UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE INVESTIGACIONES BIOMEDICAS

IDENTIFICACIÓN DE MOLÉCULAS DE LA RESPUESTA INMUNE ASOCIADAS CON LA PATOGENIA DE LA NEUROCISTICERCOSIS HUMANA

TESIS

QUE PARA OBTENER EL GRADO ACADÉMICO DE

DOCTORA EN CIENCIAS

PRESENTA

BIOL. BRENDA IRERI SÁENZ JIMÉNEZ

TUTOR: **DRA. EDDA LYDIA SCIUTTO CONDE**

COMITE TUTOR: **DRA. AGNES ODILE MARIE FLEURY**

DRA. ANAHI CHAVARRIA KRAUSER

México, D.F. 2012

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UNAM
POSGRADO
Ciencias Biológicas

COORDINACIÓN

Dr. Isidro Ávila Martínez
Director General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 05 de marzo de 2012, aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la alumna **SAENZ JIMENEZ BRENDA IRERI** con número de cuenta **400050090** con la tesis titulada **"IDENTIFICACIÓN DE MOLECULAS ASOCIADAS CON LA PATOGENIA DE LA NEUROCISTICERCOSIS HUMANA"**, realizada bajo la dirección de la **DRA. EDDA LYDIA SCIUTTO CONDE:**

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Suplente:	DR. ALFONSO ESCOBAR IZQUIERDO

Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, D.F., a 3 de mayo de 2012.

M. del Coro Arizmendi

DRA. MARÍA DEL CORO ARIZMENDI ARRIAGA
COORDINADORA DEL PROGRAMA

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Hay quienes saben casi nada de muchas cosas, hay otros que saben mucho de casi nada…

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RESUMEN

La Neurocisticercosis (NC) es causada por el establecimiento del metacestodo de *Taenia solium* en el sistema nervioso central (SNC). Es una enfermedad pleomórfica que puede cursar de forma asintomática o presentarse con sintomatología desde leve hasta severa. Se ha observado que la respuesta inmuno-inflamatoria del hospedero, así como la localización, el número de parásitos y su estado de degeneración, son factores relacionados con la heterogeneidad clínica y la severidad de la enfermedad. En este trabajo se evaluó la relevancia de la respuesta inmune local y sistémica asociada a las distintas formas clínicas y radiológicas de la NC humana. Se observó que los pacientes inflamatorios/severos los cuales presentaron líquido cefalorraquídeo (LCR) inflamatorio, con hipertensión intracraneana (HIC), además de parásitos localizados principalmente en SaBa/intraventriculares, múltiples y vesiculares, resultaron con una respuesta local caracterizada por el aumento de niveles de IL1β, IL5, IL6 e IL10 (P=0.035; P=0.002; P=0.009; P=0.0001 respectivamente), además de una supresión de la proliferación linfocitaria que se asoció con el aumento de TNFα a nivel sistémico (r= -0.5, P=0.02). En los pacientes no inflamatorios/no severos caracterizados por presentar LCR no inflamatorio, sin HIC y parásitos únicos, coloidales o calcificados, localizados principalmente en SaSu/parénquima, se estudió la relevancia de la respuesta inmuno-inflamatoria periférica asociada a la sintomatología, comparando casos epilépticos versus no epilépticos. Se observó que los casos no epilépticos, presentaron una respuesta inmuno-inflamatoria con proliferación linfocitaria específica activa, además de mayores niveles de IL5, IL10, IFNγ, TNFα, IL13 e IgG4 (P=0.0001 para IL5, IFN y TNFα; P=0.001 para IL10; P=0.045 para IL13 y P=0.063 para IgG4). Estos resultados sugieren que los casos no inflamatorios/no severos/no epilépticos presentan una respuesta activa y regulada que probablemente promueve la calcificación del parásito sin mayores secuelas como la manifestación de epilepsia.

Este trabajo muestra la importancia de la respuesta inmuno-inflamatoria a nivel local así como a nivel sistémico, asociada con la gravedad de la sintomatología y sugiere que la localización de los parásitos, la interacción entre las células residentes del sistema nervioso central y los componentes de la respuesta inmunológica pudieran determinar el tipo de respuesta inmuno-inflamatoria en los distintos compartimentos del SNC, resultando en diferencias en el tipo de daño hacia el parásito y en la patogenia de la enfermedad.

ABSTRACT

Neurocysticercosis (NC) is caused by the establishment of the *Taenia solium* metacestode in the central nervous system (CNS). Is a pleomorphic disease that may be asymptomatic or may present symptoms that vary from moderate to severe. It has been observed that the host immune-inflammatory response, as well the location, parasite number and degeneration state are factors related with the clinical heterogeneity and the disease severity. In this work, the relevance of the local and systemic immune response related with the different clinical and radiological forms of the human NC was studied. We found that inflammatory/severe patients whose presented inflammatory cerebrospinal fluid (CSF), intracranial hypertension, and multiple vesicular parasites located in SaBa/ventricles, resulted with a local response characterized by higher levels of the cytokines IL1β, IL5, IL6 and IL10 (P=0.035; P=0.002; P=0.009; P=0.0001 respectively), also suppression of the lymphocyte proliferation, which was related with higher levels of systemic TNFα (r= -0.5, P=0.02). In non-inflammatory/non-severe patients characterized by the presentation of non-inflammatory CSF, without ICH and single colloidal or calcified parasites locates in SaSu/parenchyma, the importance of the peripheral immune-inflammatory response was studied in relation with symptomatology comparing epileptic versus non-epileptic cases. Non epileptic cases presented an active specific lymphocytic proliferation and higher levels of IL5, IL10, IFNγ, TNFα, IL13 and IgG4 (P=0.0001 for IL5, IFN y TNFα; P=0.001 for IL10; P=0.045 for IL13 and P=0.063 for IgG4). This suggests that non-inflammatory/non-severe/non-epileptic patients present an active and regulated response that probably promotes parasite calcification without mayor consequences such as epilepsy manifestation.

This study shows the relevance of the immune-inflammatory response at local as well as at systemic level related with the severity of the symptomatology, suggesting that parasite location, the interaction between the resident cells of the central nervous system and the components of the immune response may determinate the type of immune-inflammatory response in the different compartments of the CNS, resulting in differences in parasite damage and disease pathogeny.

GLOSARIO DE ABREVIATURAS

BHE: Barrera hematoencefálica

BLCR: Barrera de líquido cefalorraquídeo

CFSE: Carboxi-fluoresceína diacetato succinymidil éster

CMSP: Células mononucleares de sangre periférica

ELISA: Enzyme-linked immunosorbent assay (Ensayo por inmunoabsorción ligado a enzimas)

HIC: Hipertensión intracraneana

ICAM-1: Molécula de adhesión intercelular 1

IFNγ: Interferon gama

IgG: Inmunoglobulina G

IL: Interleucina

LCR: líquido cefalorraquídeo

MCP-1: Proteína quimioatrayente de monocitos 1

NC: Neurocisticercosis

RMN: Resonancia magnética nuclear

RNA: Ribonucleic acid (Ácido ribonucleico)

SaBa: Espacio subaracnoideo de la base

SaSu: Espacio subaracnoideo de los surcos

SN: sobrenadante de las células mononucleares de sangre periférica

SNC: Sistema Nervioso Central

TAC: Tomografia axial computarizada

TGFβ: Tumoral growth factor beta (Factor de crecimiento tumoral beta)

TH: Células T cooperadoras

TNFR1: Tumoral necrosis factor receptor 1 (receptor de factor de necrosis tumoral 1)

TNFα: Tumoral necrosis factor alpha (factor de necrosis tumoral alfa)

INTRODUCCIÓN

La cisticercosis causada por el metacéstodo de *Taenia solium* es una infección que afecta a humanos y a cerdos. Se adquiere al ingerir los huevos de *T. solium,* que eclosionan en el intestino, liberando los embriones (oncosferas), los cuales penetran a través de la mucosa intestinal, alcanzan el sistema circulatorio sanguíneo y/o linfático que los distribuye en diferentes tejidos sólidos donde se desarrollan hasta metacéstodos (cisticercos), en donde pueden desplazar estructuras normales y/o generar inflamación a su alrededor (Willms et al, 2006).

En los humanos, los cisticercos se pueden localizar en músculo esquelético, sistema nervioso, ojos, tejido graso subcutáneo y corazón. Cuando se localizan en el sistema nervioso central (SNC) se le denomina neurocisticercosis (NC). Esta infección puede cursar sin sintomatología aparente o puede presentarse con distintas manifestaciones clínicas como cefalea, déficit focales, crisis convulsivas, demencia e incluso puede causar la muerte del paciente (Sotelo y Del Brutto, 2000).

La gran mayoría de los casos de NC ocurren en países subdesarrollados de Latinoamérica, Asia y África, aunque también se han reportado casos en países como Estados Unidos (Stamos et al, 1996), como consecuencia del flujo de inmigrantes de países en donde la cisticercosis es endémica (Sciutto et al, 2000). Las principales causas que propician esta parasitosis en los países en vías de desarrollo, son las relacionadas con los factores socioeconómicos tales como las inadecuadas prácticas de higiene, crianza rústica de cerdos y consumo de carne sin inspección sanitaria. Esta parasitosis podría prevenirse erradicando la práctica del fecalismo al aire libre de los humanos, con el

lavado y cocción de los alimentos, así como manteniendo a los cerdos fuera del alcance de las heces humanas.

En México, la prevalencia de la NC es de tal magnitud, que aproximadamente del 2.4%al 4.3% de las consultas neurológicas en instituciones especializadas son por pacientes con este padecimiento (Fleury et al., 2010; Jiménez-Marcial y Velázquez, 2004). Asimismo, es diagnosticada en 2-4% de las necropsias realizadas en distintas instituciones hospitalarias (Villagrán y Olvera, 1988) y es la primera causa de epilepsia de inicio tardío en México (Suastegui et al., 2009; Medina et al., 1990).

La NC presenta una gran heterogeneidad clínica. Se estima que un alto porcentaje de los casos es un hallazgo casual y puede tener un curso asintomático o clínicamente silencioso (Villagrán y Olvera, 1988; Fleury et al., 2003). La alta prevalencia de NC asintomática la sustentan diferentes estudios epidemiológicos que reportan prevalencias del 9 al 10% de NC con lesiones calcificadas y asintomáticos (Fleury et al. 2003, 2006).

Por otra parte, en los pacientes hospitalarios, el síntoma más frecuente tanto en adultos como en niños es la epilepsia, que se presentan en el 50-80% de los casos, particularmente en pacientes con cisticercos en el parénquima cerebral (Del Brutto et al., 1992; Del Brutto, 1997; Nash et al., 2001; Ferreira et al., 2002). Sin embargo, la NC presenta un cuadro clínico con síntomas y signos inespecíficos como son la cefalea, mareo, epilepsia, déficits neurológicos focales, síntomas psiquiátricos, y/o hipertensión intracraneana (HIC) que no permiten establecer su diagnóstico tan fácilmente (White, 2000; Sotelo and Del Brutto, 2000).

Patología en SNC

Además de la heterogeneidad en las manifestaciones clínicas, la NC se presenta con una gran diversidad de formas patológicas.

En el SNC los parásitos se pueden alojar en todos los compartimientos, en particular en el espacio subaracnoideo, en el sistema ventricular, en el parénquima y en la médula espinal.

Cuando se encuentran en etapa vesicular (parásito viable con membrada delgada y transparente, fluido vesicular claro y escólex invaginado) desencadenan una escasa reacción inflamatoria perilesional. Esta reacción está principalmente constituida por linfocitos, células plasmáticas y eosinófilos; que parecen participar en la eventual destrucción del parásito y su subsecuente transformación en un nódulo calcificado. La severidad de la reacción inflamatoria es altamente variable. Una vez que los cisticercos comienzan a degenerarse y se encuentran en la fase coloidal (con líquido vesicular viscoso y turbio y el escólex muestra signos de degeneración hialina y mineralización), se rodean de una gruesa cápsula de tejido colágeno con abundante infiltrado inflamatorio perilesional. El parénquima cerebral muestra una intensa gliosis reactiva de tipo astrocitítica, con proliferación de células de microglia, edema difuso, cambios degenerativos neuronales e infiltrado perivascular de linfocitos. Cuando los parásitos se destruyen (cisticercos calcificados: el parásito se transforma en un pequeño nódulo con apariencia de una calcificación), el edema disminuye, pero los cambios glióticos perilesionales suelen ser más intensos que en las etapas anteriores.

Los cisticercos del espacio subaracnoideo pueden ser pequeños cuando se localizan en la profundidad de los surcos corticales, entre dos circunvoluciones cerebrales, o pueden alcanzar hasta varios centímetros cuando estos se localizan en las cisternas de la base. Es factible que puedan desarrollarse debido a que su crecimiento no está detenido por la presión ejercida por el parénquima cerebral circundante. Estos cisticercos pueden causar síntomas neurológicos focales y aumentar la presión intracraneal; también pueden desencadenar una intensa reacción inflamatoria en las meninges, con formación de un denso exudado compuesto principalmente por fibras de colágeno, células gigantes

multinucleadas, eosinófilos y membranas parasitarias hialinizadas. Este exudado puede impedir el flujo normal del LCR en el SNC al comprometer varias estructuras cerebrales, pudiendo así participar en el desarrollo de hidrocefalia.

Los cisticercos intraventriculares pueden desencadenar una intensa reacción inflamatoria perilesional probablemente promovida por la liberación de componentes antigénicos en el LCR, produciendo ventriculitis o ependimitis crónica. Si la reacción inflamatoria ocurre cuando los cisticercos se encuentran adheridos al plexo coroideo o a la pared ventricular, la capa de células ependimarias se altera y se forman células gigantes subependimarias. Estas células tienden a agruparse y protruir hacia el interior de las cavidades ventriculares, lo cual puede ocluir el libre tránsito del LCR. Este proceso se denomina ependimitis granular y puede condicionar hidrocefalia obstructiva.

Finalmente, los cisticercos localizados en el espacio leptomeníngeo espinal también condicionan cambios inflamatorios en los nervios raquídeos de una manera similar a lo que los cisticercos meníngeos hacen con los nervios craneales.

En el mismo individuo se pueden encontrar cisticercos en distintas localizaciones y en distintos estados de degeneración.

En la mayoría de las personas infectadas con cisticercos parenquimatosos, el parásito involuciona sin tratamiento o sin mostrar síntomas clínicos (Sáenz et al., 2008), mientras que los casos con cisticercos en el espacio subaracnoideo o intraventricular siempre requieren de tratamiento cisticida para matar al parásito.

Métodos de diagnóstico de la NC

La tomografía axial computarizada (TAC) y la resonancia magnética nuclear (RMN) son herramientas de diagnóstico capaces de detectar hallazgos característicos o altamente sugestivos de una lesión causada por la presencia de cisticercos en el SNC. Estos estudios neuroradiológicos además se utilizan en el seguimiento de la respuesta del

parásito al tratamiento, ya que permiten visualizar el número, localización y estadio de desarrollo de los parásitos. La eficacia diagnóstica de cada método depende del estadio y ubicación anatómica del parásito. Así, la TAC y la RMN tienen la misma sensibilidad para la detección de la mayoría de los cisticercos parenquimatosos, mientras que la RMN es más eficaz en la detección de lesiones vesiculares ubicadas en la fosa posterior, el tallo cerebral, el espacio subaracnoideo o los ventrículos cerebrales. La TAC es el estudio de elección para evidenciar lesiones calcificadas, e incluso en algunos casos es posible diferenciar los granulomas por cisticercos de otro tipo de granulomas (Del Brutto et al., 1997).

La punción lumbar es un procedimiento auxiliar en el diagnóstico de NC que proporciona información sobre el proceso inflamatorio del paciente. En el LCR obtenido por punción se pueden observar anomalías que sugieren la presencia del parásito como el incremento de la celularidad (>5 células/ml) y/o proteínas (>40 mg/dl), hipoglucorraquia, presencia de eosinófilos y presencia de anticuerpos específicos contra el parásito (McCormick, 1985). La detección de anticuerpos en este compartimento y las características citoquímicas del LCR aunado a la sintomatología clínica y los estudios neuroradiológicos permiten en la mayoría de los casos establecer el diagnóstico de NC.

Tratamiento de la NC

Debido al pleomorfismo clínico de la NC, diferentes esquemas terapéuticos son utilizados. La caracterización precisa de la enfermedad, considerando el cuadro clínico, la viabilidad y localización de los parásitos, es fundamental para aplicar el tratamiento más adecuado de acuerdo a cada paciente. Actualmente, la terapia más común es la administración de dos cisticidas: el praziquantel y el albendazol. El albendazol es un potente cisticida que destruye del 75 al 90% de los cisticercos parenquimatosos. Es el cisticida de elección ya

que tiene mayor penetración en el LCR, presenta menores efectos secundarios y además tiene un costo inferior. El praziquantel se utiliza solamente en caso de que no haya respuesta con el manejo de albendazol (Del Brutto et al., 1999; Sotelo et al., 1988; Nash and Garcia, 2011). Dado que la destrucción del parásito se acompaña de un proceso inflamatorio importante, los cisticidas se administran junto con corticoesteroides como la prednisona o la dexametasona, principalmente en casos de encefalitis por cisticercosis, donde usualmente son necesarias dosis altas que permitan la reducción del edema cerebral. También se encuentran indicados en pacientes con aracnoiditis por cisticercosis para reducir el riesgo de hidrocefalia (Del Brutto, 1999). Además, los pacientes reciben manejo terapéutico sintomático como puede ser el uso de analgésicos, anticonvulsivos, antidepresivos, etc. Otras estrategias terapéuticas son el manejo quirúrgico que se utiliza en casos de hipertensión intracraneana donde la colocación de una válvula de derivación ventrículo-peritoneal alivia la sintomatología (White, 2000; Sotelo et al., 2001) y la exéresis de los parásitos, la cual se practica muy esporádicamente dada la eficacia de los medicamentos cisticidas.

PLANTEAMIENTO DEL PROBLEMA

Diversos factores, tanto del hospedero como del parásito, además de la exposición, participan en la heterogeneidad clínica de la NC (Fleury et al., 2003). En relación con los factores del parásito, se ha observado que los cisticercos de distintas regiones geográficas presentan una heterogeneidad genética (Maravilla et al., 2003; Vega et al., 2003) que pudiera estar asociadas a una diferente capacidad infectiva y patogénica del parásito en la NC.

Al respecto de los factores del hospedero, la edad, el género y las características de la respuesta inmuno-inflamatoria se han encontrado asociados con la heterogeneidad de la enfermedad (Rabiela et al., 1982; Rangel et al., 1987; Del Brutto et al., 1988; Del Brutto 1998; Chavarría et al., 2003; Fleury et al., 2003).

En el caso del género, las mujeres jóvenes presentan más frecuentemente formas parenquimatosas múltiples con importante inflamación y una mayor celularidad en el líquido cefalorraquídeo (LCR) que los hombres (Del Brutto et al., 1988; Fleury et al., 2004).

La edad es un factor del hospedero que también se ha relacionado con la heterogeneidad de la NC. En niños, se ha observado que es más frecuente la presencia de parásitos únicos, principalmente coloidales o calcificados y localizados en el parénquima y en el espacio subaracnoideo de los surcos (SaSu); estas características radiológicas se asocian con una prognosis benigna (Ruiz-García et al., 1997; Sáenz et al., 2006; Singhi and Singhi, 2009) y es también común en casos con NC asintomática (Fleury et al., 2003; Sáenz et al., 2006). En contraste, las formas más severas de la enfermedad con parásitos localizados en los ventrículos y en el espacio subaracnoideo de la base del cráneo, son significativamente más frecuentes en los pacientes adultos

Al respecto de la respuesta inmuno-inflamatoria, en estudios previos, se ha caracterizado el perfil inmunológico central y sistémico asociado a las formas severas de la enfermedad. Por una parte, se ha observado que los pacientes con parásitos localizados en el espacio subaracnoideo de la base (SaBa) o intraventriculares presentan, en el LCR, niveles elevados de IL5, IL6, IL10 e inmunoglobulinas G específicas (Chavarría et al., 2005). Estas moléculas habían sido reportadas anteriormente relacionadas con la sintomatología, con la actividad del parásito o con el fenómeno inflamatorio asociado al parásito (Evans et al., 1998; Rodrigues et al., 2000; Aguilar-Rebolledo et al., 2001). También se ha reportado la presencia de IFNγ e IL18 asociados a la lesión (Restrepo et al., 2001a). Al respecto de la respuesta de memoria en sangre periférica, se ha reportado por una parte producción de IL4, IL12 y TNFα en pacientes con formas activas, y aquellos con formas inactivas presentan altos niveles de IL6, IL10, IL12 y TNFα (Bueno et al., 2004), por otra parte otros autores sugieren la presencia de una respuesta celular de tipo TH1 (IFNγ, IL2, IL18) (Grewal et al., 2000; Restrepo et al., 2001). También se ha reportado un perfil de tipo TH2 (IL4, IL5 e IL13) en las formas asintomáticas de la NC (Chavarria et al., 2003, 2005).

Otro componente de la respuesta inmuno-inflamatoria es la respuesta celular proliferativa. En la NC, se ha reportado que independientemente de la severidad clínica de la enfermedad, los casos con NC sintomática no presentan proliferación celular, ni producción periférica de citocinas en comparación con los casos con NC asintomática (Chavarría et al., 2006), sin embargo algunos autores reportan que si hay proliferación en los sintomáticos o que puede ser diferente de acuerdo a la severidad de los síntomas (Restrepo et al., 2001; Bueno et al., 2004).

Al respecto de la respuesta inmuno-inflamatoria, resulta críticamente relacionada con la severidad de la enfermedad, se exacerba con el tratamiento y destrucción del

parásito y su estricto control es indispensable con el uso de en ocasiones altos niveles de esteroides sostenidos durante meses o años para evitar complicaciones que pueden terminar con la vida del paciente. Ahondar en el conocimiento de la respuesta inmuno-inflamatoria y los posibles mecanismos internos para su control contribuirán al diseño de estrategias más eficaces para su control y con menos efectos secundarios asociados que resulten en el mejor manejo de los pacientes.

HIPOTESIS

1. Existe una relación entre la respuesta inmuno-inflamatoria a nivel local y a nivel sistémico asociada a las distintas formas clínicas y radiológicas de la NC.

OBJETIVO GENERAL

1. Evaluar la participación de componentes de la respuesta inmune en la patogenia de la NC.

Objetivos Específicos

1. Determinar en sangre periférica y en líquido cefalorraquídeo el perfil de citocinas y niveles de proliferación linfocitaria específica en muestras pareadas de LCR y sangre de pacientes con NC y asociarlos con las diferentes formas clínicas y radiológicas de la enfermedad.
2. Determinar en sangre periférica y en líquido cefalorraquídeo el perfil de citocinas y niveles de proliferación linfocitaria específica de casos con parásitos únicos parenquimatosos y estudiar la importancia de la respuesta inmune en la ocurrencia de la epilepsia.

MATERIAL Y METODOS

Se describen en los artículos de los resultados

DISEÑO EXPERIMENTAL

Relación entre la respuesta inmune local y sistémica asociada con la severidad de la NC humana

Para estudiar la relación entre la respuesta local y la respuesta sistémica de pacientes con las diferentes formas clínicas y radiológicas de la NC, se colectaron muestras pareadas de sangre periférica y LCR de 31 pacientes adultos que acudieron al Instituto Nacional de Neurología y Neurocirugía, con diagnóstico de NC y que no recibieron tratamiento específico al momento del estudio (Figura 1).

Todos los pacientes se caracterizaron clínicamente por su sintomatología (con HIC o sin HIC) y radiológicamente de acuerdo al número de parásitos (único o múltiple), localización (parénquima/SaSu o SaBa/intraventricular) y estado de degeneración (vesicular, coloidal o calcificado) según la RMN o TAC. También se clasificaron de acuerdo a la neuroinflamación según el número de células leucociticas que presentaban en el LCR; cuando los pacientes presentaron 10 o más células por mm^3 se consideraron inflamatorios/severos y aquellos pacientes con menos de 10 células por mm^3fueron considerados no inflamatorios/no severos.

De la sangre periférica se obtuvieron células mononucleares (CMSP), las cuales fueron estimuladas específicamente con antígeno de cisticerco y se cultivaron para cuantificar en los sobrenadantes así como en el LCR de cada caso, los niveles de las citocinas: IL1β, IL4, IL5, IL6, IL10, IL12, IL13, IFNγ, TNFα y TGFβ por medio de la técnica de ELISA utilizando kits comerciales (BD Pharmingen, eBioscience). También en suero se midieron los niveles de IL10.

Igualmente se evaluó la capacidad proliferativa de las CMSP estimuladas con antígeno de cisticerco, a través de incorporación de timidina tritiada (Amersham Life

Sciences), en donde después de 6 días de cultivo, se obtuvo el índice de proliferación en base a la razón las cuentas por minuto obtenidas en las células estimuladas con antígeno de *T. solium* y las cuentas por minuto de células no estimuladas. Como control de la proliferación se utilizó concanavalina A (Sigma, MO).

En otro grupo de CMSP estimuladas durante 72 hrs con antígeno de cisticerco se extrajo RNA total para la búsqueda de RNAs mensajeros de marcadores de apoptosis: caspasa 3, caspasa 8 y el receptor del TNFα: TNFR1. Como control se utilizó RNA total de células sin estímulo al tiempo cero.

Para estudiar la existencia de algún componente parasitario en los plasmas y LCR de los pacientes severos, que pudieran estar favoreciendo la baja proliferación celular, se realizó un ensayo en donde se cultivaron células sanas marcadas con carboxi-fluoresceínadiacetato succinimidil éster (CFSE) (Molecular Probes, USA) en presencia de plasma o LCR de pacientes con alta y con baja proliferación celular, los niveles de proliferación celular se evaluaron por citometría de flujo en un equipo FACScan (Becton–Dickinson).

