens. The degree of stimulation was assessed as incorporation of ³H-thymidine measured as counts per minute. The results are expressed as stimulation indices when compared with the control cells incubated in the absence of antigen or mitogen and harvested at equivalent time points. The stimulation index was calculated by dividing the average ³H-thymidine incorporation of the stimulated cells by the average incorporation of the unstimulated cells from the same patient.

The proliferation assay was initially standardized with specimens from the control population. The first parameter was to determine the effect of storing the frozen cells in liquid nitrogen prior to the proliferation assay following standard methods.12 The results showed a trend for lower proliferation indices with frozen versus fresh cells from the same individual, and this reduction was more pronounced with an antigenic stimulus compared to a mitogenic stimulus. Based on these results, assays were performed on freshly drawn patient and control specimens.

A second aspect of the experimental design was to determine if there was a difference between Latin American and North American healthy controls in lymphocyte proliferative capacity. This could be possible, particularly with the parasite antigens, due to the frequency of helminthic infections in the tropics versus North America. However, for all the antigens and mitogens tested, comparable values were found for both groups. Therefore, the 11 healthy individuals were considered as one control group.

**Western blot analysis.** The L. culinaris enriched glycoproteins of T. solium metacestodes were resolved in a 15% preparative polyacrylamide gel electrophoresis system under reducing conditions.13 After transfer to a Hybond-C Nytran membrane (Amersham, Buckinghamshire, United Kingdom), a Western blot was performed using the multiscreen system (BioRad). A 1:200 dilution of the plasma from each of the patients with NCC was used, or the same dilution from a pool of three healthy controls. The antigen-antibody reaction was detected with a mouse anti-human IgG conjugated to alkaline phosphatase (Southern Biotechnology Associates, Birmingham, AL), and then developed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Sigma).14

**Statistical analysis.** Differences in the mean stimulation index between the study groups was established by the Wilcoxon signed rank sum test using EpilInfo version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA), and P values ≤ 0.05 were considered statistically significant. Correlation coefficients were calculated with Excel 97 (Microsoft Corporation, Redmond, WA).

**RESULTS**

**Patient data.** Five male and five female Hispanics who ranged in age from 25 to 50 years old (Table 1) were studied. Consistent with the literature, most patients with parenchymal cysts had reported seizures while the extraparenchymal parasites were associated with signs and symptoms of IIP. The radiologic data indicated that most patients had cysts of various stages of evolution. Four patients had calcified lesions. When viable parasites were predicted, anti-parasitic treatment with praziquantel was administered orally at dosages of 400–900 mg, three times a day for 14 days. During this study period, some of the patients with a history of seizures were controlled with an anti-epileptic medication (Dilantin®, Parke-Davis, Morris Plains, NJ), and those patients with IIP had undergone a ventriculoperitoneal shunt placement or a craniotomy. None were receiving corticosteroid treatment during the study period. The control group consisted of seven Latin American and four North American healthy individuals present in San Antonio at the time of the study.

**T cell proliferation to mitogens and recall antigens.** The T cell mitogens PHA and Con A were used to test the proliferative capacity of mononuclear cells obtained from patients with NCC. With both mitogens, the stimulation indices varied extensively among the individuals tested (Table
The difference in the mean stimulation index between the patient and the control groups was not statistically significant with either PHA or Con A induction. Interestingly, a given individual usually had a high or a low proliferation capacity with both mitogens. A positive correlation coefficient of 0.79 was found between the Con A and PHA groups.

To determine if classical T cell activation via the T cell receptor was altered in the patients with NCC, two common recall antigens were tested. Most individuals are exposed to the yeast *C. albicans* (Ca) since it is frequently present in the environment or present in the mucosa as a commensal microorganism. Sensitization to TT is typically observed through routine vaccinations, although the frequency of tetanus immunizations in Mexico may be less.

The proliferation indices for TT and Ca varied greatly (range = 1–293) among individuals in both the control and patient groups (Table 3). However, no statistically significant differences in the T cell proliferative response to these recall antigens was found between control and NCC patient groups. Similar to the mitogen responses, a positive correlation between the proliferation indices towards both environmental antigens was observed for cells from a given individual (r = 0.66). In contrast, a poor association was found between the average proliferation capacity towards mitogens versus common recall antigens for each person (r = 0.2), supporting the lack of a generalized immunosuppression in a given patient.

### T cell proliferation to parasite antigens

To analyze if a selective T cell anergy towards *T. solium* antigens is induced during the infection, the proliferation promoted by two *T. solium* cysticercus antigen preparations was tested. The GP and VF antigen preparations have been reported to be the target of a strong humoral response, but their recognition by T cells had not been previously tested. The *in vitro* cultures indicated that both GP and VF induced a significant increase in proliferation in the patients with NCC compared with controls (Table 4). The proliferative response of some control individuals to these antigens is presumably due to cross-reactive antigens or a slight mitogenic effect. The proliferative activity to the VF antigen was somewhat stronger when compared with the GP pool. The patient with the most recent documentation of a viable cyst (UH5) also appeared to have had more extensive disease. Importantly, this patient exhibited a proliferation index with GP at least six times higher than that of other patients (Figure 1).