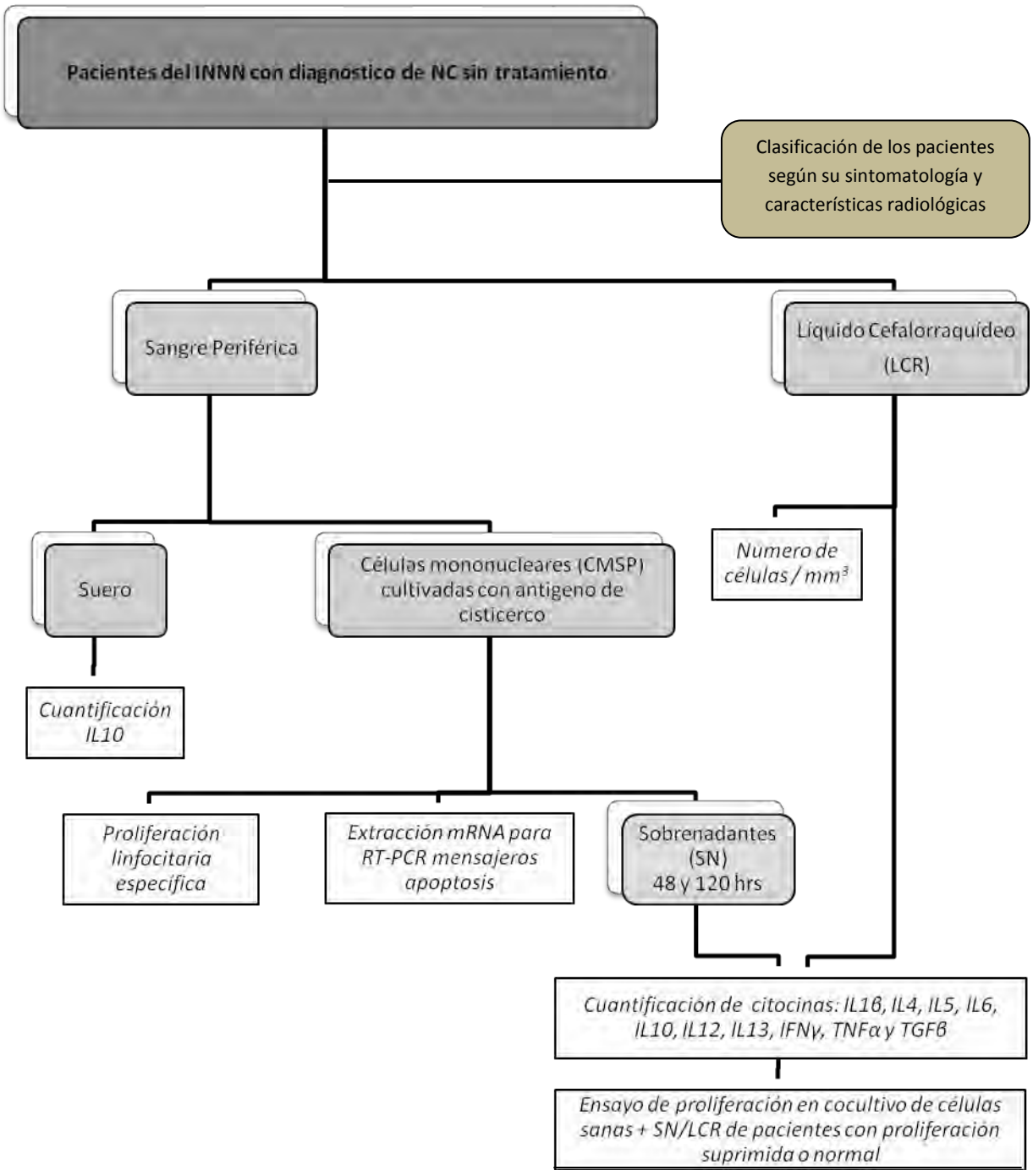

Figura 1. Esquema de la estrategia experimental en las muestras de sangre periférica y líquido cefalorraquídeo de pacientes con NC, para la determinación de la respuesta inmune local y sistémica asociada a la severidad de la enfermedad.

Estudio de la relevancia de la respuesta inmune en las formas no severas de la enfermedad

En esta parte del estudio se incluyeron a 33 casos con diagnóstico de NC única parenquimatosa por TAC o RMN que acudieron al Instituto Nacional de Neurología y Neurocirugía, el Hospital Infantil de México “Federico Gómez”, el Instituto Nacional de Pediatría o que fueron captados en la comunidad rural de Cuentepec, Morelos, México en donde se llevó a cabo un estudio epidemiológico de diagnóstico por tomografía de NC (Fleury et al., 2006).

Los pacientes se clasificaron clínicamente según su sintomatología en epilépticos o no epilépticos y radiológicamente según la localización del parásito (lóbulo frontal, parietal, occipital, temporal o ganglios basales), su laterización (hemisferio derecho o izquierdo) y el estado de degeneración del mismo (coloidal o calcificado). La edad y género de los pacientes también fueron colectados.

A todos los casos se les tomó una muestra de sangre periférica y a los pacientes hospitalarios se les tomó una muestra de LCR cuando era indicada para fines de diagnóstico y que no habían recibido tratamiento específico al momento del estudio.

De las muestras de sangre periférica se obtuvieron CMSP, las cuales fueron estimuladas específicamente con antígeno de cisticerco. En el sobrenadante de dichas células, así como en el LCR de los pacientes hospitalarios, se cuantificaron los niveles de las citocinas: IL1β, IL5, IL6, IL10, IL13, IFNγ, TNFα y TGFβ por medio de la técnica de ELISA utilizando kits comerciales (BD Pharmingen, eBioscience).

En los plasmas y LCR de los participantes, se midieron las subclases especificas de inmunoglobulinas G específicas para *T. solium* IgG1, IgG2, IgG3 e IgG4, por medio de la técnica de ELISA (Zymed Laboratories).

También se evaluó la capacidad proliferativa de las CMSP estimuladas con antígeno de cisticerco, a través de incorporación de timidina tritiada (Amersham Life Sciences), en donde después de 6 días de cultivo, se obtuvo el índice de proliferación en base a la razón las cuentas por minuto obtenidas en las células estimuladas con antígeno de *T. solium* y las cuentas por minuto de células no estimuladas. Como control de la proliferación se utilizó concanavalina A (Sigma, MO).

Con los datos obtenidos se estudió la relevancia de la respuesta inmuno-inflamatoria en la manifestación de epilepsia en casos con NC benigna. También se estudió la relación entre la localización y la lateralización del parásito con la presencia de sintomatología.

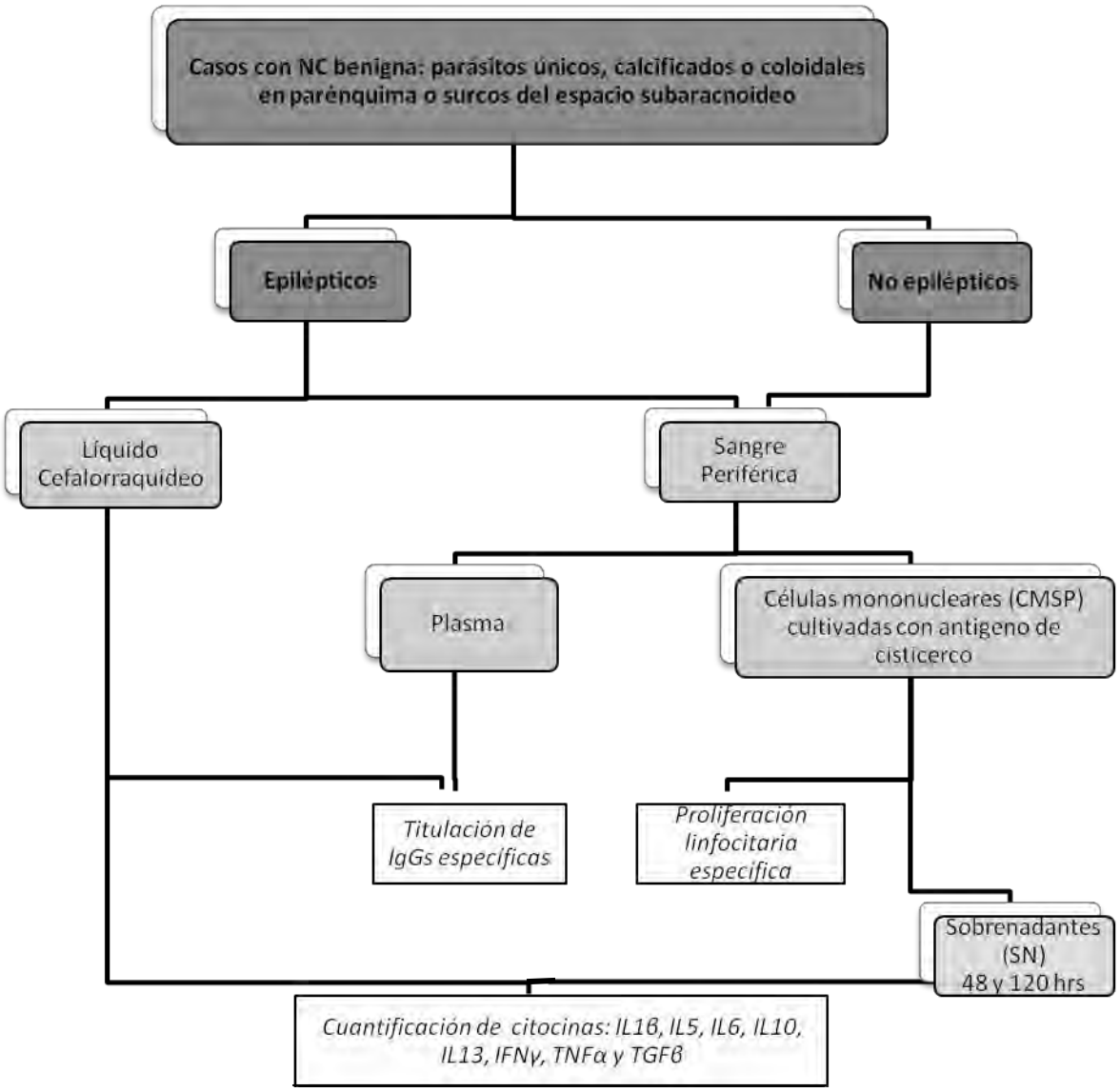

Figura 2. Esquema de la estrategia experimental para el estudio de la participación de la respuesta inmune en las formas no severas de la NC.

Consideraciones éticas

El proyecto contempla los siguientes aspectos éticos:

- Libre consentimiento informado del paciente y/o de los padres o tutores legales.
- Confidencialidad de los resultados de la investigación, tratamiento y seguimiento de los pacientes por los médicos especializados en caso de identificar enfermos.

El proyecto fue aprobado por el Comité de ética del Instituto Nacional de Neurología y Neurocirugía, el Hospital Infantil de México “Federico Gómez”, el Instituto Nacional de Pediatría y del Instituto de Investigaciones Biomédicas de la UNAM.

RESULTADOS

En este trabajo se evaluó la relevancia de la respuesta inmune local y sistémica en las distintas formas clínicas y radiológicas de la NC humana.

Por una parte se estudió la respuesta inmune local y sistémica asociada con las formas severas de la enfermedad, en particular se estudió la relación entre la respuesta de citocinas observada en los sobrenadantes de células de sangre periférica estimuladas específicamente y en LCR.

Los pacientes fueron clasificados de acuerdo a la pleocitosis en el LCR. Se observó que los pacientes con LCR inflamatorio (igual o mayor a 10 cel/mm^3) presentaron formas clínicas severas (con HIC), además de parásitos localizados principalmente en SaBa/intraventriculares, múltiples y vesiculares. Dadas las características clínicas, radiológicas y neuroinflamatorias, este grupo de pacientes se designó como inflamatorios/severos. Por otra parte, se identificó un grupo de pacientes no-inflamatorios/no-severos con LCR no inflamatorio (menos de 10 cel/mm^3), sin HIC, parásitos localizados principalmente en SaSu/parénquima, únicos, coloidales o calcificados.

El perfil inmunológico de los pacientes inflamatorios/severos se caracterizó por la presencia en el LCR de IL1β, IL5, IL6 e IL10 ($P=0.035$; $P=0.002$; $P=0.009$; $P=0.0001$ respectivamente), llamando la atención la elevada proporción de la citocina antinflamatoria IL10 sobre las citocinas inflamatorias IFNγ, TNFα e IL12 ($r=0.88$, $P<0.001$; $r=0.76$, $P<0.001$; $r=0.63$, $P<0.001$, respectivamente), en LCR de pacientes inflamatorios/severos.

También se observó una correlación negativa entre la presencia de células en LCR y la capacidad proliferativa de las CMSP estimuladas específicamente con antígeno de

cisticerco (r=-0.4, P=0.04), y con la presencia de TNFα en SN (r= -0.5, P=0.02). Además se observó una correlación positiva con la presencia de IL5 en SN (r=0.56, P=0.003).

Se exploraron las posibles causas que estuvieran involucradas en la relación de una alta celularidad en el LCR y una depresión de la proliferación linfocitaria a nivel sistémico y se descartó que pudiera ser por la presencia de un evento de apoptosis inducido por la producción de TNFα en aquellos pacientes inflamatorios. También se descartó la posibilidad de que la IL10 estuviera participando en la supresión de la proliferación linfocitaria específica a nivel sistémico ya que no se detectaron niveles elevados de IL10 en el suero de los pacientes inflamatorios y no-inflamatorios. Asimismo se exploró la posible presencia de productos de excreción/secreción del parásito que pudieran estar suprimiendo la proliferación celular en los pacientes inflamatorios, sin embargo no se observaron diferencias que pudieran sugerir la presencia de productos parasitarios en LCR o plasma de los pacientes.

Estos resultados confirman que los pacientes con NC severa presentan una respuesta inmune inflamatoria activa a nivel local y que puede ser detectada a nivel sistémico a través de la supresión de la respuesta proliferativa específica.

Por otra parte, se estudió la respuesta inmune local y sistémica en los pacientes con formas radiológicas y clínicas benignas de la enfermedad, distinguidos por la presencia de parásitos únicos en SaSu/parénquima que degeneran a un estado coloidal y posteriormente se calcifican, en ocasiones sin causar sintomatología aparente o bien la sintomatología más frecuente asociada a estas formas de la enfermedad es la epilepsia.

Se evaluaron los posibles factores que promueven la presencia de epilepsia en las formas benignas de la NC, se estudió si la localización de los parásitos en pacientes epilépticos hospitalarios con NC única parenquimatosa era diferente a la de casos no

epilépticos diagnosticados en un estudio epidemiológico en una comunidad rural de Morelos, México. Se observó que ni la localización de los parásitos en los diferentes hemisferios cerebrales ni la lateralización de los mismos resultaron asociadas a la presencia de epilepsia. De la misma manera, los pacientes epilépticos presentaron más frecuentemente parásitos coloidales y los no epilépticos presentaron más frecuentemente parásitos calcificados. También se evaluaron las diferencias al respecto de la respuesta inmune que pudieran estar asociadas con la presencia de epilepsia y se observó que los casos no epilépticos presentaron una respuesta proliferativa mayor que los epilépticos, además de mayores niveles de IL5, IL10, IFNγ, TNFα, IL13 e IgG4 ($P=0.0001$ para IL5, IFN y TNF; $P=0.001$ para IL10; $P=0.045$ para IL13 y $P=0.063$ para IgG4). Un perfil similar se observó en los pacientes con parásitos coloidales.

A nivel local, en el LCR los pacientes epilépticos presentaron un LCR no inflamatorio y niveles de citocinas no elevados.

Estos resultados sugieren que la eficiente respuesta inmuno-inflamatoria detectada en los pacientes no epilépticos promueve la calcificación del parásito sin mayores consecuencias tales como la presencia de epilepsia.

Finalmente se estudiaron las diferencias entre la respuesta inmune local y sistémica asociadas con la localización de los parásitos entre toda la cohorte de casos con NC incluidos en los dos estudios. Se observó que los pacientes con localización SaBa/intraventricular, presentaron niveles elevados de IL5, IL6 e IL10 en LCR ($P=0.018$, $P=0.048$ y $P<0.0001$ respectivamente) además de proliferación linfocitaria específica suprimida, mientras que los casos con localización SaSu/parénquima presentaron niveles elevados de IL5 e IFNγ ($P=0.002$, $P<0.0001$ respectivamente).

Tabla 1. Niveles de citocinas en SN y LCR de todos los casos estudiados según la localización de los parásitos.

Citocina	SaSu/parénquima (N=49)	SaBa/Intraventricular (N=14)	P
LCR (pg/mL)			
IL1β	9.4[a] (9.4)	9.4 (9.5)	0.142
IL5	9.4 (9.4)	26.8 (69.4)	**0.018**
IL6	16 (86.4)	53.3 (172.1)	**0.048**
IL10	9.4 (9.4)	110.7 (403.1)	**0.0001**
IL13	9.4 (12.7)	9.4 (9.4)	0.113
IFNγ	9.4 (9.4)	9.4 (9.4)	0.150
TNFα	9.4 (9.4)	9.4 (13.1)	0.361
Sobrenadante de CMSP (pg/mL)			
IL1β	82.2 (377.4)	137.6 (407.2)	0.911
IL5	207.3 (354.5)	9.4 (11.9)	**0.002**
IL6	67.9 (165.5)	35.2 (183.4)	0.48
IL10	65.1 (223.4)	41.1 (101.5)	0.293
IL13	211.6 (348.8)	117.8 (202.8)	0.116
IFNγ	170.8 (482.5)	9.4 (9.4)	**0.0001**
TNFα	403.1 (755.4)	118.1 (329.4)	0.299
Proliferación linfocitaria			
IE[b] ConA	22.1 (62.2)	4.5 (11)	0.177
IE Cisti	10.1 (37.88)	0.91 (1.2)	**0.001**

Comparación realizada por prueba no paramétrica de Mann-Whitney U. Valores de P en negritas indican diferencia significativa.

[a] Mediana (percentil 75). [b] IE, índice de estimulación.

1. Resultados de la relación la respuesta inmune local y sistémica en las formas severas de la NC.

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ORIGINAL INVESTIGATION

Neurocysticercosis: local and systemic immune-inflammatory features related to severity

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Abstract Neurocysticercosis (NC) is caused by the establishment of *Taenia solium* cysticerci in the central nervous system. Previous studies have established that neuroinflammation plays a key role in the severity of the disease. However, the relationship between peripheral and local immune response remains inconclusive. This work studies the peripheral and local immune-inflammatory features and their relationships, toward the identification of potential peripheral immunologic features related to severity. A panel of cytokines was measured in paired cerebrospinal fluid (CSF) and in the supernatant of antigen-specific stimulated peripheral blood mononuclear cells samples (SN) in a total of 31 untreated inflammatory and non-inflammatory NC patients. Increased clinical and radiologic severity was associated with an increased cerebrospinal fluid cell count. A peripheral proliferative depression that negatively correlates with CSF cellularity and TNFα and that positively correlates with SN IL5 was observed in severe NC patients. These results provide evidences to support the systemic proliferative response as a biomarker to monitor the level of neuroinflammation, of possible value in the patients' follow-up during treatment.

Keywords Neurocysticercosis · Immune response · Neuroinflammation · *Taenia solium*

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Introduction

Neurocysticercosis (NC) is caused by the establishment of the larval stage of *Taenia solium* in the central nervous system (CNS). This parasitic disease can either be asymptomatic or exhibit a great variety of clinical symptoms, ranging from headache to seizures, focal neurologic signs, and intracranial hypertension (ICH). This clinical heterogeneity is related to the parasite location, the number, and developmental stage of the parasites, which affect the intensity and characteristics of the neuroinflammatory response [1–4]. NC caused by a damaged single cysticercus located in the parenchyma or in the subarachnoid space of the sulci is frequently observed in residents of endemic areas [5, 6]. These forms occur with null or low increase of cell counts in cerebrospinal fluid (CSF) and are mostly asymptomatic

or accompanied by headaches and/or epilepsy without compromising the life of the patient. This location is also common in children, in whom it is frequently related with a benign prognosis [7, 8]. In contrast, the most severe forms of the disease occur when parasites are located in the subarachnoid space of the cerebral base or in the ventricles. In these cases, an increased cellularity in the CSF is frequently observed. Moreover, the impaired CSF circulation often leads to ICH, and the enhanced inflammatory reaction may cause arachnoiditis or even fibrosis [1, 5], both severe sequels of NC.

Considering the relevance of neuroinflammation in NC pathogenesis, increased efforts have been performed to characterize the immune-inflammatory response in NC and its role in dealing with the parasite [9–17]. All studies have been performed studying the CSF (local) or blood (peripheral) of NC patients independently. In CSF, increased levels of IL5, IL6, and IL10 were found in patients with active symptomatology compared to non-severe patients or non-NC controls [9, 18–20]. In the periphery, silent calcified NC showed increased levels of the TH2 cytokines IL4, IL5, and IL13 in the supernatants (SN) of peripheral blood mononuclear cells (PBMCs) specifically stimulated with cysticercal antigens [12, 21]. In contrast, in severe NC patients with vesicular parasites, increased production of a wider repertoire of TH1/TH2 cytokines (IL4, IL12, TNFα, IFNγ, IL2, and IL18) has been observed [11, 13, 14].

This study was designed to evaluate the local and peripheral immune-inflammatory changes that resulted from the neuroinflammation that accompanied NC. The identification of the peripheral immunologic features related with the degree of neuroinflammation could be of useful in the prognosis and follow-up of NC patients without the requirement of invasive procedures.

Materials and methods

Patients

Thirty-one Mexican patients attended at the Instituto Nacional de Neurología y Neurocirugía in Mexico City, with newly diagnosis of NC confirmed by radiologic studies (Magnetic Resonance Imaging and Computed Tomography) and without specific treatment (steroid or antiparasitic drugs) at the time of sampling, were recruited in this study from 2004 to 2007. Peripheral blood and CSF samples were collected when it was medically indicated for diagnostic purposes. Cell number and eosinophils presence in CSF and data from haematic biometry and lipid profile were recorded.

The following information was collected from the radiologic studies of NC patients: parasite number (single or multiple), stage of cysticerci [vesicular (parasites with a thin and translucent membrane and transparent fluid without apparent surrounding inflammatory reaction), colloidal (parasites with thicker walls and turbid cyst fluid surrounded by inflammatory reaction in brain tissue) or calcified (parasite debris observed as mineralized granuloma)] and CNS location [parenchyma and subarachnoid space of the sulci (SaSu), subarachnoid space of the base (SaBa) or intraventricular].

Patients were classified according to their CSF cellularity: 16 patients who presented more or equal to 10 cells per mm^3 were considered inflammatory and severe; the other 15 patients with less than 10 cells per mm^3 were considered non-severe/non-inflammatory.

Antigen preparation

Whole *T. solium* cysticerci were obtained from an infected pork's skeletal muscle from central Mexico, washed with phosphate-buffered saline solution, homogenized, and centrifuged at 25,000$\times g$ for 45 min at 4°C. Soluble antigens in the supernatant were recovered; calcium was precipitated with ammonium oxalate 0.3 M and ammonium hydroxide 1:10 and centrifuged at 25,000$\times g$ for a further 40 min at 4°C. Supernatant was recovered and filtered with 0.22-mm membrane under sterile conditions, quantified according to Lowry, and frozen at −20°C until used as a whole antigen fraction (TsAg).

Immunologic profile

Considering previous studies performed in unpaired samples from NC subjects, the following parameters were measured to evaluate the peripheral and local immune-inflammatory features and their possible relations to severity: in vitro *T. solium*-antigen specific cell proliferation, pro-inflammatory (IL1β, IL12, IFNγ, TNFα, and IL6), anti-inflammatory (IL10 and TGFβ), and TH2-type cytokines (IL4, IL5, and IL13) measured in the SN from *T. solium* specifically stimulated PBMCs and CSF, plasmatic IL10, and RNA messenger expression of apoptosis markers (caspase 3, caspase 8, and TNFR1) in *T. solium* specifically stimulated PBMCs.

Sample collection

Ten to 15 mL of peripheral blood venous was drawn into an EDTA-containing tube. Blood was diluted 1:1 with RPMI medium 1640 (Gibco BRL, NY), layered over Ficoll-Hypaque (Amersham Life Science, UK), and centrifuged at 400$\times g$ for 30 min at room temperature. Plasma was recovered and stored at −80°C until use. PBMCs were washed 3 times with 1640-RPMI and suspended in 1640-RPMI

supplemented with 10% of human AB serum (donated from The Blood Bank of Centro Médico Siglo XXI, Mexico City), 2 mM L-glutamine, 100 U/mL penicillin, 100 mg/mL streptomycin, 1% non-essential amino acids, and 1% pyruvate (Gibco BRL) and used for cell culture.

From the same patients, 2–3 mL of CSF was collected by lumbar puncture; these samples were stored at −80°C until use for cytokine quantification.

Lymphocyte proliferation

PBMCs were stimulated with concanavalin A (0.5 μg/well, Sigma, MO) or *T. solium* antigen (10 μg/well) and incubated at 37°C and in a 5% CO_2 humidified atmosphere in 96-well flat-bottom culture plates (Costar, Cambridge, MA) at a cell concentration of 1×10^5 cells per 200 mL, final volume, per well. After 6 days, cells were pulsed with 1 μCi of methyl-[^{3}H]-thymidine (Amersham Life Sciences) for 18 h. PBMCs were harvested onto glass filter papers; the amount of incorporated label was measured by counting in a 1205-β spectrometer (Wallac).

Cytokine production and titration

PBMCs were plated at a cell concentration of 2.5×10^6 cells/ml per well in 12-well cluster plates (Costar) and incubated at 37°C and in a 5% CO_2 humidified atmosphere. Cells were either stimulated or not with TsAg (10 μg/ml). After a 48 h (for IL6, IL10, TGFβ, TNFα) or 120 h (for the other cytokines) incubation, SN of the cultures were harvested and stored at −80°C until use for cytokine quantification.

ELISA was performed in 96-well, flat-bottomed microtiter plates (Nunc-Immuno Plate Maxisorp, Rosekilde, Denmark) according to the manufacturer's indications [IL1β, IL4, IL5, IL10, IL12, IL13, IFNγ (DB Pharmingen cytokine sets, CA), IL6, TGFβ (eBioscience), and TNFα (R&D DuoSet, UK)]. All assays were performed in duplicate. Assay sensitivity was 9.4 pg/mL for all cytokines.

Antibody titration

The level of anti-cysticercal antibodies were measured by ELISA in the CSF of all patients included in this study following the procedure previously described [22].

Total RNA isolation and reverse transcriptase-polymerase chain reaction apoptosis analysis

Total RNA was extracted from PBMCs without specific stimulation and with 72-h specific antigen stimulation by the Trizol method (Invitrogen). For RT-PCR analysis, 100–500 ng of RNA were reverse transcribed with Superscript II RNA H reverse transcriptase kit (200 U/sample; Invitrogen). cDNAs were amplified in each PCR assay with Platinum *Taq*DNA polymerase (5U/sample; Invitrogen), 10 μM of each primer, PCR buffer (50 mM KCl, 1.5 mM $MgCl_2$, and 20 mM Tris–HCl, pH 8.4), and 0.2 μM dNTP to a final volume of 50 μL.

Amplification was performed for 40 cycles (94° C/45 s, 56° C/45 s, 72° C/45 s) after an initial denaturation at 95°C for 4 min; final extension was performed at 72° C for 7 min. Twenty-microliter aliquots of PCR products were finally size-separated by electrophoresis in 1.2% agarose gels.

The following forward (Fw) and reverse (Rw) oligonucleotide primers were used: Caspase 3 (264 bp) Fw 5′-TTC AGAGGGGATCGTTGTAGAAGTC-3′ Rw 5′-CAAGC TTGTCGGCATACTGTTTCAG-3′; Caspase 8 (411 bp) Fw 5′-CACTAGAAAGGAGGAGATGGAAAG-3′ Rw 5′-CT ATCCTGTTCTCTTGGAGAGTCC-3′; TNFR1 (263 bp) Fw 5′-ACCAAGTGCCACAAAGGAAC-3′ Rw 5′-CTGC AATTGAAGCACTGGAA-3′; and y β-actin (306 bp) Fw 5′-TCACCCACACACTGTGCCCAT-3′ Rw 5′-CAGCGG AACCGCTCATTGCCAATGG-3′. HeLa cells UV irradiated for 10 min were used as apoptotic controls.

Proliferation of lymphocytes from healthy donors assessed in the presence of plasma and CSF samples of NC patients

PBMCs from 3 healthy donors were isolated as described previously and labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) (Molecular Probes, USA) by incubation for 10 min at 37°C in 10 μM CFSE in PBS-0.1% BSA. 10^5 cells per well were cultured in 96 cluster plates with RPMI supplemented with the selected serum or CSF (10 or 20% of CSF) from three patients with high and three patients with non-elevated proliferation levels. Cells were stimulated with α-CD3 + α-CD28 (3 μg/mL and 5 μg/mL, respectively; BD Pharmingen, Cowley, UK) to a final volume of 200 μL per well. After 72 h incubation, cultures were analyzed in a FACScan (Becton–Dickinson). Proliferation cell ratio was obtained dividing the values registered after cell stimulation by those values before cell stimulation.

Statistical analysis

Data were processed in Excel XP (Microsoft Office) and SPSS 13.0 for Windows. Pearson's tests as well as a multiple linear regression analysis were used to correlate clinical and radiologic profiles. Mann–Whitney non-parametric *U* test was used to identify differences in the immune response between groups, and Spearman correlation was used to identify the relationship between cytokine titers with CSF cell counts and proliferation index between groups. $P \leq 0.05$ was considered significant.

Table 1 Clinical and radiologic features of 31 NC patients with inflammatory and non-inflammatory CSF

	Non-inflammatory (N = 16)	Inflammatory (N = 15)
Gender (M/F)	9/7	8/7
Number of parasites (single/multiple)	7/9	3/12
Parasites stage (damaged/vesicular)	11/5	14/1
Parasites location (parenchyma/subarachnoid space at the base of the brain)	15/1	2/13
Intracranial hypertension (without/with)	15/1	10/5
CSF (cell/mm^3)	2.3 ± 2.8 (0–9)[a]	59 ± 83.1 (11–320)
Eosinophils (without/with)	12/1	7/7

[a] Mean ± SD (min–max)

Results

NC patients

The clinical and radiologic features from the 31 NC patients included in this study are shown in Table 1. Among these 31 NC patients, 25 (80.6%) did not show ICH, and 6 (19.4%) exhibited this clinical manifestation. Regarding the number of parasites, multiple cysticerci were found in 21 cases (67.7%), and 10 (32.3%) presented one single cysticercus. Sixteen patients exhibited parasites in mixed developmental stages. Vesicular parasites were present in 19 patients, while 12 of them presented only damaged or calcified parasites. Concerning their location, parasites occupied different compartments: in 17 (55%) cases, they were located only in parenchyma and/or SaSu; in the remaining 14 (45%) patients, parasites were located in SaBa or the ventricles, either associated or not with SaSu or parenchymal locations.

Increased CSF cells count is associated with increased clinical severity

Cell count in CSF was significantly higher (median (md): 59.5 cell/mm^3, percentile 75 (p75): 193.5 cell/mm^3) in patients with IHC, the most severe NC clinical sign, than in patients without this symptom (md: 6 cell/mm^3, p75: 15 cell/mm^3; $P = 0.011$). Also, CSF cell count was significantly higher in patients with parasites located in the SaBa of the brain or in the ventricles (md: 30.5 cell/mm^3, p75: 71 cell/mm^3) than in patients with parenchymal or SaSu cysticerci (md: 2 cell/mm^3, p75: 6 cell/mm^3, $P < 0.0001$). Patients with vesicular parasites exhibited higher CSF cell count than those with calcified or colloidal parasites (md: 19 cell/mm^3, p75:59.5 cell/mm^3 vs. md: 1.5 cell/mm^3, p75: 3 cell/mm^3, $P < 0.0001$). Moreover, a higher proportion of inflammatory patients presented anticisticercal antibodies in their CSF (13/15, 86.7%), than those non-inflammatory (7/16, 43.7%. $P = 0.02$, Fisher exact test).

Table 2 Peripheral and local cytokine levels in inflammatory CSF (≥10cells/mm3) and non-inflammatory CSF (<10 cells/mm^3) of NC patients

	Non-inflammatory (N = 16)	Inflammatory (N = 15)	P
Cell count	**2.0 (3.0)**[a]	**25.5 (69.0)**	**0.0001**
CSF (pg/ml)			
IL1β	9.4 (9.4)[b]	**9.4 (10.7)**	**0.035**
IL5	9.4 (9.4)	**29.7 (69.4)**	**0.002**
IL6	16.0 (19.3)	**159.5 (394.9)**	**0.009**
IL10	9.4 (9.4)	**58.3 (217.7)**	**0.0001**
IL4	9.4 (9.4)	9.4 (9.4)	NS[c]
IL12	9.4 (12.0)	16.7 (45.0)	NS
IL13	9.4 (35.5)	9.4 (9.4)	NS
IFNγ	9.4 (9.4)	9.4 (9.4)	NS
TNFα	9.4 (9.4)	9.4 (13.1)	NS
TGFβ	9.4 (9.4)	9.4 (24.1)	NS
PBMCs supernatant (pg/ml)			
IL10	26.9 (123.0)	54.0 (110.1)	NS
IL13	213.9 (325.0)	143.7 (210.2)	NS
IL1β	686.3 (957.0)	137.6 (555.6)	NS
IL4	9.4 (9.4)	9.4 (9.4)	NS
IL5	9.4. (12.1)	9.4 (14.7)	NS
IL6	51.7 (1845.1)	37.9 (154.2)	NS
IFNγ	9.4 (23.8)	9.4 (10.8)	NS
TNFα	59.4 (343.8)	118.0 (329.4)	NS
IL12	19.7 (87.0)	21.6 (92.1)	NS
TGFβ	101.8 (121.2)	124.9 (201.7)	NS
Con A SI[d]	22.0 (43.7)	5.8 (20.1)	NS
Cisti Ag SI	2.7 (18.1)	0.9 (5.5)	NS

Non-parametric Mann–Whitney U test was used for comparison. P values are indicated when they are ≤0.05. Bold values indicate higher cytokine levels

[a] Median (upper 75% percentile)

[b] Cytokine levels are expressed in pg/mL

[c] *NS*, not significant

[d] *SI*, stimulation index, defined as cpm antigen or concanavalin A-stimulated cells/cpm non-stimulated cells

Cytokine profile in inflammatory and non-inflammatory individuals

CSF was considered inflammatory when cells exceeded 10 cells per mm^3.

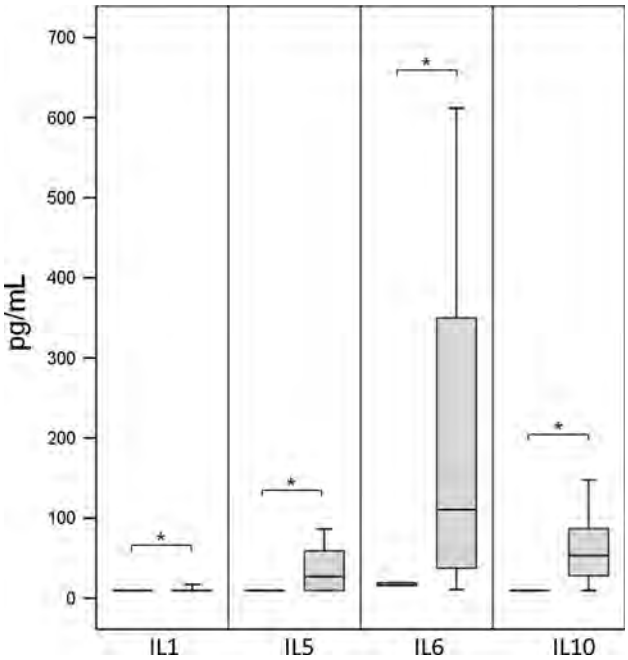

Fig. 1 Cytokine levels in inflammatory CSF (≥10cells/mm3) (*gray boxes*) are significantly higher than in non-inflammatory CSF (<10 cells/mm3) (*black boxes*). Non-parametric Mann–Whitney *U* test was performed only when $*P \leq 0.05$ is indicated. Median is indicated by the *black center line* and percentile 75 by the *top* edge of the *box*

Local and peripheral cytokine levels of patients with inflammatory (≥10 cell/mm^3; md: 25.5 cell/mm^3, p75: 69 cell/mm^3) and non-inflammatory CSF (<10 cell/mm^3; md: 2 cell/mm^3, p75: 3 cell/mm^3) were compared (Table 2). Inflammatory patients presented increased levels of IL1β ($P = 0.03$), IL5 ($P = 0.002$), IL6 ($P = 0.009$), and IL10 ($P < 0.0001$) in CSF (Fig. 1). The rest of the CSF cytokine levels, as well as the peripheral cytokines, evaluated in SN of specifically stimulated cells remained similar in both groups.

The proportion of the presence of anti-inflammatory cytokine IL10 over pro-inflammatory IFNγ, TNFα, and IL12 cytokines was evaluated. In the inflammatory patients, IL10 resulted up to six times higher than the pro-inflammatory cytokines (Table 3). In addition, positive significant correlations between CSF cell count with IL10/IFNγ, IL10/TNFα, and IL10/IL12 ratios were found ($r = 0.88$, $P < 0.001$; $r = 0.76$, $P < 0.001$ and $r = 0.63$, $P < 0.001$, respectively).

Exacerbation of immune response in inflammatory CSF

Positive correlations were found between CSF cellularity and the following cytokines in CSF: IL1β ($r = 0.43$; $P = 0.02$), IL5 ($r = 0.59$, $P = 0.001$), IL6 ($r = 0.4$, $P = 0.05$), IL10 ($r = 0.89$, $P < 0.001$), and IL12 ($r = 0.4$, $P = 0.04$). Additionally, a positive correlation was found between increased cellularity and eosinophilia in CSF ($r = 0.514$, $P = 0.006$).

Table 3 Ratios of IL10 over IFNγ, TNFα, and IL12 in cerebrospinal fluid of non-inflammatory versus inflammatory CSF of NC cases

	Non-inflammatory ($N = 16$)	Inflammatory ($N = 15$)	*P*
Ratios			
IL10/IFNγ	1.0 (1.0)[a]	6.6 (30.7)	0.0001
IL10/TNFα	1.0 (1.0)	6.1 (12.1)	0.001
IL10/IL12	1.0 (1.0)	3.2 (7.2)	0.033

[a] Median (upper 75% percentile). Non-parametric Mann–Whitney *U* test was used for comparison. *P* values are indicated when they are ≤0.05

Relation between central inflammatory response and peripheral cytokine profile

Inflammatory patients presented significant positive correlations between CSF and peripheral cytokines: CSF IL1β and SN IL4 ($r = 0.74$, $P = 0.006$), CSF IL4 and SN IL10 ($r = 0.83$, $P < 0.0001$), CSF IL10 and SN IL6 ($r = 0.833$, $P < 0.0001$), CSF IL5 and SN IL12 ($r = 0.604$, $P = 0.04$), CSF IL6 and SN IFNγ ($r = 0.693$, $P = 0.03$), and between CSF TNFα and SN IFNγ ($r = 0.636$, $P = 0.03$).

In non-inflammatory patients, significant correlation was observed in: CSF IL4 and SN TNFα ($r = 0.485$, $P = 0.049$), CSF IL10 and SN TNFα ($r = 0.520$, $P = 0.032$) and CSF IL13 and SN IFNγ ($r = 0.969$, $P < 0.0001$).

High CSF cellularity is negatively correlated with specific cell proliferation

A significant negative correlation was observed between CSF cellularity and lymphocyte-specific proliferation ($r = -0.4$, $P = 0.04$). Cell proliferation with concanavalin A showed no correlation with CSF cellularity ($P = 0.19$). Also, specific cell proliferation negatively correlated with SN TNFα ($r = -0.5$, $P = 0.02$) and positively correlated with SN IL5 ($r = 0.56$, $P = 0.003$).

Depression of the specific peripheral proliferative response and apoptosis

TNFα is a cytokine that leads cells to apoptosis [23]. Considering the negative correlation between TNFα and the cell proliferation level, the possible participation of TNFα in apoptosis was explored. The expression of the apoptotic markers TNFR1, caspase 3, and caspase 8 were evaluated in cells from patients with the highest TNFα levels and decreased proliferation index ($n = 4$), versus cells from

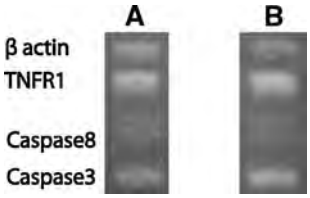

Fig. 2 Similar expression profile of three apoptotic markers in severe NC patients with increased levels of TNFα and decreased proliferation index (**a**), and in non-severe NC patients with decreased levels of TNFα and normal proliferation index (**b**). Representative PCR performed with DNA from cells harvested 72 h after *T. solium* antigen stimulation

patients with lower TNFα levels and normal proliferation index ($n = 5$). No significant differences in the expression of the mentioned apoptosis markers were observed between both groups of patients (Fig. 2).

Suppressed cell proliferation is not related with IL10 anti-inflammatory activity

The possibility that a systemic increase in IL10 was involved in the decreased specific peripheral response was discarded since this immunosuppressive cytokine was measured, and no detectable levels of IL10 were found neither in inflammatory nor in non-inflammatory NC patients (data not shown).

Excretion/secretion parasitic products and suppression of proliferative response

The presence of parasitic excretion/secretion products that could promote the suppression of the proliferative response was also explored. To evaluate this possibility, cells from non-infected individuals stimulated with αCD3/αCD28 were cultured in a medium supplemented with CSF or plasma from three NC patients with increased proliferative response, and from three NC patients with decreased *T. solium*-antigen proliferation. No differences in the T cells proliferative index from healthy donors were found between both groups (data not shown).

Discussion

This study was designed to identify systemic immunologic features that could be related to the extent of the neuroinflammation that accompanied inflammatory severe cases in NC. For this purpose, paired CSF and blood samples from clinically and radiologically well-defined NC patients without specific or anti-inflammatory treatment were studied.

Pleocytosis was considered as the main neuroinflammatory parameter of NC severity, and it was found related with a clinical severe profile characterized by the presence of ICH and vesicular parasites located in the SaBa of the brain or in the ventricles. Patients with inflammatory CSF presented increased levels of CSF IL1β, IL5, IL6, and IL10 (Fig. 1). This result was confirmed when the relation between CSF cellularity and local and systemic cytokine titers considered as a continuous variable was analyzed. Also, increased pleocytosis positively correlated with increased levels of CSF IL1 β, IL5, IL6, and IL12 and the anti-inflammatory cytokine IL10, as well as with presence of eosinophils in CSF. IL1β and IL12 are reported to be produced by activated microglia [24, 25] and also could be produced by CSF pro-inflammatory cells in response to cysticercus antigen stimulus, since IL12 level was not consistent with the two types of analysis; it is more feasible that IL12 can be produced locally by reactive local cells. IL6 could act promoting B cell stimulation [26], and both IL6 and IL5 could promote the recruitment of local cells and eosinophils [27, 28]. The presence of IL6 in CNS of NC patients has been previously associated to severity [6, 12, 19, 20]; however, their role in the destruction of the parasite remains unknown. Increased IL6 levels could also be involved in promoting TH17 cells generation [29]. This intriguing possibility should be further explored since TH17 cells may be critically related to the parasite destruction, as it occurs in the clearance of other extracellular pathogens [29]. Conversely, interleukin 10 observed in inflammatory patients could be produced by resident CNS cells or by immune cells recruited into CSF in order to regulate local inflammation, in an attempt to ameliorate its detrimental effects on the CNS [30–33]. In this sense, the proportion of this regulatory/anti-inflammatory cytokine compared with the pro-inflammatory IFNγ, TNFα, and IL12 cytokines resulted clearly elevated, suggesting that the production of IL10 is actively participating in regulating the inflammatory phenomena elicited.

In inflammatory individuals, local inflammatory TNFα, IL1β, and IL6 as well as the anti-inflammatory/Th2 cytokines IL4, IL10, and IL5 were found to be correlated with a subset of pro-/anti-inflammatory cytokines produced in SN of antigen-specific stimulated PBMCs. Opposing correlations about pro- and anti-inflammatory roles of the cytokines were found. These observations support the premise that systemic inflammatory mediators can induce cytokine synthesis within the brain in response to a stimulus elicited by the cysticercus establishment, and vice versa. However, in order to understand the relationship between the local and systemic immune components, further studies about the activation and effector mechanisms, as well as the cell types involved in the NC inflammatory response, should be studied.

Regarding non-inflammatory individuals, anti-inflammatory IL4, IL10, and IL13 cytokines in CSF positively

correlated with peripheral pro-inflammatory TNFα and IFNγ. This group of patients presented low CSF cellularity, and probably IL4, IL10, and IL13 are produced locally by resident cells such as microglia, astrocytes, and neurons as mediators of the TH1 response in CNS [30]. Thus, it seems that in non-inflammatory individuals, a local anti-inflammatory profile associated with a peripheral inflammatory response prevails.

An interesting finding was the significant association between the increased CSF inflammatory reaction and the depressed systemic-specific proliferative response. This finding was consistent with the previously reported significant association between severe neurocysticercosis and a decrease in the proliferative cell response [10, 18]. This result points to a tight relation between local and systemic immunity elicited against the parasitosis, reflected in the presence of anti-inflammatory cytokines in CNS that could act as modulators of the CNS damage caused by neuroinflammation.

Considering the possible relevance of cell proliferation inhibition by the inflammatory phenomena of NC pathogeny, possible factors that could contribute in controlling the systemic proliferative response were explored. The up-regulation of IL10 was considered as a possibly involved mechanism, since it has been reported for other parasitic infections [34–36]. This hypothesis was discarded because no increased peripheral IL10 levels were detected, neither in supernatant of specifically stimulated cells nor in plasma from inflammatory and non-inflammatory NC patients (data not shown). Taking into account the possible existence of suppressive parasite excretion/secretion factors [37–39], their presence in sera and CSF was evaluated. As shown in this study, no evidences of suppressive factors were found. Finally, since it is well documented that cell apoptosis is promoted by TNFα [23, 40, 41], the possibility of apoptotic events in inflammatory NC patients underlying the suppression of specific proliferative response was explored. Neither TNFR1, caspase 3, nor caspase 8 overexpression was observed in inflammatory NC patients with increased TNFα levels and decreased proliferation index compared with patients with decreased TNFα levels and normal proliferation index (Fig. 2).

In summary, increased neuroinflammation with a mixed pro-/anti-inflammatory local cytokine profile (IL1β, IL5, IL6, IL10, and IL12) is associated with a depressed PBMCs proliferative response, which in turn is associated with increased levels of SN TNFα and decreased levels of IL5 in SN. These cytokine and proliferation profiles characterize the immune response of severe NC patients. The absence of peripheral immunologic changes in the less neuroinflammatory NC patients revealed that the management of the infection is restrained to the CNS. Peripheral immunologic features that were modified in the most neuroinflammatory patients could be useful in the management and follow-up of NC patients. Mechanisms and cellular components involved in signaling exchange between CNS and the periphery deserve further study.

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Ethical standard The present study fulfilled all regulations for research with human subjects required by Mexican laws and International regulations. It also complied with all ethical aspects considered in the General Rules of Health for Clinical Investigation. The protocol was approved by the ethics committee of the Instituto Nacional de Neurología y Neurocirugía, México, and written informed consent was obtained from all individuals. All participants volunteered to enter the study. Patients were informed that sera and CSF samples obtained during their hospital studies would be used for this work. All results were confidential.

Conflict of interest The authors declare that they have no conflict of interest.

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2. Resultados de la relevancia de la respuesta immune en las formas no severas de la NC.

En preparación

FACTORS RELATED WITH EPILEPSY IN INDIVIDUALS WITH A SINGLE PARENCHYMAL DAMAGED CYSTICERCUS

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1. Introduction

Neurocysticercosis (NC) is caused by the establishment of the metacestode of *Taenia solium* in the central nervous system (CNS). NC may exhibit a variety of clinical manifestations, ranging from completely asymptomatic to a severe symptomatology that endangers patient's life [1]. Symptoms may vary from headaches, epilepsy and dementia to intracranial hypertension [2]. Cysticerci location (parenchyma, subarachnoid space, ventricles, sulci) and the intensity of the host inflammatory response against the parasite are the two most important factors related to the severity and prognosis of the disease [3,4].

Parasites located in the subarachnoid space at the base of the brain or in the ventricles usually lead to a severe clinical profile, characterized by intracranial hypertension due to an obstruction of cerebrospinal fluid (CSF) circulation and/or as a consequence of an exacerbated inflammatory response [3].

In contrast, when parasites are located in the cerebral parenchyma or in the subarachnoid space of the sulci, cysticerci commonly degenerate to a colloidal stage and then calcify without provoking apparent symptomatology. Indeed, calcified parenchymal cysticerci are frequent autopsy findings [5, 6]. Asymptomatic NC caused by a single parenchymal, calcified cysticercus is also highly frequent among the open population. It occurs approximately in 9% of the inhabitants of rural communities in central Mexico, as reported in two independent epidemiological studies based on computed tomography scan [7, 8]. However, parenchymal cysticerci may cause symptoms in certain circumstances, being epilepsy the most severe and the most frequently observed symptom [9, 10], particularly in children [11]. In these cases, it has been observed that epilepsy is significantly associated with the presence of damaged (colloidal and calcified) parasites [12].

The symptoms caused by a single parenchymal cysticercus could result from the parasite particular location. It is well known that the susceptibility to epilepsy depends on the structure involved; i.e., lesions located in highly epileptogenic areas such as the temporal lobe could be more prone to cause epilepsy than those lesions in extra-temporal regions [13]. The immunological mechanisms that mediate cysticercal destruction could also play an important role in this process. In this sense, it is reported that inflammation and immune mediators play a significant role in epileptogenesis [14].

This study was designed to explore whether the specific inmuno-inflammatory response elicited against one single parenchymal, damaged cysticercus could participate in the occurrence of epilepsy.

2. Materials and Methods

2.1 NC cases

Thirty-three non-treated NC patients with confirmed diagnosis of single parenchymal, damaged cysticercus by computed tomography (CT) scan or magnetic resonance imaging (MRI) were included. Patients were classified according to their symptoms. One group included 11 epileptic patients attended between years 2006 and 2009 at the Instituto Nacional de Neurología y Neurocirugía (INNN), the Hospital Infantil de México “Federico Gomez” or at the Instituto Nacional de Pediatría, all of them located in Mexico City. Another group included 22 non-epileptic NC patients, one patient from INNN presenting headache at the time of the diagnosis, and 21 patients from a rural community in Mexico, identified in a CT scan epidemiological study [8].

Peripheral blood samples from all NC patients and, when available, CSF samples from symptomatic patients were obtained to be included in this study, which lasted from

2004 through 2008. Age and gender of each patient were recorded. None of the patients received any treatment (steroid or anti-parasitic drugs) at the time of sampling.

2.2 Characterization of the disease

From radiological studies (CT scan or MRI) performed in each NC patient, the following additional information was collected: stage of the cyst [colloidal: (parasites with thicker walls and turbid cyst fluid surrounded by inflammatory reaction in brain tissue) or calcified (parasite debris observed as mineralized granuloma)], CNS specific location (frontal, parietal, occipital, temporal lobes or basal ganglia), and lateralization (right or left hemisphere).

Symptoms associated with the disease were established by interrogatory and clinical examination of the patients, and recorded either as epileptic or as non-epileptic.