#### Analysis of the humoral immunity

The glycoproteins of the *T. solium* metacestodes have been reported to be very immunogenic, eliciting *T. solium* specific antibodies in patients with NCC. The persistence of this immunity once the parasite has died varies among patients. In this study, we evaluated the specificity and intensity of the anti-cystercial IgG response to individual glycoproteins by Western blot analysis in patients with NCC (Figure 1). All patients had variable antibody titers against the GP proteins of molecular weights 10–12, 16, 18, 24, and 28 kD. Patient UH5 exhibited the strongest response. At a higher dilution of UH5 serum, the UH5 lane had distinct bands at the appropriate molecular weight of 10–12, 16, 18, 24, and 28 kD. Strong

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**Table 1**

<table>
<thead>
<tr>
<th>Patient identity</th>
<th>Brain biopsy</th>
<th>Presence of cysts (stage and year studied)</th>
<th>Antihelminthic</th>
<th>Symptoms</th>
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<tbody>
<tr>
<td>HU12</td>
<td>+</td>
<td>+(V/D, 1994)</td>
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<td>-</td>
<td>+(C, 1994)</td>
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<td>+</td>
</tr>
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<td>-</td>
<td>+(C, V/D, 1985)</td>
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<td>+</td>
</tr>
<tr>
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<td>-</td>
<td>+(V/D, 1992)</td>
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<tr>
<td>UH20</td>
<td>-</td>
<td>+(C, 1994)</td>
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</table>

* The clinical information of the 10 patients with NCC studied is indicated. Three had NCC confirmed by brain biopsy. Computerized tomography and/ or magnetic resonance imaging of the brain was used to determine the characteristics of the cyst, including its presence (+) or absence (−), location (parenchymal or extraparenchymal), and apparent stage of viability. For the latter, the cysts were classified as calcified (C) based on the presence of calcified lesions, or as viable/degenerating (V/D) when low-density lesions with or without annular enhancement were observed. The year the radiologic study was done is indicated. Some of the patients were treated with praziquantel during the year when the first radiologic study was performed. In all patients, the symptoms such as increased intracranial pressure (IIP), headaches (HA), or seizures occurred at least one month prior to the study. In contrast, within the last month prior to the blood collection and analysis these symptoms were controlled in all patients. Some data were not available (NA). None of the patients were being treated with corticosteroids for at least 10 months prior to the study period.

† These patients with seizures were taking Dilantin (Parke-Davis, Morris Plains, NJ) at the time of the study.

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**Table 2**

<table>
<thead>
<tr>
<th>Mitogen-induced stimulation index*</th>
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<td><strong>PHA range</strong> (n = 11)</td>
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<td>Controls</td>
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<tr>
<td>NCC</td>
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<tr>
<td>P value</td>
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<tr>
<td>p value †</td>
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</tbody>
</table>

* Values are the average stimulation index ± standard error of the mean. PHA = phytohemagglutinin, Con A = concanavalin A; NCC = neurocysticercosis.

† Correlation coefficient between PHA and Con A groups.

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**Table 3**

<table>
<thead>
<tr>
<th>Recall antigen-induced proliferative response in patients with NCC†</th>
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<tbody>
<tr>
<td><strong>TT range</strong> (n = 11)</td>
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<tr>
<td>Controls</td>
</tr>
<tr>
<td>NCC</td>
</tr>
<tr>
<td>P value</td>
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<tr>
<td>p value †</td>
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</tbody>
</table>

* Values are the average stimulation index ± standard error of the mean. NCC = neurocysticercosis; TT = tetanus toxoid; Ca = Candida albicans extract.

† Correlation coefficient between TT and Ca groups.
responses were also exhibited by UH17, UH18, and UH13. The rest of the patients had lower or undetectable antibody titers. The control sera presented a faint reactivity with the cysticercal antigens. The most common reactivity was directed towards proteins of 10–12, 16, 18, 24, and 28 kD. In comparison, the preimmunized sera from the patients with NCC and a pool of three healthy controls were negative. The GP and VF antigens were used previously as antigens for the serologic diagnosis of NCC, but not to assess their capacity to induce cell-mediated immunity. In this study, both antigens induced the proliferation of leukocytes, and this stimulation was more pronounced for the VF fluid when compared with the GP fraction. This may be due to the higher complexity or potential mitogenic effects of the crude VF antigen versus the GP enriched fraction.

In summary, the present study indicated that most patients have an active peripheral cellular and humoral immune response. It is possible that immunosuppression is most apparent at the level of the local cerebral microenvironment where such suppression may help maintain the chronic persistence of the viable metacestode.

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