2.3 Ethical considerations

The present study fulfilled all regulations and ethical aspects considered for Clinical Research by Mexican laws and International regulations. The protocol was approved by all the participant health institutions' Ethics Committee. It does not perform human experimentation. All participants and parents or guardians (in case of minors) received an explanation of the study objectives and the specific procedures included in the study. Afterwards, they gave signed informed consent. All participants volunteered to enter the study. Results were confidential.

2.4 Sample collection

Six to ten ml of peripheral blood was drawn into an EDTA-containing tube. Blood was diluted 1:1 with RPMI media 1640 (Gibco BRL, NY), layered over Ficoll-Hypaque (Amersham Life Science, UK) and centrifuged at 400 x *g* for 30 min at room temperature. Plasma was recovered and stored at –20 ºC until use for antibody quantification. Peripheral blood mononuclear cells (PBMCs) were recovered, washed 3 times with RPMI-1640 and suspended in the same media supplemented with 10% of human AB serum (donated from the blood bank of Centro Médico Nacional Siglo XXI, Mexico City), 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin, 1% non-essential amino acids and 1% pyruvate (Gibco BRL). In the case of symptomatic patients, for whom CSF sampling was indicated by practitioners, 1-3 ml of CSF was collected by lumbar puncture; these samples were stored at –80 °C until use for cytokine quantification.

2.5 Antigen preparation

Whole *Taenia solium* cysticerci were obtained from skeletal muscle of infected pigs from central Mexico and washed with phosphate-buffered saline solution (PBS). *T. solium* antigens (TsAg) were obtained following the procedures previously described [15]. Aliquots were stored at –20 ºC until use.

2.6 Immune-inflammatory profile

The following features were measured to define an immunological profile related to epilepsy in NC: *T. solium* specific IgG subclasses (IgG1, IgG2, IgG3, IgG4) in plasma and CSF; in vitro TsAg specific cell proliferation; pro-inflammatory (IL1β, IFNγ, TNFα, and IL6),

anti-inflammatory (IL10) and TH2 type cytokines (IL5 and IL13) measured in the supernatant (SN) of *T. solium* specifically-stimulated PBMCs from all patients and also in CSF samples, when available.

2.7 Lymphocyte proliferation

PBMCs were stimulated either with Concanavalin A (ConA) (0.5 µg/well; Sigma, MO) or TsAg (10 µg/well), incubated at 37 °C and in a 5% CO_2 humidified atmosphere in 96-well flat-bottom culture plates (Costar, Cambridge, MA) at a cell concentration of 1 x 10^5 cells per 200 µL of final volume per well. After 6 days, the cells were pulsed with 1µCi of methyl-[^{3}H] thymidine (Amersham Life Sciences) for further 18 h. PBMCs were harvested onto glass filter papers; the amount of incorporated label was measured by counting in a 1205-β spectrometer (Wallac).

2.8 Cytokine production and titration

PBMCs were placed at a cell concentration of 2.5 x 10^6 cells/ml per well in 12-well cluster plates (Costar), incubated at 37° C and in a 5% CO_2 humidified atmosphere. Cells were either stimulated or not with TsAg (10 µg/ml). After 48 h (IL6, IL10, TNFα) and 120 h (IL1β, IL5, IL13, IFNγ,) of incubation, SN of cultures were harvested and stored at –80 °C until use for cytokine quantification.

Sandwich ELISA of SN and CSF was performed in 96-well, flat-bottom microtiter plates (Nunc-Immuno Plate Maxisorp, Rosekilde, Denmark) according to the manufacturer's indications: BD Pharmingen cytokine sets, CA, for IL1β, IL13, IFNγ, and

eBioscience for IL5, IL6, IL10, TNFα. All assays were performed in duplicate. Assay sensitivity was 9.4 pg/ml for all cytokines.

2.9 IgG subclass antibody detection by ELISA

Plasma and CSF antibody levels were measured by indirect ELISA. TsAg (1 µg/100 µl/well) was incubated overnight at 4 °C in carbonate buffer, pH 9.5. Wells were washed, incubated with 1:50 diluted plasma or 1:10 diluted CSF for 1 h at 37°C. Bound immunoglobulins were developed using rabbit anti-human IgG1, IgG2, IgG3 or IgG4 coupled to biotin (1:1000, Zymed Laboratories), streptavidine alkaline phosphatase conjugate (1:3000; Zymed), and p-nitrophenyl phosphate (Sigma) as substrate. Plates were read at 405 nm after 30 min of incubation. All assays were performed in duplicate.

2.10 Statistical analysis

Data were processed in Excel XP (Microsoft Office) and SPSS 13.0 for Windows. Chi-square test was used to compare frequencies. The nonparametric Mann-Whitney U-test was used to identify differences in the immune response between groups.

3. Results

3.1 General description of NC cases

Table 1 describes the 33 NC cases included in this study, 22 non-epileptic (5 male/ 17 female) and 11 epileptic NC patients (8 male/ 3 female).

Regarding epileptic patients, 4 presented generalized seizures, 2 presented both generalized and partial seizures and 5 presented partial seizures.

The ages of non-epileptic cases ranged between 7-78 years old (43 ± 21.4). Only one patient presented headache, while all the others were asymptomatic. The age of epileptic NC patients ranged from 2-42-years old (20.3 ± 13.7).

Significant differences in age and gender were found between the two groups, being epileptic patients younger ($P = 0.016$) and more frequently males ($P = 0.006$).

3.2 Epilepsy is associated with colloidal stage, but not with parasite location or lateralization

Table 1 shows that neither parasite location ($P > 0.2$) nor lateralization ($P = 0.7$) were found to be associated with the presence of epilepsy. Nevertheless, epileptic patients presented single calcified or colloidal parasites (4 and 7 patients respectively), while non-epileptic cases presented only calcified cysts (Table 1). The association between epilepsy and the presence of colloidal parasites was significant ($P < 0.0001$).

3.3 Systemic specific immune-inflammatory response is lower in epileptic than in non-epileptic cases

Higher levels of *T. solium* antigen lymphocyte proliferative response were found in non-epileptic cases compared to the epileptic ones ($P = 0.047$) as well when stimulated with ConA. Non-epileptic cases also exhibited significantly higher levels of IL5, IL10, IL13, IFNγ and TNFα in the SN of *T. solium* specifically-stimulated PBMCs, when compared to epileptic patients (Table 2).

3.4 Cytokines in CSF from epileptic patients

Epileptic patients presented non-inflammatory CSF according to their cell count (N=11; range 0-7, mean 3 ± 2.6 cell/mm^3). None of the IgG subclasses or cytokines were increased (data not shown) with respect to the expected normal values reported elsewhere [16].

3.5 Comparison of the immune-inflammatory profile between patients bearing a colloidal versus a calcified cysticercus

Comparing the measured immunological parameters between patients with colloidal versus calcified parasites, significantly higher SN levels of IL5, IL10, IFNγ, and TNFα; serum IgG4, and higher specific proliferation index were found in patients with calcified parasites compared with those patients with colloidal cysticerci (Table 3).

4. Discussion

This study was performed in epileptic and in non-epileptic NC patients affected by a single damaged cysticercus located in the cerebral sulci or in the parenchyma of the brain.

Parenchymal NC is the most frequent form of the disease in the open population [7, 8, 12], and also in the pediatric patients [11, 17]. Although this particular location has a benign prognosis compared with those cases in which parasites are located in the ventricles or in the subarachnoid space at the base of the brain [18], it can be accompanied by repeated seizures affecting the patients' quality of life [19, 20].

This study points to the relevance of an effective immune-inflammatory response in patients with calcified single parenchymal cysticerci without later seizure onset. Indeed, an increased specific proliferative response, significantly higher levels of TH1/TH2 cytokines (IFNγ, TNFα / IL5, IL13) and the anti-inflammatory cytokine IL10, were observed in non-epileptic compared with epileptic patients (Table 2). No detectable evidences of specific immunity related with the parasite destruction were found in patients with colloidal parasites that presented epilepsy. Thus, it is likely that in non-epileptic patients, a proper parasite antigen presentation probably promote adequate lymphocyte recruitment into the brain parenchyma that led to cysticercus destruction without any subsequent symptomatology. Similar differences in the immunological profile were observed between patients with calcified versus colloidal parasites (Table 3). In this regard, it has also been reported seizures related with colloidal or early damaged *Taenia crassiceps* cysticerci [21]. The involvement of pro-inflammatory cytokines as a seizure mediator was lately discarded [22].

In a previous study, comparing a wider group of patients harboring viable or degenerating parasites in different locations, it was found that symptomatic patients, including epileptic and patients with intracranial hypertension or focal deficits, showed a depressed peripheral cellular immune response [23]. In the cited work, symptomatic patients presented mainly vesicular cysticerci. In the present study, even though all symptomatic patients exhibited damaged parasites, it is possible that the release of parasitic antigens persists when the parasite is dying, promoting the depression of the specific immunity and the local inflammation that accompany epileptic forms of NC. It is noteworthy that the results of a meta-analysis showed that cysticidal treatment of colloidal parasites is significantly associated with a reduction of seizure recurrence [24], pointing to the relevance of a complete parasite destruction in controlling epilepsy.

The possibility that a cysticercus promotes epilepsy due to its localization in the cerebral parenchyma was also evaluated. It is well known that temporal lobe structures, particularly hippocampus, amygdale and piriform cortex, are more susceptible to epileptogenesis-triggering brain injuries [25]. Additionally, it has been reported that the left hemisphere presents an enhanced vulnerability to epileptogenesis [26]. Despite all these considerations, no significant differences, neither in lobe localization or lateralization between epileptic and non-epileptic cases, were found.

In this study, epilepsy was more frequent in younger patients, a finding in agreement with the higher frequency of epilepsy in the pediatric population than among adults [27-29]. The difference in gender found in our study should be taken with caution, since most of non-epileptic patients included in this study came from an epidemiological study in which more women than men participated [8].

In conclusion, the low specific immunity elicited in some individuals carrying a single cysticercus in the parenchyma is associated to the presence of seizures. An improvement in the specific immunity to promote cysticercal calcification to control seizure occurrence in these patients will be a task to follow in the next future.

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Table 1. Clinical and radiological description of 33 NC cases

	Epileptic N = 11	Non-epileptic N = 22	*P*
Sex (M/F)	8 / 3	5 / 17	**0.006**
Age (Mean ± SD)	20.3 [a] ±13.7 [b]	43±21.4	**0.016**
Degeneration state			
Calcified/Colloidal	4/11	22/0	**0.0001**
Localization			
Frontal	4 (40%)	8 (38.1%)	1
Parietal	5 (50%)	7 (33.3%)	0.7
Occipital	1 (10%)	2 (9.5%)	0.28
Temporal	0 (0%)	1 (4.8%)	1
Basal Ganglia	0 (0%)	3 (14.3%)	0.53
Right/Left Hemisphere	4 / 10	6 / 11	0.7

Features from different clinical variables were compared by Chi-square test.

[a]Mean value. [b]Standard deviation.

Table 2. Systemic immune-inflammatory response in epileptic and non-epileptic individuals with a single parenchymal damaged cysticercus

	Epileptic *N* = 11	Non-Epileptic *N* = 22	*P*
IL1β	791.5[c] (1590.4)[d]	81.6 (92.9)	0.393
IL5	9.4 (23.6)	**329.2 (528.5)**	**0.0001**
IL6	864.7 (1863.5)	68.3 (85.9)	0.143
IL10	13.0 (58.5)	**201.2 (247.6)**	**0.001**
IL13	97.1 (223.1)	**280.8 (355.6)**	**0.045**
IFNγ	9.4 (9.4)	**318.5 (773.3)**	**0.0001**
TNFα	29 (92.5)	**723.7 (770.4)**	**0.0001**
IgG1	0.1 (0.40)	0.1 (0.1)	0.76
IgG2	0.07 (3.61)	0.08 (0.1)	0.98
IgG3	0.009 (0.15)	0.07 (0.09)	0.32
IgG4	0.013 (2.04)	0.07 (0.08)	0.063
SI TsAg[a]	1.9 (39.7)	**20.3 (35.7)**	**0.047**
SI ConA[b]	0.8 (48.2)	**26.2 (83.2)**	**0.035**

Immune-inflammatory features of supernatant between epileptic and non-epileptic cases were compared by the non-parametric Mann-Whitney U-test. Cytokine levels in SN of specifically-stimulated PBMCs are measured in pg/ml, IgG subclasses in optical densities. Bold values indicate levels/titers significantly higher. [a]SI TsAg and [b]SI ConA (cpm-antigen-stimulated cells/cpm-not-stimulated cells) is the stimulation index of the lymphoproliferative response induced by *Taenia solium* whole antigen (TsAg) or ConA, respectively. [c]Median of the cytokine or the antibody levels. [d]75% upper percentile values.

Table 3. Systemic immune-inflammatory response in individuals with one single parenchymal damaged cysticercus

	Colloidal *N* = 7	Calcified *N* = 26	*P*
IL1β	1059[c] (3355)[d]	81.6 (95.8)	0.19
IL5	9.4 (56)	**300.5 (454.7)**	**0.004**
IL6	1832.9 (1987.4)	68.3 (88.3)	0.08
IL10	9.4 (66.1)	**193.4 (237.7)**	**0.058**
IL13	63.7 (303.4)	217.2 (350.8)	0.13
IFNγ	9.4 (9.4)	**267.2 (697.5)**	**0.001**
TNFα	51.1 (236.3)	**697 (762.9)**	**0.016**
IgG1	0.13 (1.3)	0.1 (0.1)	0.61
IgG2	0.07 (2)	0.08 (0.1)	0.91
IgG3	0.001 (0.2)	0.07 (0.09)	0.24
IgG4	0.006 (0.8)	**0.07 (0.09)**	**0.02**
SI TsAg[a]	1.1 (20.3)	**20.3 (38.1)**	**0.02**
SI ConA[b]	0.8 (62.7)	25.8 (72)	0.06

Immune-inflammatory features between patients bearing one colloidal/degenerating or calcified cysticercus were compared by the non-parametric Mann-Whitney U-test. Cytokine levels are measured in pg/ml, IgG subclasses in optical densities. Bold values indicate levels/titers significantly higher. [a]SI TsAg and [b]SI ConA is the stimulation index of the

lymphoproliferative response induced by *Taenia solium* whole antigen (TsAg) or ConA, respectively. [c]Median of the cytokine or the antibody levels. [d]75% upper percentile values.

DISCUSIÓN

Este trabajo es el primer estudio realizado analizando la relación entre la respuesta local y sistémica así como su relación con la severidad de la enfermedad en el mismo conjunto de pacientes con NC.

Diversos reportes sobre el estudio de la respuesta inmune en las distintas formas de la NC se reportan diferencias entre las observaciones realizadas, probablemente como consecuencia de diferencias en la clasificación de los pacientes incluidos en los diferentes estudios. En este trabajo se clasificaron a los pacientes de acuerdo a la celularidad del LCR, la presencia de hipertensión intracraneal (HIC), del estadio de los parásitos y su localización, características determinantes en la severidad y la inflamación asociada a la enfermedad.

Se observó que los eventos inmuno-inflamatorios que ocurren en el SNC como consecuencia de la presencia del parásito en este compartimiento, promueven la producción de citocinas periféricas, e influyen en la capacidad proliferativa de las células mononucleares. Los pacientes inflamatorios/severos caracterizados por un aumento en la celularidad del LCR (igual o mayor a 10 cel/mm^3), manifestación de HIC y múltiples parásitos vesiculares localizados principalmente en SaBa/intraventriculares, presentaron una respuesta a nivel local determinada por el aumento de niveles de IL1β, IL5, IL6 e IL10, en comparación con los pacientes no severos/no inflamatorios. A nivel sistémico se observó una supresión de la proliferación linfocitaria que se asoció con el aumento del número de células en el LCR y con el aumento de TNFα en el sobrenadante de las células proliferadas específicamente. Se encontraron correlaciones entre los niveles de las citocinas detectadas en el LCR y en los respectivos sobrenadantes de las células proliferadas. Aunque modestos, estos hallazgos periféricos podrían ser relevantes para el manejo de los pacientes con formas severas de la NC, considerando que a partir de la

respuesta inmuno-inflamatoria periférica se puede saber lo que está ocurriendo a nivel local, sin necesidad de realizar procedimientos invasivos como la punción lumbar.

Por otra parte en los pacientes no severos/no inflamatorios caracterizados por presentar LCR no inflamatorio (menos de 10 cel/mm^3), sin HIC y con parásitos únicos localizados principalmente en SaSu/parénquima en estadio coloidal o calcificado, no se observaron niveles aumentados de las citocinas estudiadas ni a nivel local, ni a nivel sistémico. Es factible que la respuesta del hospedero en este compartimiento sea capaz de matar al parásito a través de un fenómeno inmuno-inflamatorio localizado no detectable a nivel sistémico, ni en el LCR.

Aunque en muchos casos, las formas no-inflamatorias/no-severas caracterizadas por NC única parenquimatosa cursa sin sintomatología, la epilepsia es el principal síntoma asociado a estas formas de la enfermedad (Singh et al., 2010; Sáenz et al, 2006). La presencia de parásitos únicos en el parénquima es la forma más común en la población abierta y en comunidades rurales de los países con cisticercosis endémica, y son hallazgos frecuentes en estudios de autopsias (Villagrán y Olvera, 1988). Se desconocen las causas que conducen a presentar o no epilepsia, sin embargo se sabe que la epileptogénesis cuenta con un componente inmunológico (Friedman y Dingledine, 2011). En este trabajo reportamos evidencias que permiten sostener que la respuesta inmuno-inflamatoria podría estar involucrada en la epilepsia asociada a las formas sintomáticas no-inflamatorias/no-severas de la NC. Los pacientes epilépticos con parásitos únicos parenquimatosos presentaron un perfil inmunológico diferente de aquellos que no presentaron epilepsia. Asimismo, en esta serie de pacientes se observó además que ni la localización ni la lateralización de los parásitos resultaron en la ocurrencia de epilepsia.

Los casos no epilépticos con parásitos únicos calcificados parenquimatosos presentaron una respuesta inmuno-inflamatoria (mayores niveles de IL5, IL10, IFNγ y

TNFα en el sobrenadante de células estimuladas específicamente e IgG4 en plasma) y proliferación linfocitaria específica activa, mientras que los casos epilépticos presentaron niveles bajos de las citocinas e inmunoglobulinas estudiadas. Estos resultados sugieren que la epilepsia en pacientes no-inflamatorios/no-severos se asocia a la incapacidad del individuo de generar una respuesta inmune efectiva en contra del parásito, lo cual se asocia a que estos pacientes hayan presentado principalmente parásitos coloidales, los cuales probablemente estén muriendo por cuestiones biológicas propias del parásito y no por acción de la respuesta inmune del hospedero.

Comparando los niveles de citocinas y de proliferación linfocitaria entre los pacientes de ambos estudios con parásitos localizados en SaSu/parénquima versus los pacientes con parásitos localizados en SaBa/intraventrícular, se observó que estos últimos presentaron mayores niveles de IL5, IL6 e IL10 en LCR, además de supresión en la proliferación linfocitaria específica, características igualmente encontradas en las formas inflamatorias de la enfermedad, lo que comprueba que la localización de los parásitos es un factor importante para severidad de la enfermedad. Por otra parte, los pacientes con parásitos localizados en SaSu/parénquima presentaron mayores niveles de IL5 e IFNγ en SN, citocinas producidas principalmente por los pacientes no epilépticos. De esta manera además de la localización, otros factores tales como la exposición del hospedero, el número de parásitos y la sintomatología, participan en la respuesta inmune en contra del parásito en las formas no-inflamatorias/no-severas de la enfermedad.

Una explicación por la cual los casos parenquimatosos asintomáticos presentan una respuesta sistémica activa es porque se trata de casos provenientes de una comunidad rural con alto grado de exposición a los antígenos del parásito y muy probablemente las células de memoria son capaces de activarse con mayor eficiencia que en los casos parenquimatosos hospitalarios.

Dado que en los pacientes no-inflamatorios/no-severos se observa una respuesta local y sistémica diferente a la de los casos inflamatorios/severos, es posible que la localización del parásito este involucrada de manera importante en el transporte de antígenos a los nódulos linfáticos, así como la entrada de células efectoras al lugar de la infección, presentándose de manera diferente la acción de la respuesta inmuno-inflamatoria en torno al parásito en el parénquima y el espacio subaracnoideo de los surcos, que en los ventrículos y el espacio subaracnoideo de la base. Ante esta premisa, se ha observado que existe expresión diferencial de moléculas de adhesión y quimiocinas en las células epiteliales de la barrera hematoencefálica (BHE) y la barrera de líquido cefalorraquídeo (BLCR), lo que podría estar condicionando la entrada de distintos tipos celulares a las diferentes regiones, y por lo tanto se desataría un tipo de respuesta diferente en cada compartimiento. Por ejemplo se ha observado que las células T de memoria interactúan con la P-selectina de las células epiteliales de los plexos coroideos y que al entrar al LCR son capaces de diseminarse a los espacios meníngeos y perivasculares (Kivisakk et al., 2003), sin embargo también hay estudios que sugieren que las células T entran directamente a través de los vasos meníngeos mediante la interacción con las moléculas de adhesión (Bartholomäus et al., 2009). También se ha observado que la expresión de CCL20 en plexos coroideos es importante en la regulación del reclutamiento de células TH17 y de células T reguladoras, a través de la interacción con la CCR6, la cual se expresa en distintas poblaciones celulares bajo diferentes condiciones de estimulo (Jordan, 2008; Yamazaki et al., 2008; Reboldi et al., 2009). De esta forma, la interacción entre las células de la respuesta inmune y las células residentes del SNC es determinante para el tipo de respuesta que se desataría en el sitio de infección.

La respuesta inmunológica en el grupo de pacientes inflamatorios/severos se caracterizó por la presencia de niveles elevados de IL1β, IL5, IL6 e IL10 en el LCR y también se observó que la celularidad en el líquido cefalorraquídeo correlacionaba positivamente con los niveles de estas citocinas. Resulta de interés dilucidar el papel de cada una de estas citocinas dentro del fenómeno inflamatorio producido. Dado que estas citocinas se encuentran en el LCR inflamatorio, posiblemente están siendo producidas por linfocitos proinflamatorios o en el caso de la IL10, que podrían ser secretada por células T reguladoras. Sin embargo, también es factible que estas citocinas puedan ser producidas por células residentes del SNC tales como la microglia activada, astrocitos y neuronas (Benveniste, 1997) o por las células epiteliales de los plexos coroideos (Schwerk et al., 2011). Es por eso que resulta de interés el poder definir la fuente de producción de estas citocinas, de tal suerte que se pudiera controlar de manera terapéutica el proceso inflamatorio en las formas severas de la enfermedad, promoviendo la producción de citocinas anti-inflamatorias y suprimiendo la producción de aquellas pro-inflamatorias en los distintos tipos celulares involucrados.

La presencia de IL1β en el LCR podría estar promoviendo el reclutamiento de leucocitos en el SNC. Se ha observado que esta citocina induce la expresión de la proteína quimioatrayente de monocitos 1 (MCP-1) por parte de los astrocitos, además de la expresión de molécula de adhesión intercelular 1 (ICAM-1) en las células endoteliales (Proescholdt et al., 2003; Shaftel et al., 2007). La IL5 e IL6 pudieran estar participando en el reclutamiento de eosinófilos, además de la estimulación de linfocitos B y de otras células efectoras (Ghaffar et al., 1998; Karlen et al., 1998; Lotz et al., 1998). Es de destacarse la acrecentada proporción de IL10 en comparación con las citocinas proinflamatorias IFNγ, TNFα e IL12 en el LCR, que sugiere la importante participación de la IL10 en la regulación del fenómeno inflamatorio ya que se sabe que las células

residentes del SNC son importantes productoras de citocinas reguladoras tales como la IL10, cuya principal función es suprimir los efectos detrimentales en el cerebro con el fin de mantener la homeostasis (Aloisi et al., 2001; Strle et al., 2001; Vitkovic et al., 2001; Tiemessen et al., 2003). En este sentido, es de interés estudiar el origen de la IL10 y los mecanismos que promueven su producción para la regulación del fenómeno inflamatorio local.

Tanto en estudios previos (Chavarria et al., 2006) como en el presente, se encontró que la supresión de la respuesta proliferativa de células estimuladas específicamente, se asocia con la severidad de la enfermedad. Los casos con NC no-severa/no-inflamatoria y no epilépticos, presentaron niveles incrementados de proliferación celular específica, mientras que los pacientes sintomáticos inflamatorios/severos, presentaron niveles suprimidos de proliferación. En los pacientes inflamatorios/severos se observó que la supresión en la proliferación linfocitaria específica se asocia con el aumento de niveles de TNFα en los sobrenadantes de las células de sangre periférica con estimulo antigénico. Se sabe que la apoptosis es promovida por esta citocina proinflamatoria (Liu, 2005; Xu and Shi, 2007) y es por eso que se exploró la posibilidad de la presencia de apoptosis en los pacientes severos con proliferación linfocitaria suprimida y niveles elevados de TNFα en el sobrenadante, sin embargo no se detectó sobre-expresión de RNA mensajero de TNFR1, caspasa 3, ni caspasa 8 en células periféricas estimuladas específicamente. A nivel local, la inducción de apoptosis en el SNC es un mecanismo muy importante para las células residentes para mantener la homeostasis, de tal manera que la microglia y las neuronas expresan moléculas de la superfamilia del TNF que participan en la vía de señalización de muerte (Choi and Beneviste, 2004; Kraft et al., 2009), es por esto que no se puede descartar de manera contundente que se esté induciendo apoptosis a nivel local

y que esté siendo observado a nivel periférico a través de una supresión en la proliferación linfocitaria específica.

La supresión de proliferación linfocitaria periférica en los pacientes severos, pudiera ser también consecuencia de un fenómeno de anergia o tolerancia periférica consecuencia de la estimulación antigénica como se ha reportado en otras infecciones helmínticas como filariasis y esquistosomiasis (Smith et al., 2004; Babu et al., 2006). Se ha reportado también que en este evento podrían estar participando las células T reguladoras, posiblemente a través de la producción local de IL10 y la inducción de anergia en el SNC (Goverman, 2009). En la esclerosis múltiple se ha estudiado la importancia de las células T reguladoras en la supresión de las células TH1 y TH17 y se ha reportado que las T reguladoras son capaces de migrar al SNC sin ser detectadas en la periferia (Fritzsching et al., 2011). De esta forma, resulta de interés estudiar la presencia de las células T reguladoras a nivel local y a nivel sistémico y la manera en la que interactúan con las células residentes del SNC, así como el mecanismo por el cual pudieran ser capaces de inducir anergia ante la presencia de parásitos en el SNC.

En conclusión los pacientes con NC parenquimatosa presentan una respuesta sistémica activa caracterizada por la presencia de citocinas TH1/TH2 y una aumentada proliferación linfocitaria específica, mientras que los pacientes con NC severa presentan una respuesta de tipo inflamatorio en el LCR, la cual puede ser identificada a nivel sistémico por la supresión de la proliferación linfocitaria específica y que es regulada al menos por la presencia de IL10 a nivel local.

A partir de los resultados, resulta de interés estudiar la posible relevancia de los mecanismos por los cuales las células de la respuesta inmune pudieran atravesar la BHE y la BLCR, así como la posible interacción entre las células residentes de los distintos compartimentos del SNC con los componentes de la respuesta inmune que pudieran

llegar al sitio de la infección. De igual manera, dado que la supresión de la capacidad proliferativa de los linfocitos es un factor que se observó involucrado en la severidad de la enfermedad, el estudio de los factores que pudieran reactivar a los linfocitos para que logren proliferar nuevamente, es una alternativa terapéutica para que los pacientes con NC severa sean capaces de resolver la enfermedad de manera satisfactoria.

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ANEXOS

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Impact of *Taenia solium* neurocysticercosis upon endocrine status and its relation with immuno-inflammatory parameters

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ABSTRACT

Neurocysticercosis (NC) is a parasitic disease caused by the infiltration of the larval stage of *Taenia solium* in the central nervous system. Clinical presentations are heterogeneous and particularly depend, on the age and gender of the host. We designed a clinical study to evaluate the hormonal changes associated with neurocysticercosis and the relationships between disease heterogeneity, endocrine and immunological status. A total of 50 patients and 22 healthy subjects were included. A precise clinical and radiological description of disease for each patient was recorded. A broad hormonal profile was assessed for each participant and, in a sub-group of patients, immunological features were also evaluated. Compared with controls, all patients had lower dehydroepiandrosterone (DHEA) concentration; male patients also had lower concentrations of 17β-estradiol and higher concentrations of luteinising hormone (LH). In the clinically severe patients, lower concentrations of progesterone and androstenedione were found in women. Higher concentrations of follicle stimulating hormone (FSH) and lower concentrations of testosterone were found in men when compared with the less clinically severe patients. Significant correlations were found between estradiol and IL-10 in male patients, and between dehydroepiandrosterone (DHEA) and IL-1β, and androstenedione and IL-17 in female patients. To our knowledge the present study constitutes the first demonstration that the presence of *T. solium* larvae in the central nervous system can modify the host environment by the induction of endocrine and immunological changes. These results provide a stimulating background to analyse the repercussions of these changes on the course of the disease and on patient reproductive health.

1. Introduction

Human neurocysticercosis (NC) is a parasitic disease caused by the installation of the larval stage of *Taenia solium* in the CNS. NC exhibits a clinically heterogeneous picture ranging from asymptomatic to a severe neurological syndrome (Sciutto et al., 2000; Garcia et al., 2003).

Cysticerci localization in the CNS and the intensity of the inflammatory reaction are two factors clearly related to the clinical severity of the disease (Fleury et al., 2004; Chavarría et al., 2005). Inflammatory reaction intensity is influenced by the host's sex and age; compared with men, women exhibit a higher inflammatory reaction (Del Brutto et al., 1998; Fleury et al., 2004). Young women more frequently present a severe clinical presentation caused by multiple degenerating cysticerci in the parenchyma (Rangel et al., 1987). The prevalence of damaged cysticerci is significantly higher in women than in men (Romero et al., 2007). Children present a lower NC frequency and a lower frequency of severe forms of the disease (Sáenz et al., 2006). Also, vesicular parasites are more frequent in elderly hosts (Fleury et al., 2004).

Cysticercosis prevalence can be affected by the hormonal status of the host. Castration and pregnancy in pigs increase cysticercosis prevalence (Morales et al., 2002). Once infected, cysticercosis modifies the hormonal levels of pigs with increased concentrations of testosterone and 17β-estradiol (E2) (Peña et al., 2007). Moreover, in vitro studies have shown that human chorionic gonadotropin (hCG) effectively promotes cysticercal evagination and *T. solium* tapeworm elongation (Díaz-Orea et al., 2007). The finding that

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T. solium is also able to synthesise sex steroid hormones adds complexity to the host-parasite interactions (Jiménez et al., 2006; Valdez et al., 2006; Fernández Presas et al., 2008; Romano et al., 2008).

The present study investigated whether hormones involved in reproduction and stress are affected by the presence of parasites in the human brain. Immunological changes accompanying different clinical and radiological forms of human NC were also explored.

2. Materials and methods

2.1. Patients

Fifty NC patients attending the Instituto Nacional de Neurología y Neurocirugía (INNN) in Mexico City, Mexico and 22 healthy subjects (controls) were recruited between August 2005 and October 2007. The controls were employees of the INNN who did not suffer from any diseases and had not taken any medication.

None of the NC patients had previously received any specific cysticidal treatment or anti-inflammatory drugs (corticosteroids). A venous sample was taken between 7:00 and 10:00 am from each participant. A sample of CSF was also collected from patients who required a lumbar puncture for medical reasons. Cysticercus number, stage and localisation were radiologically defined and the clinical manifestations of all patients were retrieved.

Patients were clinically classified regarding the presence or absence of intracranial hypertension (headache, nausea or vomit and papilledema upon fundoscopic examination), one of the most severe clinical NC presentations. Although other symptoms (for example uncontrolled generalised seizures or chronic meningitis) can also be clinically severe, in this study none of the patients presented such symptoms. For this study, patients with intracranial hypertension were considered as "clinically severe". Radiologically, patients were classified regarding the presence or absence of multiple vesicular parasites localised in the subarachnoid space at the base of the skull, which represents one of the most severe radiological presentations.

2.2. Ethical considerations

This study fulfilled the research regulations for human beings required by Mexican laws and International regulations as well as ethical aspects considered in the General Rules of Health for Clinical Investigation. The protocol was approved by the INNN Ethical Committee. Patients were instructed on the aims of the study and gave informed consent.

2.3. Hormonal profiles

Shortly after blood collection, serum was separated and maintained at −20 °C until required. The concentration of the following hormones was measured by radioimmunoanalysis (RIA) using ^{125}I tracers kits: testosterone (TESTO-CT2), 17β-estradiol (E2, ESTR-CTRIA), luteinising hormone (LH) (RIA-gnost hLH), follicle stimulating hormone (FSH) (RIA-gnost hFSH), prolactin (RIA-gnost PROL), cortisol (CORT-CT2), all from CIS-bio International (Gif sur Yvette, France) and dehydroepiandrosterone sulphate (DHEA-S, "coat-a-count" kit, Diagnostic Products Corp., Los Angeles, USA) which were measured in men. E2, progesterone (PROG-CTRIA, CIS-bio International), DHEA-S, LH, FSH, prolactin, androstenedione (A4) (RIAZENco Zentech, Liege Science Park, Belgium) and cortisol concentrations were measured in women. The detection limits were: E2, 8 pg/mL; progesterone, 0.05 ng/mL; cortisol, 4.6 nmol/L; A4, 0.05 ng/mL; testosterone, 0.1 nmol/L; DHEA-S, 5 ug/dL; LH 0.15 mUI/mL; FSH, 0.10 mUI/mL and prolactin, 5 uU/mL.

2.4. In vitro cytokine titration

A total of 2.5×10^6 of peripheral blood mononuclear cells (PBMC) per mL per well were stimulated with 30 μg/mL of *T. solium* antigen and plated in 12 cluster plates (Costar), and incubated at 37 °C in a 5% CO_2 humidified atmosphere. After 48 h (for IL6, IL10, TGFβ, TNFα) and 120 h (for IL1β, IL17, IFNγ), the culture supernatants (SN) were harvested and stored at -80 °C until required for cytokine quantification.

Cytokine titration in SN and CSF was determined using a commercial sandwich ELISA kit (BD Pharmingen Cytokine Sets, CA, USA; eBioscience; R&D Duo-Set, UK). Sensitivity levels were 9.4 pg/mL for all cytokines.

2.5. Statistical analysis

All data were recorded in Excel software and analysed using SPSS version 10 software. Quantitative variables were expressed as mean and S.D., or median and 25th–75th percentile values, and compared using the two-tailed Student's *t*, ANOVA, Mann–Whitney or the Kruskal–Wallis tests. Qualitative variables were expressed in percentages and compared using χ^2. Parametric or non-parametric statistics were employed according to the size of the sample and data distribution. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. General characteristics of patients and controls

Fifty patients participated in this study; 21 women (18–61 years, mean: 37.3 ± 11.8 years) and 29 men (21–65 years, mean: 41 ± 12.5 years). Twenty-two healthy non-NC subjects were also included as controls, 13 women (mean: 35.5 ± 10.1ranging from 25–52 years) and nine men (mean: 37.1 ± 9.8 ranging from 30–60 years). Age was not significantly different between patients and controls ($P = 0.33$ for men and $P = 0.67$ for women).

Eight women (six patients and two controls) were considered as potentially menopausal based on their clinical story and hormone profiles, and were not considered for the female analysis of LH, FSH, E2 and progesterone. Thereafter, for sex steroid hormone analysis the mean age of female patients was 31.2 ± 7, while the mean age of female controls was of 32.9 ± 8.8 ($P = 0.58$).

3.2. Clinical and radiological presentation of patients

As shown in Table 1, intracranial hypertension was not present in 31 of the 50 (62%) patients included in this study. Severe radiological presentation with multiple vesicular parasites located in the basal cisterns or in the ventricular space was demonstrated by 27 of the 50 (54%) NC patients. No significant differences between men and women were found regarding clinical or radiological criteria. In addition, clinically severe forms occurred at an age significantly older than the non-severe presentations (mean: 43.9 ± 14.1 versus 36.7 ± 10.3, $P = 0.04$).

3.3. Differences in hormonal status between patients and controls

As shown in Table 2, male NC patients exhibited significantly lower concentrations of E2 (mean 21 ± 10 versus 30.4 ± 8.2, $P = 0.02$) and DHEA (mean 132.4 ± 107 versus 251.6 ± 102, $P = 0.006$), and higher LH concentrations (mean 6.5 ± 11 versus 2.2 ± 2.2, $P = 0.02$) than healthy controls.

Table 1
Clinical and radiological descriptions of neurocysticercosis (NC) patients included in this study.

	Women (n = 21)	Men (n = 29)	P
Age (years)	7.3 ± 11.8[a]	41 ± 12.5	0.29
Clinical presentation			
Not severe	12 (57.1)[b]	19 (65.5)	0.57
Severe	9 (42.9)	10 (34.5)	
Radiological presentation			
Not severe	7 (33.3)	16 (55.2)	0.16
Severe	14 (66.7)	13 (44.8)	
Number of cells/mm^3 in the CSF	25.8 ± 38.6	34.9 ± 40.9	0.44

[a] Mean ± S.D.
[b] Number of patients (percentage of patients).

Table 2
Hormonal differences between neurocysticercosis (NC) patients and healthy controls.

Hormones	Men			Women		
	Patients	Controls	P	Patients	Controls	P
LH (mUI/mL)[b]	6.5 ± 11[a]	2.2 ± 2.2	**0.02**	4.6 ± 6.4	7.5 ± 6.7	0.28
FSH (mUI/mL) [b]	7.8 ± 15	4 ± 1.9	0.13	3.6 ± 2.8	3.4 ± 2.3	0.86
Prolactin (uU/mL)	370.5 ± 373	182 ± 107	0.15	407.6 ± 483	381.1 ± 372	0.87
DHEA (ug/dL)	132.4 ± 107	251.6 ± 102	**0.006**	95.4 ± 99	158.3 ± 69	**0.05**
Cortisol (nmol/L)	221.7 ± 208	257 ± 83	0.47	304.6 ± 252	432.4 ± 191	0.13
Estradiol (pg/mL) [b]	21 ± 10.5	30.4 ± 8.2	**0.02**	46.3 ± 54.2	85.7 ± 78.6	0.15
Progesterone (ng/mL)[b]	ND	ND		1.8 ± 5.7	6 ± 11.7	0.15
Testosterone (nmol/L)	16.4 ± 9.5	17.2 ± 4.9	0.78	ND	ND	
Androstenedione (ng/mL)	ND	ND		0.45 ± 1.3	1.5 ± 2.5	0.19

LH, luteinising hormone; FSH follicle stimulating hormone; ND: not determined.
Bold indicates significant differences ($P \leqslant 0.05$).
[a] Mean ± S.D.
[b] Hormones levels in pre-menopausal women only.

In women, DHEA concentrations were significantly lower in NC patients than in healthy controls (P = 0.05). No significant differences were found in the other measured hormones.

3.4. Differences in hormonal concentrations between clinically and radiologically severe and non-severe NC patients

In clinically severe male patients, significantly decreased serum testosterone concentrations (9.7 ± 6 nmol/L versus 19.6 ± 9 nmol/L, P = 0.007) and significantly increased FSH concentrations (14.5 ± 26 mUI/mL versus 4.7 ± 2 mUI/mL, P = 0.05) were observed compared with non-severe patients (Table 3). Moreover, lower E2 concentrations were detected in severe (16.3 ± 9 pg/mL) than in non-severe patients (23.4 ± 10 pg/mL) albeit non-statistically significant (P = 0.09). No significant differences in other hormonal concentrations were found between radiologically severe and non-severe male patients (Table 3).

In women, clinically severe patients exhibited significantly lower progesterone (0.07 ± 0.1 ng/mL) and A_4 concentrations (0.08 ± 0.09 ng/mL) than non-severe patients (3.5 ± 7.9 ng/mL, P = 0.03, and 0.74 ± 1.7 ng/mL respectively, P = 0.01).

Different hormone concentrations were also found when comparing healthy controls with severe and non-severe patients of both sexes, classified both clinically and radiologically. As shown in Fig. 1, male patients displayed a significant decrease in DHEA and E2 concentrations as clinical severity increased (P = 0.018 and P = 0.015 compared with controls, respectively). Increased radiological severity in men was also accompanied by a significant increase in LH (P = 0.038) and a nearly significant decrease in E2 (P = 0.07). In women, a significant decrease in progesterone concentrations was observed as clinical severity increased compared with controls (P = 0.027).

No significant correlation was found between CSF cellularity and the studied hormones regardless of patient classification according to sex or disease severity.

3.5. Relation between cytokine profile and NC presentations

As Table 4 shows, radiologically severe NC patients (multiple vesicular parasites located in basal cisterns or ventricles) presented higher IL-1β concentrations in SN (50–127 pg/mL) with respect to non-severe patients (23.4–23.4 pg/mL, P = 0.003). Radiologically severe patients also exhibited higher IL-10 concentrations in CSF (34–225 pg/mL versus 9.4–41 pg/mL; P = 0.004). No statistically significant differences in the other cytokines were found between severe and non-severe patients.

3.6. Correlations between hormones and cytokines

Correlations between the serum hormonal and cytokine concentrations in SN or CSF were assessed. A significant negative correlation between E2 and IL-10 in CSF was detected in male patients (n = 14, r = −0.53, P = 0.049). Among women, significant positive correlations were observed between DHEA and IL-1β in SN (n = 7, r = 0.77; P = 0.04) and between A4 and IL-17 in SN (n = 8, r = 0.86; P = 0.006).

No significant correlations were found between other pairwise tested hormones and cytokines.

4. Discussion

In this study, differences between the hormonal profiles (sex-steroid hormones, gonadotropins, prolactin and cortisol) that accompany NC of different clinical and radiological severity were assessed. It is relevant to examine this aspect of NC as the disease

Table 3
Differences in the hormonal levels between clinical and radiological severe and non-severe neurocysticercosis (NC) patients.

Hormones	Radiological			Clinical		
	Severe	Non-severe	*P*	Severe	Non-severe	*P*
Men						
LH (mUI/mL)	10 ± 15.8[a]	3.8 ± 2.4	0.17	9.4 ± 17.3	5 ± 5.7	0.48
FSH (mUI/mL)	11.5 ± 23	5.1 ± 2.4	0.8	14.5 ± 25.9	4.7 ± 2.4	**0.05**
Prolactin (uU/mL)	360 ± 151	379 ± 490	0.9	256 ± 172	428 ± 434	0.27
DHEA (ug/dL)	148 ± 128	121 ± 91	0.66	110.4 ± 88	143 ± 116	0.47
Cortisol (nmol/L)	220 ± 198	223 ± 221	0.98	205 ± 190	229 ± 220	0.78
Estradiol (pg/mL)	20.7 ± 13.7	21.2 ± 8	0.89	16.3 ± 9	23.4 ± 10	0.09
Testosterone (nmol/L)	14 ± 11.7	18.3 ± 7	0.25	9.7 ± 5.7	19.6 ± 9	**0.007**
Women						
LH (mUI/mL)[b]	3.6 ± 6.1	7.8 ± 7	0.11	4.6 ± 6.7	4.4 ± 6.6	1
FSH (mUI/mL)[b]	3.2 ± 2.8	5.4 ± 2	0.2	4.2 ± 2.6	3.0 ± 3.1	0.38
Prolactin (uU/mL)	360 ± 381	494 ± 664	0.6	582 ± 638	252 ± 229	0.37
DHEA (ug/dL)	104 ± 106	78.9 ± 87	0.6	92.1 ± 109	97.9 ± 96	0.92
Cortisol (nmol/L)	267 ± 250	386 ± 258	0.28	222 ± 269	379 ± 222	0.28
Estradiol (pg/mL)[b]	50.4 ± 69	36 ± 23.8	0.66	50.0 ± 79.4	43 ± 21.1	0.23
Progesterone (ng/mL)[b]	2.7 ± 7	0.1 ± 0.09	0.49	0.07 ± 0.1	3.5 ± 7.9	**0.03**
Androstenedione(ng/mL)	0.6 ± 1.5	0.1 ± 0.07	0.45	0.08 ± 0.09	0.74 ± 1.7	**0.01**

LH, luteinising hormone; FSH, follicle stimulating hormone; DHEA, Dehydroepiandrosterone.
Bold indicates significant differences ($P \leqslant 0.05$).
[a] Mean ± S.D.
[b] Hormones levels determined only in pre-menopausal women.

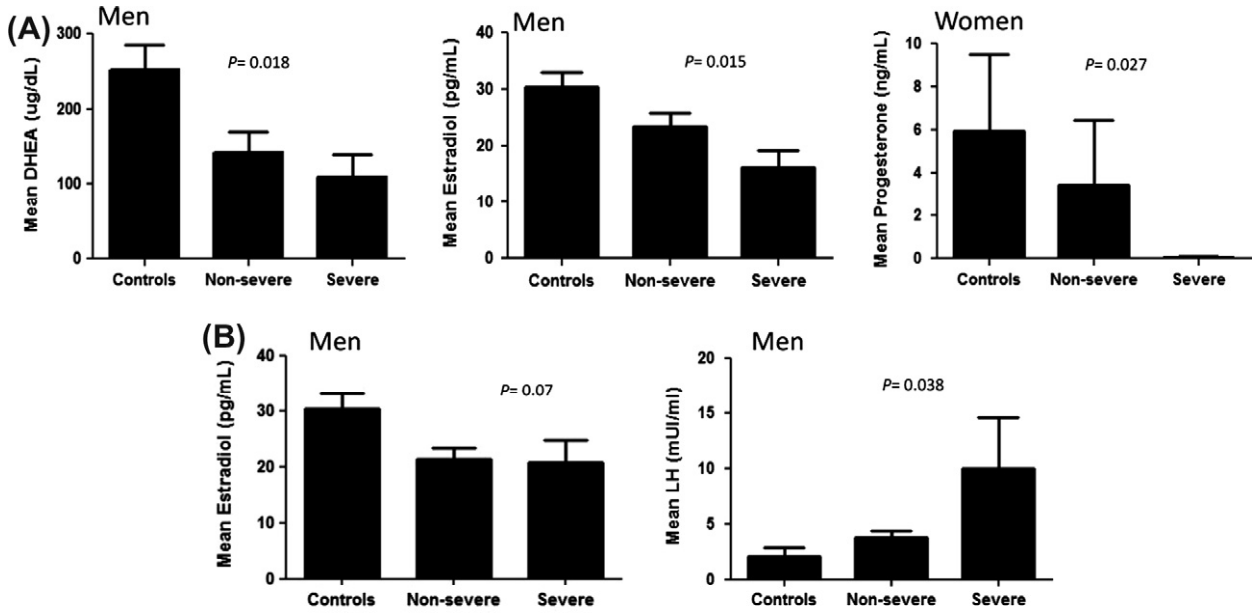

Fig. 1. Dehydroepiandrosterone (DHEA), 17β-estradiol, progesterone and luteinizing hormone (LH) concentrations in controls and in neurocysticercosis (NC) patients classified by (A) clinical and (B) radiological criteria according to disease severity. Data indicate media ± S.E.M.

affects the CNS, co-exists with clear immune-inflammatory changes, and presents gender- and age-associated differences both in clinical and radiological terms.

Alterations of the hormonal status during infections are not rare. Among the factors that may account for those are stress and infection-associated cytokine release (Papadimitriou and Priftis, 2009; Pérez et al., 2009).The stress response is mediated by the stress system that involves the CNS and peripheral organs (Chrousos, 2009). Due to the presence of inflammation, pain and other severe symptoms, neurological diseases cause chronic stress that result in the impaired function of several systems. Disruption of gonadal functions by chronic stress has been described and is the result of central and peripheral actions of hormones, proinflammatory cytokines and adipokines that inhibit the hypothalamus–pituitary–gonadal (HPG) axis at various levels (Kyrou and Tsigos, 2008; Chrousos, 2009). On the other hand, it is also known that chronic stress can have a major impact on different neurological diseases, for example neurodegenerative and mental disorders, and plays a significant role in susceptibility, progress and outcome of these diseases. The hypersecretion of glucocorticoids occurring in these diseases is probably one of the mechanisms involved (Chrousos, 2009; Sotiropoulos et al., 2011). Moreover, in infectious and neurological diseases, alterations of the hypothalamus–pituitary–adrenal (HPA) axis, the HPG axis, or a direct involvement of gonads or adrenal glands may occur.

Alterations in gonadal steroid production have been described in several parasitic infections and diverse hosts. Changes in E2 concentration have been shown in mice challenged with *Taenia crassi-*

Table 4
Cytokine profile in neurocysticercosis (NC) patients with severe and non-severe clinical and radiological presentation.

Cytokine	Radiological			Clinical		
(pg/mL)	Severe	Non-severe	*P*	Severe	Non-severe	*P*
IL-1 SN	88.5 (50–127)(9)[a]	23.4 (23.4–23.4)(*4*)	**0.003**	51.2 (31–93)(*7*)	55.9 (23–676)(*6*)	0.94
CSF	35.6 (23–49) (*14*)	23.4 (23.4–23.4) (*12*)	0.14	35.6 (23–49)(*10*)	23.4 (23–25) (*16*)	0.34
IL-6 SN	252 (56–692) (*18*)	117.8 (21–579)(*13*)	0.49	98.5 (34–429)(*13*)	379.9 (51–748) (*18*)	0.13
CSF	124 (9–243) (*14*)	14 (9–177)(*12*)	0.41	38.2 (9–184)(*9*)	92.0(9–272) (*17*)	0.44
IL-10 SN	59.6 (9–569)(*18*)	9.4 (9.4–353)(*13*)	0.39	9.4 (9.4–592) (*13*)	36.8 (9–207)(*18*)	0.77
CSF	69 (34–225) (*14*)	9.4 (9.4–41) (*13*)	**0.004**	54.3 (19–69) (*9*)	112 ± 158 (*18*)	0.67
IL-17 SN	19.7 (9–31) (*11*)	15.8 (10–25)(*4*)	1	15.7 (10–29) (*8*)	19.9 (9–26) (*7*)	0.87
CSF	35.6 (9–85) (*13*)	9.4 (9.4–9.4)(*7*)	0.22	13.3 (9–21) (*8*)	9.4 (9–13) (*12*)	0.38
IFN-γ SN	45.8 (23–78) (*9*)	167.4 (58–193)(*4*)	0.15	48.2 (39–93) (*7*)	92.8 (23–178)(*6*)	0.94
CSF	175 (23–207) *14*)	23.4 (23–146)(*12*)	0.23	181.6 (23–207)(*10*)	23.5 (23–182)(*16*)	0.45
TGF-β SN	37.6 (9–85) (*11*)	9.4 (9.4–212)(*4*)	0.18	20.5 (9–71)(*8*)	77.5 (9–280) (*7*)	0.46
CSF	9.4 (9.4–9.4) (*13*)	9.4 (9.4–9.4) (*7*)	1	9.4 (9.4–9.4)(*8*)	9.4 (9.4–9.4) (*12*)	0.68
TNF-α SN	9.4 (9.4–9.4) (*11*)	9.4 (9.4–9.4) (*4*)	0.85	9.4 (9.4–9.4) (*8*)	9.4 (9.4–9.4) (*7*)	0.71
CSF	9.4 (9.4–9.4) (*13*)	9.4 (9.4–9.4) (*7*)	0.82	9.4 (9.4–9.4) (*8*)	9.4 (9.4–9.4) (*12*)	0.77

SN, culture supernatants after specific stimulation of mononuclear cells with *Taenia solium* antigens.
Bold indicates significant differences ($P \leqslant 0.05$).
[a] Median (25–75% percentile values) (*number of samples tested*).

ceps (Larralde et al., 1995), as well as in *Plasmodium vinckei petteri* infections induced in mice (Barthelemy et al., 2003), in *Ligula intestinalis* infections induced in fish (Trubiroha et al., 2010), and in human filariasis (Mavoungou et al., 2005). Variations in testosterone concentrations have been described in infections with *Plasmodium chabaudi chabaudi* in mice, *Mycobacterium leprae* and *Mycobacterium tuberculosis* in humans (Barthelemy et al., 2004; Leal et al., 2006; Rey et al., 2007). In addition, ovarian dysfunction was reported in a woman diagnosed with NC (Choudhry S, Mejía J, Bahtiyar G, Mejía J, Sacerdote A., 2009. Endocrine Disruptor Effects of Central Nervous System Cysticercosis; Induction of Polycystic Ovarian Syndrome. Presented at the Endocrine Society 91st Annual Meeting, June 2009, Washington D.C., USA).

The data found in this study point to the involvement of the HPG axis and to a probably primary gonadal failure in NC patients. This latter alteration seems to predominate in men, who showed a decrease in E2 with an increase in LH, together with decreased testosterone and E2 levels, and the increase in FSH detected in severe patients. These alterations may be the result of an inhibitory effect of some molecules participating in the inflammatory reaction that accompanies progression of the disease (Kalyani et al., 2007). For example, significantly higher IL-1 levels in the SN of severe patients can act negatively on testicular function (Tsigos et al., 1999; Garcia et al., 2006). No significant negative correlation between IL-1 and testosterone was found in this study, but this may be due to the small number of patients (six) in which both features were measured. Also, it is possible that the lack of correlation between this cytokine and testosterone may be in part attributed to the fact that cytokines were measured in vitro, when blood immune cells are no longer in their natural environment where they are exposed to steroid concentration changes caused by the disease. On the other hand, decreased testosterone concentrations may be participating in the maintenance of the inflammatory reaction since testosterone is considered an anti-inflammatory hormone, as it reduces co-stimulatory factors expressed by antigen-presenting cells, induces the suppression of inflammatory cytokines (Klein, 2004), and increases the anti-inflammatory cytokine IL-10 (Liva and Voskuhl, 2001). This is an important result since inflammation is the most critical sign of NC severity. The findings of the present study present new prospects to manage and control the inflammatory response in NC. Decreased E2 levels found in the entire group of male patients in the presence of preserved levels of testosterone may be the consequence of diminished testosterone aromatisation to E2 by the P-450 aromatase enzyme, as aromatisation is influenced by different cytokines (Lambard et al., 2005).

In women, clear involvement of ovarian function (decrease of progesterone and A4 concentrations) was found in severe patients. Considering our results, it could not be ascertained whether NC directly affects the ovarian functions or the HPG axis. However, some findings argue in favour of an HPG axis engagement since changes in ovarian hormones did not correlate with changes in pituitary hormones. Mediators involved in the patients' inflammatory reaction, either local or systemic, may account for suppression of the HPG axis. Support for this premise comes from the correlation of A4-IL-17 found in female patients, and the raised IL-1β and IL-10 levels registered in SN and CSF, respectively, in the entire group of severe patients. On the other hand, evidence of a direct effect on ovaries is supported by the normal prolactin levels, which suggest that at least part of the pituitary gland was working properly. Additionally, the lack of significant gonadotropin differences between patients and controls may be due to the natural cyclic hormonal variations in women, which introduced a large dispersion in the results. The significant variations in progesterone levels suggest that most NC patients, particularly the severe cases, could present ovulation failure and thus probably be infertile during the active infection.

Regarding the adrenal function, significantly decreased DHEA concentrations were found in both male and female patients together with no significant changes in cortisol levels. Such findings are partly in line with data from patients with leprosy (Leal et al., 2003) and tuberculosis (Botasso et al., 2007). Serum cortisol concentrations found in patients and controls in the present study cannot be considered as basal because non-invasive methods should be employed to obtain these measurements (Romano et al., 2010). However, cortisol levels showed no differences between patients and controls, which at least discard the premise of a profound compromise of the glomerular layer of the adrenal gland. DHEA production may have been affected by cytokines released by immunocompetent cells that can act on the hypothalamus, on the pituitary or even directly on the adrenal glands. A shift in adrenal steroid synthesis, away from adrenal androgens and toward the essential cortisol pathway may occur, as enzymes involved in steroidogenesis are modulated by different cytokines (Herrmann et al., 2002). The link between cytokine and hormone synthesis is illustrated by the positive correlation between IL-1 and DHEA found in women. It is also possible that the normal levels of cortisol are related to the chronicity of the clinical distress experienced by patients (Van den Berghe, 2002). Although information on duration of symptoms before inclusion of the patients in this study was not available, the time between the onset of symptoms and hospital consultation is usually long.

One point deserving attention is the poor association between the hormonal profile and radiological criteria. Instead, results indicate that the clinical criteria of severity are more clearly related to hormonal changes. The poor correlation between clinical and radiological features is striking upon patient examination. In fact, it is common to see patients with a large parasite burden and few symptoms, and patients with a small parasite load but with significant clinical distress when critical cerebral areas are affected. This result could indicate that the ailment caused by the neurological affliction may be more important than the parasitosis itself in the genesis of hormonal alterations.

It is also interesting to note that the most salient differences were recorded in men. Data obtained on female menstrual phases were not consistent enough. However, the clear differences in progesterone concentrations found in the study strongly suggest that the reproductive cycle was altered.

To the best of our knowledge, the present study constitutes the first known demonstration of immuno-endocrine alterations in NC patients. Results of this study show the complex immune-endocrinological relationships that underlie NC pathogenesis, and provide a stimulating background to analyse the repercussion of these changes on the course of the disease and in patient reproductive health.

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Regulatory T Cells in Central Nervous System: in Health and Disease

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Abstract: Regulatory T cells participate in several immune responses including autoimmune reactions inducing self-tolerance, tumor immunity, transplantation tolerance and microbial infection. Nevertheless, regulatory T cells actions seem to be different when they are in the central nervous system (CNS), since they interact with resident cells of the CNS, according to the particular conditions elicited in this compartment. This review focuses on the role of regulatory T cells in health, autoimmune and other CNS diseases, pointing out their interactions with resident CNS cells.

Keywords: Brain tumor, central nervous system, cerebral vascular diseases, experimental autoimmune encephalomyelitis, multiple sclerosis, neuroinflammation, regulatory T cells.

INTRODUCTION

Central nervous system (CNS) was initially considered a privileged immune place because of the absence of an immune response in this compartment; this concept was sustained by four CNS characteristics: 1) the presence of the restrictive blood-brain barrier, 2) the graft acceptance, 3) the low level expression of major histocompatibility complex class II (MHCII) and adhesion molecules, and 4) the lack of conventional lymphatic drainage and thus a reduced monocytes and lymphocytes circulation [1, 2]. However, this concept was very restrictive and recently CNS has been proposed as a site of selective and modified immune reactivity [3]. It is becoming clear that immune response actively occurs in CNS and there are several evidences of the interaction between cells and molecules of the immune system and the resident cells of CNS, such as neurons, microglia, oligodendrocytes, astrocytes and endothelial cells. These cells can produce inflammatory mediators, induced in response to pathological conditions like tissue injury or infection and to neurotrophic factors, in order to promote brain protection and prevent further damage produced by the inflammatory response [4-6]. An impairment of immunological homeostasis in CNS microenviroment may have important consequences because of the vast metabolic activity of this compartment. Thus, the study of regulatory mechanisms of inflammation in the CNS is of great relevance, as they can exert protective or destructive effects depending on the inflammatory mediators' induced and released by the resident cells and the immune cell populations, including the different subsets of T helper cells. For example, Th1 and Th17 cells principally produce inflammatory cytokines that keep an active immune response and could produce local damage; albeit Th2 and regulatory T cells can produce anti-inflammatory cytokines, which mainly participate in CNS protection through the regulation of the inflammatory response (Table **1**). Recently, several studies have shown the role of regulatory T cells (Treg) in immune responses elicited in the CNS, particularly in autoimmune diseases, cancer, vascular diseases, infections and brain plasticity. This review examines the role of Treg cells in different CNS alterations, emphasizing on their interactions with resident CNS cells and their regulatory mechanisms.

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REGULATORY T CELLS

Suppressor T cells were first proposed to exist in mice in the early 1970s [7, 8] and were thought to cause suppression by secreting antigen-specific factors. This hypothesis could not be proved and the study of these cells was forgotten until the mid-90´s when Sakaguchi and colleagues described a suppressor CD4+ T-cell population crucial in the control of autoreactive T cells *in vivo*, characterized by the constitutive expression of the activation marker the α-chain of interleukin-2 receptor (CD25) [9]. Since then, several groups have shown that CD4+CD25+ T cells exert hyporesponsive and suppressive functions [10-16]. The term "suppressor T cell" was replaced by the term "regulatory T cell" since Treg cells might both enhance or suppress immune responses [17]. Treg cells are a heterogeneous cell population, which represent 5–10% of the CD4+ T lymphocytes in healthy adult mice and humans [18].

Until now different types of Treg cells have been described. They can be classified in two main groups: thymus-derived Treg cells known as natural occurring Tregs and those induced outside of the thymus (Table **2**).

Natural Occurring Tregs

Thymus-derived regulatory T cells, known as naturally occurring CD4+CD25+ Treg cells, arise early in development in this organ. They constitutively express the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), a negative regulatory factor critical for its induction and function [19, 20], the glucocorticoid-induced TNF receptor (GITR), a protein which engages with their development, survival, and

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Table 1. Main Effects on the CNS of Cytokines Produced by Regulatory T Cells

Cytokine	Target Cell	Effects
IL-10	Neuron	1) Trophic support and survival after neurotoxic effects of glutamate *in vitro* [139]. 2) Increases the expression of Bcl-2 and Bcl-xL, blocks cytochrome c release and caspase cleavage under stress conditions [140]. 3) Neuroprotective effects in spinal cord injury [140]. 4) Neuroprotective effect on the initiation of "ictal" epileptiform discharges [141]. 5) Regulates gene expression in cells of the Hypothalamic-Pituitary-Adrenal axis [142, 143]. 6) Promotes survival by blocking the effects of pro-apoptotic cytokines and by promoting expression of cell survival signals [144]. 7) Increases survival of both cortical and cerebellar granule neurons [145, 146].
	Oligodendrocyte	1) Promotes endogenous remyelination in EAE [147]. 2) Promotes survival by blocking the effects of pro-apoptotic cytokines and by promoting expression of cell survival signals [144, 148].
	T helper cells	1) Reduces Th1 pro-inflammatory cytokine production [144, 149]. 2) Suppresses cytokine receptor expression [144]. 3) Inhibits receptor activation [144]. 4) Induces anergy in brain-infiltrating T cells by inhibiting cell signaling through the costimulatory CD28-CD80/86 pathway [144].
	Microglia, Astrocytes,	1) Down regulation of MHC class II expression and inhibition of the antigen-presentation capacity [149]. 2) Promotes survival by blocking the effects of pro-apoptotic cytokines and by promoting expression of cell survival signals [144, 150, 151]. 3) Affects production of inflammatory mediators in LPS-activated co-cultures of microglial and astro-glial cells [152].
TGF-β	Neuron	1) Participates in functional neuronal connectivity [153]. 2) Anti-apoptotic effects by regulating the expression and ratio of apoptotic (Bad) and anti-apoptotic proteins (Bcl-2, Bcl-x1) [154]. 3) Neuroprotective activity against N-methyl-D-aspartate receptor-mediated excitotoxicity [155]. 4) Protects from cell death involving phosphorylation of MKK4, JNK, c-Jun(Ser63), and enhancement of AP-1 binding [156]. 5) Neuroprotective activity against ischemia-induced neuronal death [157].
	Microglia	1) Reversion of activated state [158]. 2) Affects the production of inflammatory mediators in LPS-activated co-cultures of microglial and astroglial cells [152].
	Astrocytes	1) Recovery of the astroglial membrane resting potential [158]. 2) Affects the production of inflammatory mediators in LPS-activated co-cultures of microglial and astroglial cells [152].
	T cells	1) Suppresses TNFα and IFN-γ production [159]. 2) Suppresses cytotoxic T cell response [159]. 3) Downregulatory effects on T cell proliferation [160].

activation [21, 22] and the costimulatory molecule CD28, needed for the generation, maintenance and survival of CD4+CD25+ T cells [23, 24].

They also express the forkhead/winged helix transcription factor Foxp3, necessary for Treg development, as it has been described to suppress transcription from nuclear factor of activated T cells and nuclear factor κB response elements [25-28]. FoxP3 also participates in the transcriptional suppression of key T-cell cytokine genes, including those encoding IL-2, IL-4, and IFN-γ [29, 30]. Others markers reported in human naturally occurring CD4+CD25+ Treg cells are the chemokine receptors CCR4 and CCR8 [31], the expression of E3 ubiquitin ligase, GRAIL, related with T cell regulatory phenotype [32] and recently a correlation has been described between the expression of Foxp3 and CD25 and low levels or absence of CD127, the α chain of the IL-7 receptor [33].

They are anergic to antigenic stimulation, and also, they are characteristic for maintaining the immunological tolerance inhibiting proliferation and cytokine production of autoreactive T cells, as well as of CD8+ T cells and established Th1 and Th2 cells [34-36].

Naturally occurring CD4+CD25+ Treg cells may produce TGF-β and IL-10 under certain *in vivo* and *in vitro* conditions and also they can mediate suppression by cell-to-cell contact via their TCR, acting in a cytokine independent

Table 2. Main Features of the Different Regulatory T Cells Populations

Cell Type	Cell Markers	Origin	Mechanism of Cell Suppression	Main Effects
Natural Occuring Treg cells	CD25(high) [47] CTLA-4 [19, 20] GITR [21, 22] CD28 [23] Foxp3 [25-28]	Thymus [9]	Autoantigen recognition via TCR [24]	Suppression of autoreactive T-cell responses [34-36]. Induction of adaptative Treg cells [41-44].
Adaptative or Induced Treg cells	CD25(-/low or +) [41, 48] Cell surface TGB-β [39, 48] CTLA4 [48] Foxp3 [41, 48]	Periphery [24] Co-stimulation of TCR and TGF-β [48]	TGF-β (++) and IL-10 production [13, 14, 38, 39]	Suppression of pathological immune responses [49-53]. Anergy induction. Suppression of T cell activation and cytokine production [49-53].
Tr1 or IL-10 Treg cells	CD25 (high) [62] CTLA-4 [62]	Periphery [55, 57]	IL-10 (++) and moderate TGF-β Production [54, 55, 61- 63] or Antigen recognition via TCR [56, 63].	Suppression of pathological immune responses [49-53]. Maintenance of immunological tolerance [62]. Suppression of T cell activation and cytokine production [49-53, 57].

way, however antigen presenting cells (APC) are not directly required for delivery of the suppressive signal [36-40]. Several studies have described the capacity of these cells to induce conventional T cells to exert regulatory functions on other CD4+ cells by a phenomenon called infectious tolerance by inducing high level production of IL-10 and/or TGF-β in other cells, acting in a contact-independent way [41-44]. Another characteristic of these cells is that they do not produce IL-2, however *in vivo* studies have demonstrated that the presence and binding of IL-2 is crucial for the differentiation and/or survival of the regulatory CD4+CD25+ T cells [45-47].

Induced T Regulatory Cells

Other types of T regulatory cells are those that acquire a functional regulatory phenotype outside of the thymus particularly at the site of inflammation. These cells have been termed induced or adaptive T regulatory cells. Some studies suggests that these cells are initially conventional T cells expressing lower or no levels of CD25 ($CD25^{low/-}$) and the expression of CD25 can be upregulated according to the conditions present in the environment, in this regard CD4+CD25-Foxp3- cells may differentiate to CD4+CD25+ Foxp3+ Treg cells by TCR costimulation besides TGF-β induction [41, 48]. In addition, Treg cells induction might be triggered by low antigen exposure, antigen presented in an inappropriate milieu due to altered TCR signal transduction, oral tolerance or in the presence of IL-2. Once induced, they could act by the same mechanisms as naturally occurring Treg cells [49-53].

Tr1 Regulatory Cells

Another subset of regulatory T cells is the Tr1 or IL-10 producing Treg cells. These are induced in response to antigenic stimulation and produce large amounts of IL-10 [54, 55], they express high levels of CD25, do not express Foxp3 and little to no IL-2 [56, 57]. T cell proliferation is inhibited by naturally occurring CD4+CD25+ Treg cells [56, 58]. Induction of IL-10 Treg cells is done by antigenic stimulation and immunosuppressive drugs *in vitro* [59, 60]. Some authors demonstrated that they act through the production of IL-10 and also TGF-β mainly locally at the site of inflammation [54, 61, 62], however, some other evidences indicate that inhibition of T cell proliferation is done by a cell contact-dependent way despite the IL-10 production [56, 63].

CNS REGULATORY T CELLS IN HEALTH

CNS Regulatory T Cells

Regulatory T cells can reach CNS in pathologic and non pathologic conditions. Since they share the expression of CD25 with conventional activated T cells [64]; it is possible that they are able to pass through the intact blood brain barrier like activated T cells. CNS is considered a site of selective and modified immune reactivity for its limited exchange of immune components and also for the local environment that interacts with immune cells and their mediators, in order to maintain homeostasis. For years, it was thought that the presence of immune components in CNS was detrimental. Recently, some observations suggests a protective role of the immune response in CNS, Treg cells play a critical task in normal conditions, like brain plasticity, and in pathological immune processes such as infections, vascular diseases, development of tumors and neurodegenerative disorders (Table **3**), as well those involving autoimmune processes like multiple sclerosis [65-67].

In normal conditions, natural occurring Treg cells can reach CNS and monitor for the presence of effector T cells. In pathological processes, it has been observed that recruit-

Table 3. Regulatory T Cells Properties in Different Diseases

Condition	Effects of Treg Cells	Consequence	Potential Therapy
Healthy brain	Immunological surveillance [161]	Possible immediate regulatory function *in situ* [162]	-
Brain plasticity	Enhancement of suppressive activity [65, 85, 86]	Limits beneficial autoimmune cell response [65, 85, 86]	Treg cell depletion
MS, EAE	Decreased cell-cell suppression activity [93-95] IL-10 production [92]	Impairment of Treg cell function leading a Th1 response [93-95]	Treg cell induction
Stroke	Suppress inflammatory milieu [112-114]	Attenuate damage [112, 113]	Treg cell induction
Brain tumors	Enhancement of suppressive activity locally and peripherally [118, 121, 122]	Avoids antitumor immunity [118]	Treg cell depletion
Infections: ECM	IL-10 and TGF-β production [131, 132] Enhancement of suppressive activity [132]	Augmentation of infected cells [127-130]	Treg cell depletion
Infections: Coronavirus	Suppression of Th1 response [133, 134]	Anti-inflammatory response minimizing collateral effects of the anti-viral immune response [134].	Treg cell induction

MS: Multiple Sclerosis, EAE: Experimental Autoimmune Encephalomyelitis, ECM: Extracerebral malaria

ment of Treg cells is regulated by expression of adhesion molecules: P-selectin (CD62P), ICAM-1 [68], CD103 (αEβ7 integrin), local chemokine production such as CCL17 and CCL22 [69], and increased levels of chemokine receptors like CCR5 [70], CCR2, CCR4 and CCR8 [69, 71]. Suppressive function of Treg cells occurs mainly locally since an increased number of these cells has been found in association with the inflammation site [72-74], but also a considerable inhibition of effector cells has been observed in secondary lymphatic organs [75]

CNS Cells Induce Regulatory T Cells

Several studies have demonstrated that resident CNS cells participate in the regulation of T-cell responses and CNS inflammation [76, 77]. It has been established that CNS cells such as microglia and astrocytes, which are thought to be the immune effector cells of the CNS, as well as neurons, participate in the induction and action of Treg cells that down modulate inflammation in the CNS. Some studies *in vitro* showed that CD4+CD25- T cells can be induced to become CD4+CD25+ Treg cells by expressing Foxp3 and cell-surface TGF-β in a cell-cell contact dependent manner and by TGF-β production [41, 48]. Recently, Liu and colleagues demonstrated the role of neurons in inducing autoimmune CD4+ T cells to become Treg cells [73]. They showed that neurons can induce proliferation of autoreactive CD4+ T cells in an antigen independent but cell-to-cell dependent way, initially by neuron TGF-β action over T cells TGF-βR which leads to triggering of signal pathways mediated by the B7-CD28 co-stimulatory interaction. In addition, TGF-β production is induced in CD4+ T cells, exerting an autocrine antiproliferative action and consequently CTLA4 and CD25 expression is detected. Converted CD4+CD25+ TGF-β+CTLA4+ T cells have a regulatory function both *in vitro* and *in vivo.* In this way, neuron-induced Treg cells are able to suppress proliferation of autoimmune CD4+ T cells and inhibit progression of experimental autoimmune encephalomyelitis (EAE). It was also reported that these converted Treg presented Foxp3 upregulation. The exact mechanism by which suppression occurs remains to be studied.

Astrocytes can also induce T cells to become Treg cells. It is known that astrocytes are in close contact with endothelial cells and are part of the BBB. They are the most abundant glial cell population and in order to help maintain homeostasis they participate actively in CNS immune responses as they are potential CNS APC, also they have the capacity to produce cytokines such as TNF-α, IL-6, IFN-β, GM-CSF and TGF-β and several chemokines (CCL2, CCL5, CCL20, CXCL8 and CXCL10) [76]. Some studies have demonstrated an astrocyte regulatory role in neuroinflammation. It has been shown that astrocytes can suppress T cell proliferation, growth and inflammatory cytokine production by the release of TGF-β2 [77, 78]. Also, astrocytes can induce Treg cells, able to suppress autoreactive T cell proliferation. Suppression mechanisms are not well established yet, but they seem to be cell-contact dependent, TGF-β and IL-10 independent [79].

Microglia cells have an important activity in CNS immune surveillance. In natural culture conditions, microglia expresses IL-10 [80]. Relatively high expression of TGF-β and IL-10 in microglia might thus be important in maintaining a normal physiological microenvironment [81, 82]. When activated, microglia express MHCII and adhesion/costimulatory molecules, it also secretes several chemokines and cytokines. The relationship between Treg cells and microglia has been poorly studied, but it has been suggested that activated microglia attracts Treg cells to the injured site with the macrophage-derived chemokine CCL22 production, which interacts with its receptor CCR4 on Treg cells [31, 83, 84].

Regulatory T Cells in Brain Plasticity

The suppressive effect of Tregs cells has been studied in mice adaptation to stress. It is known that the adaptative immune response is active in learning and memory processes [85, 86]. Recently it has been demonstrated that adaptation to acute psychological stress is impaired by the suppressive function of Treg upon effector T cells involved in plasticity processes, since mouse depleted from Treg cells, showed significant improvement to withstand psychological stress [65]. Adaptive autoimmunity through CNS autoantigen production contributes to damage repair controlled by Treg cells. In this sense, it is of great importance maintaining an immunological balance between autoimmune and Treg cells.

REGULATORY T CELLS IN CNS PATHOLOGIES

Neurodegenerative Diseases

Multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), are inflammatory, demyelinating diseases of the CNS characterized by the action of autoreactive T cells generally with a Th1 and also Th17 phenotype; this inflammatory process leads to myelin destruction [87-89]. Presence of Treg cells in CNS of EAE mice and in MS human brain correlates with disease recovery since they suppress autoreactive T cells effector functions through direct cell contact [68, 90, 91] and also through IL-10 production [92]. Although MS patients showed no difference in the number of peripheral blood CD4+CD25+ T cells when compared with healthy controls, they presented a selective decrease in the suppressive activity of CD4+CD25+ Treg cells [93-95]. Some efforts have been done in order to elucidate the reason why CD4+CD25+ and Treg cells functions are altered in MS. Some evidences suggests that the impairment in the suppressive function lies on abnormalities in Foxp3 expression in CD4+CD25+ Treg cells, also a disequilibrium has been reported in the homeostatic composition during the neogenesis of Treg which lead to a declination of Treg CD4+CD25+CD31+ in MS patients [96].

CD31 (PECAM-1) distinguishes recent thymic emigrants from peripherally expanded T cells; diminished thymic release of CD31+ Treg cells may be distressing functional properties of this T cell population in MS [96]. In MS patients, Tr1 cells functionality appears to be impaired since IL-10 level production is insufficient to suppress proliferation in stimulated cells, probably due to alterations in the IL-10R and CD46 signaling pathways [97, 98].

Effector T cells in MS and EAE comprises as well the newly Th17 subset, characterized by the production of IL17, IL17F and IL22 [99, 100]. Their importance in autoimmune disease inflammation has been demonstrated, as Th17 cells are primed before Th1 cells in EAE and the increased number of Th17 cells in CNS resulted in the exacerbation of EAE [101, 102]. Also some observations suggest that the presence of IL17 leads to an inhibition in the suppression functions of Tregs [70, 103].

In neurodegenerative pathologies such as Alzheimer (AD) and Parkinson (PD) disease, little has been explored. A higher activity but not increased numbers of Tregs has been described in the MPTP mouse model of Parkinson disease and in AD patients, induction of Tregs and attenuation of neuroinflammation was also observed [104, 105]. As neurodegeneration increases in these diseases, neurotransmitter levels drop, especially dopamine in PD and acetylcholine, glutamate and serotonin in AD [106, 107]. Some observations on the relation between neurotransmitters and regulatory T cells have been made; particularly it has been shown that dopamine can reduce the suppressive function of Treg cells on effector T cells [108]. However, additional studies need to be performed in order to elucidate its role over the immune cells that participate in these pathologies.

Vascular Diseases

Stroke is a vascular disease that initiates an inflammatory response mediated by microglia and endothelial activation, causing breakdown of the BBB and further recruitment of leukocytes [109]. The significance of Tregs has been highlighted in the ischemic brain since in patients as well as in experimental stroke, increased numbers of Tregs are found after cerebral ischemia [110, 111]. Presence of CD4+CD25+ Foxp3+ contributes to regulate of inflammatory brain damage, reducing pro-inflammatory cytokine levels, modulating lymphocyte recruitment and microglia activation [112]. Treg suppression of effector inflammatory mechanisms occurs locally in the brain ischemic zone. Recently, Liez and colleagues proposed that Treg suppression is mediated by IL-10 production [112], but other groups have observed some antigen specificity, since mucosal tolerized mice to E-selectin induce generation of E-selectin-specific Tregs [113] and after stroke, these cells are restimulated and accumulate at the site of inflammation where E-selectin is overexpresed, regulating inflammatory response, attenuating damage and promoting neuronal survival [114]. A similar phenomenon has been reported with MBP-tolerized animals which are more prone to elicit a suppressive response after stroke, suggesting that antigen-specific regulatory T cells may be restimulated earlier in the damaged site [115].

Brain Tumors

In brain tumors, the role of Treg is different; there is an increase in the number and suppressive function of Treg cells both in tumor infiltrating lymphocytes as well as in peripheral blood of patients with glioblastoma multiforme, promoting further tumor development and less patient survival [116, 117]. In the mouse experimental glioma model, it has been shown that depletion of Treg through the blockade of markers such as CD25+ or CTLA-4, results in enhanced antitumor immunity and prolonged mice survival [118- 120]. The reason why Tregs are abundant nearby tumors remains unclear, but as brain tumors have mechanisms to induce immune tolerance such as IL-10 and TGF-β production [121, 122], it is presumed that tumor cells are themselves involved in Treg cells recruitment and activation that favor tumor growth. In this sense, it seems that FoxP3 induces the expression of the regulatory enzyme heme oxygenase-1 (HO-1) in tumor infiltrating CD4+CD25+ cells, which increases during glioma progression [123, 124]. Expression of HO-1 has been shown to be elevated and is responsible for the induction of pro-inflammatory cytokines in infiltrated macrophages/microglia cells, contributing to neoangiogenesis, neoplastic outgrowth and tissue damage [125, 126]. Other

possibility is, as it is known that Treg cells can recognize tissue specific self-antigens and as many tumors express self-antigens, Treg can suppress the activation and expansion of different antitumor effector cells as they do it in autoimmune responses. In astrocytomas, tumorogenic enviroment could be promoted by astrocyte induction of Treg cells, as it has been seen in other conditions [79].

Infectious Diseases in CNS

In infections, Treg play an important role in the control of the immune responses to pathogens. In experimental cerebral malaria (ECM) it has been demonstrated that depletion of Tregs cells before and during infection, leads to a complete clearance of the parasites because of the enhanced proliferative response against parasitized red blood cells [127-130]. Immune impairment observed in malaria infection points out to be a consequence of the suppressive function of Tregs cells during early stages of infection, and it seems to be through an IL-10-dependent way [131]. In humans, increased levels of TGF-β and the presence of CD4+CD25+ Foxp3+ Treg cells are associated with higher rates of parasite growth *in vivo* [132], probably *Plasmodium* parasites promote the suppressive action of Treg cells upon Th1 effector cells.

Recently, a protective role of Treg cells has seen in the neurological viral infection: murine coronavirus-induced acute encephalitis, where tissue damage is present due to an exacerbated Th1 specific immune response. Suppressive activity of regulatory T cells participates in controlling the inflammatory phenomena, however, it is not enough to minimize the collateral effects of the exacerbated anti-viral inflammatory reaction [133, 134]. Further studies are still required to evaluate why Treg cells response is insufficient and what are their main mechanisms of action in infections.

REGULATORY T CELLS: BENEFICIAL OR NOT?

There is noteworthy evidence about the intimate relation between resident CNS cells and Treg cells. Recent studies have demonstrated the critical participation of such T cells also in functioning of the normal healthy brain, and specifically in processes of learning and memory [85, 86]. As described above, Treg cells prevent CNS autoimmune responses such as MS and EAE, but the beneficial effect of autoimmune cells is not restricted to CNS self-repair. Autoimmune T cells are also important in tumor rejection and in reducing neuronal injury after a CNS insult, directed by activation of autoimmune T cells through antigen-presenting cells at the lesion site but at the same time restricted by Treg cells mediating the development of an autoimmune disease, namely Treg cells are indispensable in maintaining the homeostasis between risk and need [135-137]. The ability of CNS resident cells to induce Treg cells and interactions between both populations depends on the microenviromental immune-inflammatory conditions. As seen on Table **3** in some cases Treg cells presence results highly beneficial, but in others pro-inflammatory T cell response is insufficient due to early Treg suppression.

In the last years, the importance of the homeostasis between autoreactive and regulatory T cells in CNS has been proposed to be relevant in protection and restoration of this compartment by a phenomenon termed "neuroprotective autoimmunity", since T cells can have a bivalent role in neuroprotection but also in neurodegeneration. A suitable homeostasis between the autoimmune and the regulatory T cells is a fundamental requirement for maintaining the balance between the need for adaptive autoimmunity as a way to induce tissue regeneration and the need to avoid any unnecessary risk of autoimmune disease [138].

Thus, beneficial role of Tregs is fundamental in healthy conditions, but under active immune-inflammatory conditions their role depends on the pathology and the specific requirements to alleviate immune response, as it has been reviewed in this paper.

CONCLUSION

Regulatory T cells in CNS in healthy conditions are important for homeostasis, and in pathological circumstances, Treg suppression is fundamental in the immune-inflammatory phenomena. But depending of the microenvironmental conditions this suppression could result beneficial or could be detrimental to the host Fig. (**1**). For example in

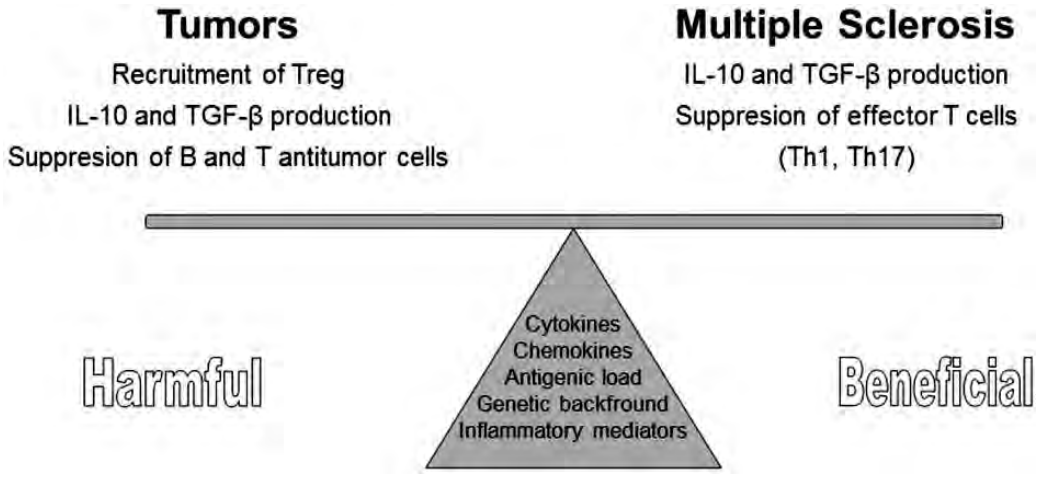

Fig. (1) Role of regulatory T cells in CNS in two different pathological circumstances, where depending of the microenvironmental conditions (listed in the triangle) Treg suppression could be beneficial in multiple sclerosis or detrimental in brain tumors.

tumor immune response, while the presence of effector T lymphocytes, including CD8+ and non-regulatory CD4+ helper T-cells may be beneficial, suppression of regulatory T cells promotes tumor survival and growth Fig. (**1**).

On the other hand, it is believed that the interaction of regulatory T cells with CNS resident cells to modulate immune response could be a component of this so called immune-privileged site, as resident CNS cells direct Treg recruitment and can even induce the switch of effector T cells to regulatory T cells Fig. (**1**).

Knowledge of Treg biology leads to a promising therapeutic use of these important cells in the control and treatment of several autoimmune and infection diseases. But it is also important to consider that this therapeutic use must be applied in a disease-specific manner, since Tregs probably have a dual effect depending on the pathology and other variables such as age, gender, genetic background, disease progression, among others.

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ABBREVIATIONS

AD	=	Alzheimer
APC	=	Antigen presenting cells
CNS	=	Central nervous system
CTLA-4	=	Cytotoxic T lymphocyte-associated antigen 4
EAE	=	Experimental autoimmune encephalomyelitis
ECM	=	Experimental cerebral malaria
GITR	=	Glucocorticoid-induced TNF receptor
MHCII	=	Major histocompatibility complex class II
MS	=	Multiple sclerosis
PD	=	Parkinson
Treg	=	Regulatory T cells

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Neurocysticercosis: detection of *Taenia solium* DNA in human cerebrospinal fluid using a semi-nested PCR based on HDP2

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Human neurocysticercosis (NC) is caused by *Taenia solium* larvae lodged in the central nervous system. This disease is usually diagnosed by radiology but the results are not always clear-cut and so immunological assays are often also used. A semi-nested PCR, based on the non-coding HDP2 sequence of *T. saginata*, has now been developed for detecting DNA from *T. solium* cysticerci and confirming NC. This PCR, which amplifies a 171-bp *T. solium* product, allowed the specific detection of just 174 attograms of *T. solium* DNA.

The efficacy of the PCR was tested using cerebrospinal fluid (CSF) from neurological patients, including 46 confirmed Mexican cases of NC and 32 patients from non-endemic Spain. Eighteen of the confirmed cases [including 10 (71%) of the 14 with vesicular extraparenchymal cysticerci and four (17%) of the 24 with damaged cysticerci] and two (33%) of the six patients with 'uncertain' diagnosis (in whom a diagnosis of NC could not be established by radiological and immunological studies) were found PCR-positive. The 36 patients known to have neurological problems other than NC were found PCR-negative.

The HDP2 PCR offers a new tool in the diagnosis of NC and in exploring the pathogenesis of this serious disease.

Neurocysticercosis (NC), caused by *Taenia solium* cysticerci, is increasingly recognised as a cause of severe neurological disease world-wide (Nicoletti *et al.*, 2005; Marconi *et al.*, 2006). Human infection with the cysticerci may be asymptomatic or cause non-specific pleomorphic manifestations such as headaches, seizures and intracranial hypertension (Fleury *et al.*, 2004). The fate of the cysticerci is also heterogeneous: some evolve towards a colloidal stage before calcifying whereas others disappear without any evident radiological signal (Martinez *et al.*, 1995). The diagnosis of NC is mainly based on the results of radiological studies, computerized axial tomography (CAT), and/or nuclear magnetic resonance (NMR) (Del Brutto *et al.*, 2001; Del Brutto, 2005; Garcia *et al.*, 2005). Such methods do not

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always give a clear-cut result, however, and so, in spite of their limited sensitivity and specificity, immunological assays (either antibody- or antigen-detection) also still often contribute to NC diagnosis (Larralde *et al.*, 1989; Wilson *et al.*, 1991; Harrison *et al.*, 1998; Bobes *et al.*, 2006).

The detection of parasite-specific DNA sequences in the cerebrospinal fluid (CSF) could also support NC diagnosis, in particular in cases in whom radiological and immunological procedures have failed to provide any convincing evidence. In this study, a specific method, based on a semi-nested PCR, was designed to detect the genomic DNA (gDNA) of *T. solium* cysticerci in CSF samples from neurological patients. The potential usefulness of the PCR in NC diagnosis was evaluated using test samples of CSF from NC cases and from other neurological patients from endemic and non-endemic areas.

PATIENTS AND METHODS

Patients and Samples

CSF samples were obtained by lumbar puncture from 32 Spaniards (all with neurological problems other than NC) and 56 Mexicans [46 confirmed cases of NC, six patients with an uncertain diagnosis (possibly NC), and four patients with neurological problems other than NC]. For each confirmed or suspected case of NC, attempts were made, by CAT and/or NMR, to determine the stage of the cysticerci (vesicular or damaged) and the location of the cysticerci in the central nervous system (in the subarachnoid space at the base of the brain or in the sulci, parenchymal, or intraventricular), and the treatment received at the time of the lumbar puncture was recorded.

Detection of Specific Antibodies and HP10 Antigen

The levels of anti-*T. solium* antibodies and HP10 antigen in each Mexican CSF sample were evaluated (Larralde *et al.*, 1989; Harrison *et al.*, 1998; Bobes *et al.*, 2006).

Semi-nested PCR

Each CSF sample was also tested for *Taenia*-specific DNA in a semi-nested PCR, with primers based on the oligonucleotide sequence of the HDP2 non-coding DNA originally identified in the genome of *T. saginata* (Gonzalez *et al.*, 2000; GenBank accession AJ133764).

DNA PURIFICATION AND CONTROLS

The total gDNA from each CSF sample was isolated by a single extraction with guanidinium thiocyanate acid (Casas *et al.*, 1995) and resuspended in 50 µl water that had been de-ionized and sterilized in a Milli-Q® unit (Millipore, Billerica, MA). The *T. solium* gDNA used as a positive control was extracted, using the QIAGEN genomic DNA kit (QIAGEN, Hilden, Germany), from metacestodes collected from naturally infected, Mexican pigs. This positive control and human gDNA (Sigma) were used to optimise the amount of template and the annealing temperatures in the first and second rounds of PCR.

PRIMER DESIGN

The Lasergene software package (DNASTAR, Madison, WI) was used to design two primers based on HDP2: PTs7S35F22 (5′-CTT CTC AAT TCT AGT CGC TGT GGT CAG-3′) and TSAR10 (5′-CGA GGA ATA GAT GGA TGA AGGG-3′). These primers, which allow the amplification of part of the HDP2 sequence that is specific for *T. solium* and *T. saginata*, and the PTs7S35R1 primer (5′-GGA CGA AGA ATG GAG TTG AAG GT-3′) described by Gonzalez *et al.* (2000), were then synthesised by Roche (Basel, Switzerland).

As a positive amplification control, a specific primer set based on conserved sequences of the human β-actin gene — 5′-TCA CCC ACA CAC TGT GCC CAT

CTA CGA-3′ and 5′-CAG CGG AAC CGC TCA TTG CCA ATGG-3′ — was also employed (Takenouchi *et al.*, 2003).

PCR

The DNA amplification was performed in two steps. The first PCR was carried out in 50-μl mixture containing PCR buffer (Roche), 0.5% glycerol, 200 μM of each of the deoxynucleoside triphosphates (Biotools B&M Labs, Madrid), 0.5 μM of each of four primers (PTs7S35F22, TSAR10, and the two primers for the human β-actin gene), 2.5 U Expand High Fidelity Taq DNA polymerase (Roche) and 20 μl gDNA from a CSF sample. The thermocycler used, a GeneAmp® PCR System 2700 (Applied Biosystems, Foster City, CA), was set to give 3 min at 94°C, then 35 cycles, each of 15 s at 94°C, 30 s at 56°C and 30 s at 72°C, before a final 7 min at 72°C. A *Taenia*-specific amplification product of 200 bp (the only band seen when the template was 1 ng purified *T. solium* DNA) and a human β-actin product of 306 bp (the only band seen with 1 ng human gDNA as the template) were expected with each sample positive for cysticercal DNA.

The second PCR was carried out, in fresh tubes, with the same reagents as the first PCR but in a final volume of 25 μl, the primers PTs7S35F22 (0.5 μM) and PTs7S35R1 (0.5 μM), and 2 μl DNA template from the first reaction diluted 4:1000 in DNAse-free water. For this reaction, the thermocycler was set to give 3 min at 94°C, then 35 cycles, each of 15 s at 94°C, 30 s at **63**°C and 30 s at 72°C, before a final 7 min at 72°C. A *Taenia*-specific amplification product of 171 bp was expected. Amplicons were separated by electrophoresis in 3% agarose (MS8; Pronadisa, Madrid) gels and visualized by ultra-violet trans-illumination after ethidium-bromide staining. In order to avoid contamination, sample preparation, reaction set-up, PCR amplifications, and amplicon detection were all performed in separate areas, under clean conditions.

In other runs, to determine the minimal amount of parasite DNA detectable using the semi-nested PCR assay, different known amounts (10 ng to 44 attograms) of purified *T. solium* gDNA were employed as template.

RESULTS

The semi-nested PCR assay was able to detect as little as 174 attograms of *T. solium* gDNA (data not shown).

In the first PCR, although a 306-bp band corresponding to the β-actin gene was observed in all the CSF samples, none of the test samples appeared to give the 200-bp band seen when purified *T. solium* gDNA was the template [Fig. 1(a)].

Variable amplicon patterns were observed after the second PCR: (1) bands of 171 and 150 bp; (2) just a 171-bp band; (3) just a 150-bp band; or (4) no bands [Fig. 1(b)]. Sequencing of the amplicons indicated that, although the 150-bp amplicon was of human origin (data not shown), the 171-bp (GenBank accession EF043036) was *Taenia*-specific and similar to a partial fragment of *T. saginata* HPD2 DNA (GenBank accession AJ133740). In fact, the 171-bp consensus sequence for *T. solium* HPD2, obtained using the semi-nested PCR and CSF samples from all of the confirmed NC cases, showed 85% identity with the corresponding *T. saginata* sequence, differing in just 25 nucleotides (Fig. 2). The 150-bp amplicon had homology with clone RP5-907D15 of the 20q13.2-13-33 human chromosome that contains the STX16 gene for syntaxin 16, a novel gene (LOC149773) with a CpG island complete sequence (GenBank accession AL050327).

None of the PCR based on negative-control samples showed any evidence of contamination. In addition, the 306-bp fragment of the β-actin gene produced, in the first PCR, using each of the CSF

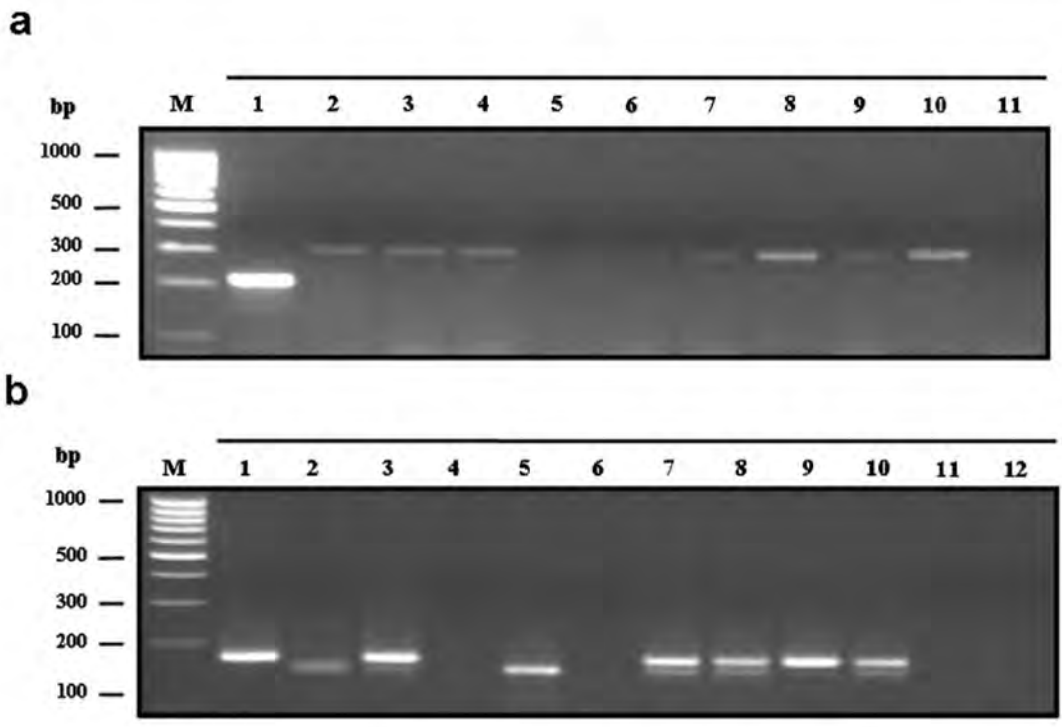

FIG. 1. The amplicons from the first PCR round (a) and the full HPD2 semi-nested PCR (b) after their separation by electrophoresis in 3%-agarose gels and ethidium-bromide staining. The lanes contained molecular-weight markers (lane M), *Taenia solium* genomic DNA (lane 1), human genomic DNA (lane 2), samples of cerebrospinal fluid from patients (lanes 3–10) and negative controls for the primary and second PCR (lanes 11 and 12, respectively). A band of <171 bp was observed in lanes 2, 3, 5, 7, 8 and 10 whereas the *Taenia*-specific band, of 171 bp, was observed in lanes 1, 3, 7, 8, 9 and 10.

samples indicated that the activity of PCR inhibitors in the samples was negligible. The specificity of the semi-nested PCR seemed to be very good (100%), as each of the CSF samples from the 36 patients known to have neurological problems other than NC yielded the 306-bp band (β-actin gene) in the first PCR and either the 150-bp band (15 samples) or no band (21 samples) in the second PCR. The only CSF samples that gave the 171-bp amplicon in the second PCR came from confirmed or possible cases of NC. The probability that an NC case would be found PCR-positive did, however, vary with the condition and/or location of their cysticerci (see Table). Patients with

```
T. sag.  CTTCTCAATT  CTAGTCGCTG  TGGTCAGAAC  GACTTTGTAG  TCTCGGTTCG  AGTACACTCT  60
T. sol.  ..........  ..........  ........C.  C.....AC..  ..........  ........T.  60

T. sag.  AACAGAAGTC  AAGAGAGGGA  TAAAAATATA  GCTCCAATGT  TGACATGGTT  CATACAGTGA  120
T. sol.  ..T..C....  .C......A.  .G....C..C  .T....T...  C..T......  ..C..G....  120

T. sag.  ATTTAGCTGT  ACCAGCACCT  AACCATCCAC  CTTCAACTCC  ATTCTTCGTCC              171
T. sol.  ...C.....C  ..T....T..  GG......T.  ..........  ...........              171
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FIG. 2. Alignment of the partial nucleotide sequence of *Taenia saginata* HDP2 (*T. sag.*; GenBank accession AJ133764) with that of the 171-bp amplicon produced in the semi-nested PCR (*T. sol.*; GenBank EF043036). *Taenia solium* distinct nucleotides are indicated by the specific nucleotides.

vesicular cysticerci (63% PCR-positive), in particular those with cysts in the subarachnoidal space at the base of the brain or in the ventricles (71% PCR-positive), were those most likely to be found PCR-positive (see Table). Among the NC cases with vesicular parasites in the subarachnoid space at the base of the brain ($P=0.1$), those with damaged cysticerci ($P=1$), and those where there was uncertainty about the presence of vesicular cysticerci ($P=1$), there was no evidence that corticosteroid treatment prior to lumbar puncture reduced the chances of positivity in the semi-nested PCR.

With the radiological confirmation of NC taken as the 'gold standard', the HDP2 PCR and the HP10-antigen assay exhibited sensitivities that were similar (39% *v.* 33%; $P>0.05$) and much lower than that of the antibody-detection assay (80%). Antibody detection not only gave high sensitivity but was also the only method tested that was likely to give a positive result when the samples came from NC cases with damaged cysticerci (see Table). The HDP2 PCR detected cysticercal DNA in some neurological patients who appeared negative for the HP10 antigen and specific antibodies, including two patients who were considered possible NC cases although not showing good radiological evidence of NC.

DISCUSSION

The semi-nested, HDP2-based PCR developed in the present study detected from 0% to 71% of confirmed NC cases, depending on parasite stage (vesicular or damage) and location. As expected, given previous observations (Meri *et al.*, 1999; Almeida *et al.*, 2006), cysticercal DNA was detected in the CSF of most of the cases (10/16) who had vesicular parasites in their central nervous system. The PCR was most likely to give a positive result when the CSF sample being tested came from a patient with the most severe form of NC, with parasites in the subarachnoid space at the base of the brain or in a ventricle (Del Brutto, 2005). A correlation between disease severity and PCR positivity could be clinically useful but, given the variability in the clinical manifestations of *T. solium* infection (Vega *et al.*, 2003), needs to be evaluated further. It is unclear why six (37%) of the patients with vesicular NC were found PCR-negative, although this may reflect genetic

TABLE. *The results of testing samples of cerebrospinal fluid from 88 neurological patients in the HDP2 PCR and in assays for HP10 antigen and* Taenia-solium-*specific antibodies*

Diagnosis	No. and (%) of samples: Investigated	Found positive: In HDP2 PCR	For HP10 antigen	For anti-*Taenia solium* antibodies
CONFIRMED NEUROCYSTICERCOSIS	46	18 (39)	15 (33)	37 (80)
Vesicular	16	10 (63)	14 (88)	16 (100)
Ventricle or basal subarachnoid space	14	10 (71)	14 (100)	14 (100)
Parenchyma or subarachnoid sulci	2	0 (0)	0 (0)	2 (100)
Damaged cysticerci	24	4 (17)	0 (0)	16 (67)
Uncertainty about presence of vesicular cysticerci	6	4 (67)	1 (17)	5 (83)
Uncertain (possibly NC)	6	2 (33)	0 (0)	1 (17)
NEUROLOGICAL PROBLEM OTHER THAN NEUROCYSTICERCOSIS				
Mexican patient	4	0 (0)	0 (0)	0 (0)
Spanish patient	32	0 (0)		

differences in the *T. solium* strains involved (Vega *et al.*, 2003). Further studies to investigate temporal changes in the presence and concentration of parasite gDNA in the CSF are also required. Interestingly, the two patients investigated who had parenchymal NC were found negative in the PCR and in the HP10-antigen assay, a finding in agreement with previous data that indicate that parenchymal cysticerci have restricted access to the CSF (Fleury *et al.*, 2007). Although *Taenia*-specific antibodies can be detected in the CSF of most NC cases, assays for the detection of such antibodies cannot be used to differentiate between severe and mild/recovering cases (Fleury *et al.*, 2004), limiting their clinical usefulness.

The highly specific HDP2 PCR could well be useful in the diagnosis of NC and in following the progression of the disease and its post-treatment follow-up. This assay revealed *T. solium* DNA in the CSF of four of the 24 investigated patients who, by radiology, only appeared to harbour damaged parasites. It is possible, however, that these four PCR-positive patients (like six of the other PCR-positives — four with radiological uncertainty about the presence of vesicular cysticerci and two considered 'uncertain' NC cases) had vesicular parasites that were not radiologically detectable (see Table). It is also possible that the parasite DNA detected in these cases could be related to the perilesional oedema that is occasionally observed in association with cysticerci that, radiologically, appear to be damaged (Nash *et al.*, 2001, 2004).

In summary, in combination with the immunological assays available, the HDP2 PCR could be a useful tool in supporting NC diagnosis, in particular when radiological methods have failed to establish the presence of *T. solium* cysticerci in the central nervous system. It could also help in following the pathogenesis of NC and in post-treatment follow-up.

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Human and porcine neurocysticercosis: differences in the distribution and developmental stages of cysticerci

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Summary

OBJECTIVE To describe and compare the clinical impacts of neurocysticercosis (NC) caused by *Taenia solium* in humans and pigs.

METHODS Comparative study of the brains of 16 asymptomatic pigs and 35 human NC cases (15 asymptomatic and 20 symptomatic).

RESULTS In humans, cysticerci were more frequently located in the ventricles and subarachnoid space at the base of the brain (11.8% *vs.* 1.6%; $P = 0.001$ and 25.9% *vs.* 0%; $P < 0.0001$, respectively) while in pigs, cysticerci were more frequently found in the parenchyma (44.4% *vs.* 7.6%; $P < 0.0001$). In human brains, 75.9% of the cysticerci were calcified, while in pigs all cysticerci were in the vesicular stage.

CONCLUSION The duration of infection and the host–parasite relationship (such as immune reactivity and brain haemodynamics) differ between humans and pigs. This may account for the different distribution and stage of the cysticerci among humans and pigs.

keywords neurocysticercosis, cysticerci, pig cysticercosis, human cysticercosis, *Taenia solium*

Introduction

Cysticercosis caused by metacestodes of *Taenia solium* (cysticercus) is an infection that commonly affects both pigs and humans and less often other animal species such as dogs, monkeys, wolfs or certain felines (Cadigan *et al.* 1967; Esch *et al.* 1990; Ito *et al.* 2002). In humans and pigs, the parasite is frequently located in the central nervous system (CNS), causing neurocysticercosis (NC) (De Aluja & Vargas 1988; Fleury *et al.* 2006a; Prasad *et al.* 2006).

Human NC may be asymptomatic (Briceño *et al.* 1961; Villagran & Olvera 1988; Fleury *et al.* 2003, 2006a) but it can also be manifested by a wide variety of unspecific signs and symptoms (i.e. seizures, headache, etc.) and in some cases may seriously compromise human life (Sotelo & Del Brutto 2000; Fleury *et al.* 2006b). The location of the parasite within the host's brain (parenchyma, subarachnoid, ventricles or spinal cord), its size, number and developmental stage (vesicular, colloidal, granular-nodular, nodular and calcified), as well as the variability in the intensity of the host's immuno-inflammatory reaction contribute to the differences in NC severity (Rabiela *et al.* 1982; Escobar 1983; Del Brutto & Sotelo 1988; Fleury *et al.* 2004). The level of exposure (i.e. amount and frequency of egg ingestion), host (i.e. age, gender) and parasite factors are likewise probably involved in the modulation of NC severity although its mechanisms are not known (Rangel *et al.* 1987; Del Brutto & Sotelo 1988; 1998; Chavarría *et al.* 2003; Fleury *et al.* 2003; Maravilla *et al.* 2003; Vega *et al.* 2003; Saenz *et al.* 2006).

In pigs, the most common intermediary host of *T. solium,* cysticerci, may be located in different organs, including the CNS. In contrast to their much studied effects upon human health, the impact of intracranial cysticerci upon pig health has been superficially explored. Recently, excessive salivation, blinking and tearing, and in some cases subconjunctival nodules in infected pigs have been reported (Prasad *et al.* 2006). However, no clinical signs of intracranial hypertension were found, which is the most severe clinical complication in human NC (Hernández *et al.* 1973; Rodríguez and Boleaga 1982).

To determine the role of anatomical factors involved in the clinical differences of NC in humans and pigs, this paper describes and compares the distribution, location and developmental stages of cysticerci in infected brains of pigs and humans.

Material and methods

The study included 22 brains of apparently asymptomatic adult pigs, naturally infected with cysticerci. The animals originated from the states of Morelos and Guerrero, Mexico. All pigs were adult, over 12 months of age. For anatomical and histological studies, brains were fixed in 10% formaldehyde and sliced in 10 to 12 coronal sections of approximately 10 mm thickness 1 month later (Figure 1). In each brain, the number of parasites, their distribution (frontal, parietal, occipital, temporal lobe and cerebellum), location [subarachnoid space of the sulci (SaSulci), subarachnoid space at the base of the brain (SaBase), parenchymal and ventricular (IV)] and degenerative stages (vesicular, colloidal, granular-nodular or calcified) were recorded (Sciutto *et al.* 2000). Only parasites clearly embedded in the tissue or attached to the leptomeninges were counted, while few loose parasites were not considered as their precise location could not be determined.

The same data were collected from 35 human cases of NC (Figure 2) – 20 symptomatic (with intracranial hypertension, dementia, stroke and/or seizures) and 15 asymptomatic (NC was an autopsy finding); all data were obtained from the necropsy pathology records of the *Hospital General de México* (Mexico City, DF, Mexico) between 1989 and 1999. Possible similarities and differences in the distribution, location and developmental stage of the cysticerci between pigs and humans were recorded and statistically evaluated.

Data were processed in EXCEL 7.0 (Microsoft) and SPSS 10.0 for Windows. Cumulative percentages of each group and percentages of each group category were calculated. Intergroup comparisons were made using the Mann–Whitney *U*-nonparametric test.

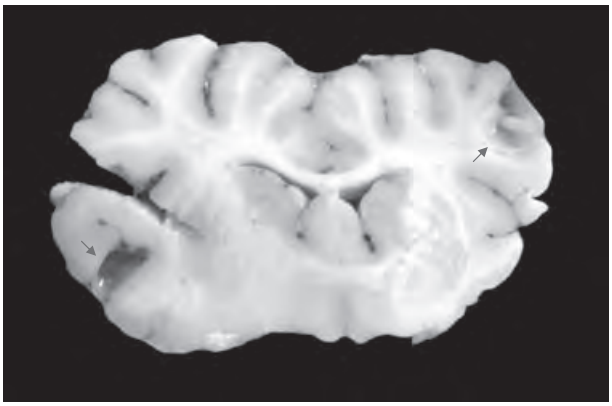

Figure 1 Coronal view of a pig brain with two cysticerci localized in the subarachnoid space of the sulci (arrows).

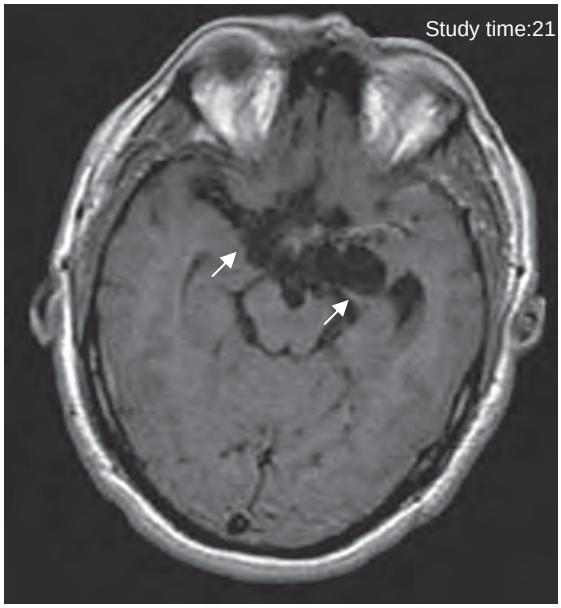

Figure 2 Magnetic resonance imaging of a human brain with multiple parasites localized in the subaracnoideal cisterns at the base of the brain (arrows).

Results

Porcine neurocysticercosis

A total of 588 parasites were counted in 22 pig brains. The parasitic load varied from 2 to 196 cysticerci per brain, with an average of 27 ± 42.8 cysticerci. In the case with the highest parasite load, all 196 cysticerci were in the vesicular stage. In rest of the pigs, 96.2% of parasites were vesicular (566/588); only one adult sow presented 22 of its 46 cerebral parasites in a calcified stage (3.7%). All parasites located in the muscles of this sow were calcified.

Human neurocysticercosis

Necropsy brain studies reported 170 cysticerci found in 35 symptomatic and asymptomatic adult human NC cases. The individual parasite loads ranged from 2 to 16 cysticerci per brain, with an average of 4.8 ± 3.8 parasites. Cysticerci were located mostly in the SaSulci (93, 54.7%) and in the SaBase (44, 25.9%), followed by the ventricles (20, 11.8%) and the parenchyma (13, 7.6%). Most of the 106 parenchymal or SaSulci parasites were situated in the parietal lobe (55, 32.3%), followed by the frontal (37, 21.8%), temporal (9, 5.3%), occipital (4, 2.3%) and the cerebellum (1, 0.6%); 129 parasites were colloidal or calcified (75.9%), whereas only 41 (24.1%) were vesicular. Fifteen

of the 35 human cases were asymptomatic. Eighty-five per cent (17/20) of the symptomatic cases, for whom intracranial hypertension, dementia, stroke and/or seizures were reported, died from NC complications (Table 1, I *vs.* II). Symptoms in human NC were associated with the presence of cysticerci in the SaBase ($P < 0.0001$), while hosts with parasites in the parenchyma or in the SaSulci were mostly asymptomatic ($P = 0.01$ and $P = 0.02$, respectively).

Differences between pig and human neurocysticercosis

To compare the distribution of cysticerci between humans and pigs, only pigs with parasite loads that were not significantly different from the loads found in humans were selected (2–16 parasites). Parasite distribution, location and developmental stages of 124 cysticerci in 16 pig brains were compared with 49 cysticerci in 15 asymptomatic human brains and with 121 cysticerci from 20 symptomatic human brains. As in Table 1, differences of cysticerci location and developmental stages between pigs and humans were significant, regardless of the symptomatology in humans. Cysticerci in the parenchyma were more frequent in pigs than in humans ($P2 = 0.001$, $P3 < 0.0001$), the majority being vesicular ($P2$ and $P3 < 0.0001$). A significant higher frequency of cysticerci in ventricles and SaBase was also found in asymptomatic and symptomatic humans ($P2 = 0.01$, $P3 = 0.001$ for intraventricular and $P2 = 0.024$, $P3 < 0.0001$ for SaBase). In addition, most of the cysticerci in human brains were calcified (75.5% in asymptomatic humans and 76% in symptomatic humans, $P2$ and $P3 < 0.0001$). With respect to cysticerci distribution, occipital parasites were more frequent in pigs ($P2 = 0.014$, $P3 < 0.0001$), and frontal parasites were more frequent in pigs and in asymptomatic humans than in symptomatic human cases ($P1 = 0.01$ and $P3 < 0.0001$).

Number of cysticerci in human and porcine neurocysticercosis

Figure 3 shows that, parasite loads were similar ($P = 0.17$) in asymptomatic pigs (median = 7.5; percentile 75 = 10.75) and symptomatic humans (median = 4.5; percentile 75 = 9.25), whereas asymptomatic humans had significantly fewer parasites (median = 2; percentile 75 = 4) than pigs ($P = 0.001$) or symptomatic humans ($P = 0.006$).

Discussion

The distinctive clinical profiles exhibited by humans and pigs could be due to the major differences found between the parasite developmental stage and anatomic distribution of cysticerci in the brain. These differences may arise from a number of causes, all in need of verification.

The predominance of vesicular cysticerci in pigs could reflect a better adaptation of the parasite to its porcine than to its human host. The difference may be a sign of a favourable bias of the parasite towards the more permissive

Table 1 Description and differences in distribution, location and degenerative stages of cysticerci between asymptomatic pigs and asymptomatic/symptomatic human neurocysticercosis

	I; Asymptomatic human 15 cases, 49 cysticerci		II; Symptomatic human 20 cases, 121 cysticerci		III; Asymptomatic pig 16 cases, 124 cysticerci		*P*1; (I *vs.* II)	*P*2; (I *vs.* III)	*P*3; (II *vs.* III)
Distribution									
Frontal	17†	40.5%	20	31.2%	54†	44.3%‡	0.01	NS	<0.0001
Parietal	20	47.6%	35	54.7%	36	29.5%	NS	NS	NS
Occipital	2	4.8%	2	3.1%	25	20.5%	NS	0.014	<0.0001
Temporal	2	4.8%	7	11%	5	4.1%	NS	NS	NS
Cerebellum	1	2.3%	0	0%	2	1.6%	NS	NS	NS
Location									
Parenchyma	8	16.3%	5	4.1%	55	44.4%	0.01	0.001	<0.0001
SaSulci	34	69.4%	59	48.8%	67	54%	0.02	NS	NS
Intraventricular	5	10.2%	15	12.4%	2	1.6%	NS	0.01	0.001
SaBase	2	4.1%	42	34.7%	0	0%	<0.0001	0.024	<0.0001
Developmental stage									
Vesicular	12	24.5%	29	24%	124	100%	NS	<0.0001	<0.0001
Colloidal and calcified	37	75.5%	92	76%	0	0%			

†Total number of cysticerci; ‡percentage of cysticerci exhibiting different distribution or location or degenerative stages.

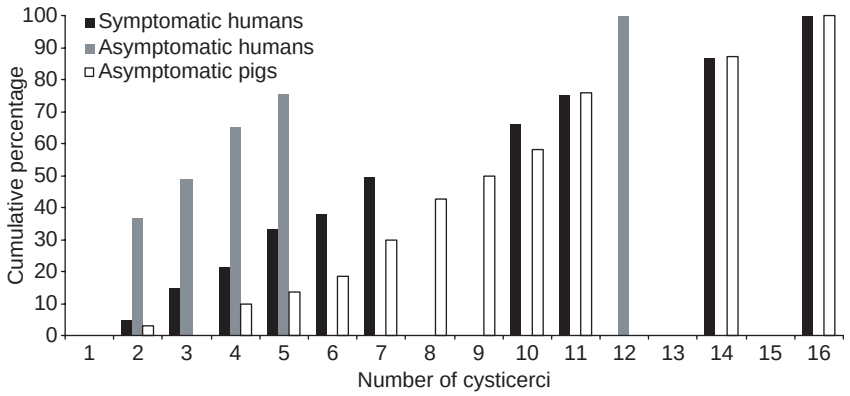

Figure 3 Cumulative percentage of the number of cysticerci per group. The cysticerci number did not significantly differ between the 20 symptomatic humans and the 16 asymptomatic pigs. A lower number of cysticerci were found in the 15 asymptomatic humans than in pigs and symptomatic humans.

and accessible intermediate porcine host instead of the more restrictive and progressively inaccessible human host (Hoberg 2001).

The higher frequency of parasite destruction and calcification in humans reveal the aggressive interactions that can be the cause of a prominent immuno-inflammatory reaction in the CNS, which may promote parasite destruction, CNS injury and clinical expression (Riley & White 2003; Fleury *et al.* 2004; Takayanagui & Odashima 2006). Some interspecies differences in the immuno-inflammatory response surrounding the cysticerci have been reported: eosinophils are more frequent in porcine NC, while a lower proportion of plasma cells are observed in humans (Rabiela *et al.* 1982; De Aluja & Vargas 1988; Alvarez *et al.* 2002; Londono *et al.* 2002). Different cell populations surrounding the parasite could be mounting different immune responses in the two host species.

Another likely source of differences in the proportions of calcified cysticerci in pigs and humans may be related to differences in the age of their infections. In humans, NC may take years to develop symptoms (Dixon & Lipscomb 1961). Rural pigs of Mexico are rarely kept for more than a year after birth before being slaughtered for human consumption (De Aluja *et al.* 1998, 1999). Thus, the many calcified cysticerci found in humans may be the remains of a protracted heavy immunological attack from their human host, while the vesicular cysticerci of the pig may not have been subjected to prolonged immune response from their porcine host.

In pigs, cysticerci were more frequently located in the parenchyma than in humans, while in humans cysticerci were more commonly present in the ventricles and SaBase. A number of factors may be involved in these location differences. Anatomic and vascular brain differences between species may result in different ways of entry of the parasites to the CNS (Strauch *et al.* 2007). Differential expression of adhesion molecules in the vessels of the blood–brain barrier and in the endothelium of the choroid plexus may also be involved (Alsam *et al.* 2003; Masocha *et al.* 2004). What the determinants of the location of the cysticerci in the CNS are merits further exploration, as location of the cysticerci in human NC is clearly related to the severity of the disease (Fleury *et al.* 2003; Saenz *et al.* 2006). In agreement with previous reports, we observed a significant higher frequency of parasites in the SaBase in symptomatic human patients than in asymptomatic ones ($P < 0.0001$, Table 1).

Larger number of parasites in pigs than in humans could reflect exposure differences (dose, number and frequency of egg ingestion) and/or restrictive immunity. However, apparently, parasite number is not an important determinant of interspecies symptom differences as it was similar in symptomatic humans and asymptomatic pigs (Figure 3). Considering that the human brain is almost seven times larger than the porcine brain, it seems that humans provide cysticerci with a more restrictive access to the CNS (Nieuwenhuys *et al.* 1998). Anatomic, vascular and immunological differences between species could also be involved.

In summary, there are clear differences between porcine and human NC. More thorough knowledge of their causes could contribute to the understanding of NC pathogenesis and its better management.

Acknowledgements

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Human Neurocysticercosis: Rightward Hemisphere Asymmetry in the Cerebral Distribution of a Single Cysticercus

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ABSTRACT: The distribution of single cysticerci between cerebral hemispheres was studied in 227 adult cases of calcified and vesicular neurocysticercosis (NC). A rightward lateralization of calcified cysticerci was significant only in women, whereas vesicular cysticerci were equally distributed in both hemispheres. Factors related with the differences in the inflammatory response and in the regional cerebral blood flow between genders could be involved.

Human neurocysticercosis (NC) is a severe and frequent neurological disease in developing countries (Sciutto et al., 2000; Garcia et al., 2003; Fleury et al., 2006), and it is considered as a re-emergent disease in developed ones due to the increase of immigration (DeGiorgio et al., 2005). NC is caused by the larval stage of the cestode parasite *Taenia solium* (cysticercus), which may develop in the central nervous system of its human host. Although cerebral lateralization is well recognized in several brain physiological functions, and the brain is progressively recognized as capable of modulating the local and systemic immune response (Tarkowski et al., 1995; Fu et al., 2003; Meador et al., 2004), a possible asymmetric distribution of cysticerci or of any other brain parasites, i.e., toxoplasmosis, malaria, has not been investigated. The present study examines the hemisphere distribution of single cysticerci in the human brain.

In total, 227 human adult NC cases with a single cysticercus were included. The anatomic status of the parasites (189 calcified, 38 vesicular), their hemispherical location (132 right, 95 left), and the diagnostic tool used (radiology in 172 and autopsy in 55) were recorded. Ninety-four men and 133 women were included. All the patients with radiological diagnosis had a CT scan. In a subgroup of them (50 cases), magnetic resonance imaging (MRI) was also used. In all of these 50 cases, CT scan and MRI results were consistent. Only cases with a single lesion closely resembling those described for *T. solium* cysticerci

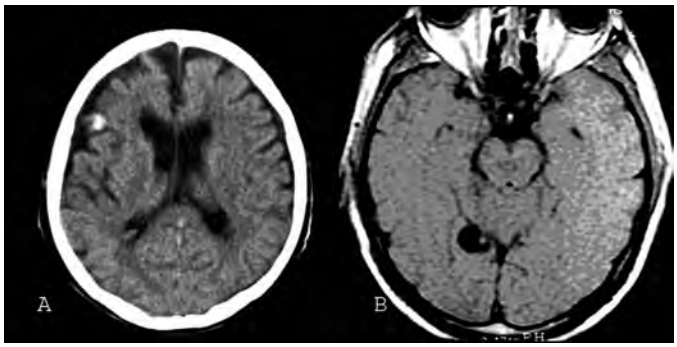

FIGURE 1. (**A**) CT scan. Single calcified cysticerci located in right hemisphere. Small hyper dense nodules in CT (<2 mm) were considered as calcified cysticerci because of showing the typical radiological features of calcified cysticerci and considering the high NC prevalence in Mexico. (**B**) MRI. Single vesicular NC located in the right hemisphere.

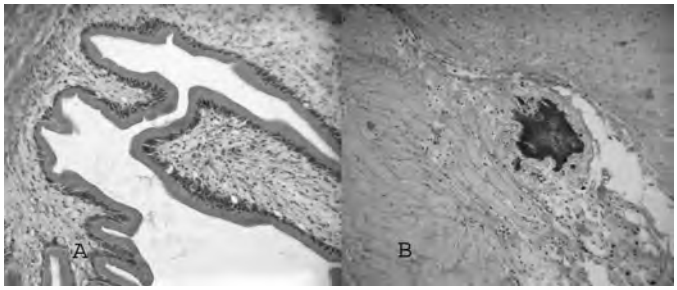

FIGURE 2. (**A**) Microscopic identification of live cysticerci is based on the identification of the 3-layered structure containing fluid. (**B**) The microscopic diagnosis of calcified cysticerci must rest on clinical and epidemiological data because specific structures are absent. Other possible sources of calcified nodules in the brain, such as old tuberculomas or meningiomas should be excluded, because the best morphologic diagnosis of such lesions is of consistency with calcified cysticerci.

(Rodriguez-Carbajal and Boleaga-Duran, 1983; Villagran and Olvera, 1988) were included. The inclusion criteria were: reliable diagnosis by CT scan and/or MRI (Fig. 1), or macro- and microscopic findings at autopsy (Fig. 2).

Distribution of single cysticerci in the cerebral hemispheres (right, left) was evaluated with respect to stage of the parasite (calcified, vesicular), sex (women, men), and method of diagnosis (CT scan or necropsy). Results were analyzed with the SPSS software. Significant differences in the frequency of parasites in each hemisphere were evaluated using a nonparametric χ^2 test.

Table I shows that there is a right-side localization of calcified cysticerci in the brain hemisphere ($P = 0.002$), which was statistically significant in CT scan and autopsy results in women only ($P = 0.03$, and $P = 0.003$, respectively). In contrast with calcified cysticerci, no statistically significant lateralization of vesicular cysticerci was found in either women or men using either the CT scan, or in autopsy cases.

Evidence is presented of a statistically significant preferential bias towards the right hemisphere of single calcified cysticerci in women. Vesicular cysticerci did not show a hemisphere preference. Right-side bias of single calcified cysticerci may result from the more frequent entry of the parasite into the right than into the left hemisphere or from a differential management of the host–parasite relationship between hemispheres. The fact that vesicular parasites locate equally in the right and left hemisphere suggests equal entry of parasites into both hemispheres and emphasizes the differential management hypothesis. It is possible that the right hemisphere may calcify incoming parasites more effectively, or the left hemisphere might destroy them more effectively without sequel, or both. Because it is only observed in women, this bias could be promoted by increased inflammatory response observed in NC females and its particular hormonal local environment (Fleury et al., 2004; Chavarria et al., 2005; Soucy et al., 2005). Difference in regional cerebral blood flow (rCBF) between women and men recently reported (Van Laere et al., 2001; Pirson et al., 2006) could be involved. In this respect, it was reported that rCBF was increased in the right sensorimotor cortex and decreased in the left temporal cortex in women. It is possible that a higher rCBF in right hemisphere in women promotes a higher cysticerci calcification rate due to the arrival of a significant number of immunocompetent cells. At present, there is no way of discerning which immune mechanism involved in the host–parasite relationship would be responsible for this symmetry bias of females. Further research is necessary to evaluate lateralization in the natural course of other immunoinflammatory confrontations in the brain and to understand the reasons of such an observation.

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TABLE I. Distribution of single calcified and vesicular cysticerci in 227 human NC cases.

	Calcified				Vesicular			
Diagnosis	Total	Right	Left	*P**	Total	Right	Left	*P*
Radiological								
Men	48	28	20	0.25	21	10	11	0.83
Women	88	54	34	0.03	15	4	11	0.07
Histopathological								
Men	23	11	12	0.8	2	2	0	—
Women	30	23	7	0.003	0	0	0	—
Totals	189	116	73	0.002	38	16	22	0.33

* *P*: *P* value of the difference between right/left parasite localization in each cases group.

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Ribeiroia ondatrae Cercariae Are Consumed by Aquatic Invertebrate Predators

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ABSTRACT: Trematodes amplify asexually in their snail intermediate hosts, resulting in the potential release of hundreds to thousands of free-living cercariae per day for the life of the snail. The high number of cercariae released into the environment undoubtedly increases the probability of transmission. Although many individual cercariae successfully infect another host in their life cycle, most fail. Factors that prevent successful transmission of cercariae are poorly understood. Microcrustaceans and fish have been observed to eat cercariae of some species, although the possibility that predation represents a significant source of mortality for cercariae has been largely unexplored. We tested the cercariophagic activity of several freshwater invertebrates on *Ribeiroia ondatrae*, a trematode that causes limb deformities in amphibians. Individuals of potential predators were placed into wells of multiwell plates with 10–15 cercariae, and numbers of cercariae remaining over time were recorded and compared with numbers in control wells that contained no predators. Of the species tested, *Hydra* sp., damselfly (Odonata, Coenagrionidae) larvae, dragonfly (Odonata, Libellulidae), larvae, and copepods (Cyclopoida) consumed cercariae. In some cases, 80–90% of the cercariae offered to damselfly and dragonfly larvae were consumed within 10 min. In most cases, predators continued to consume cercariae at the same average rates when offered cercariae together with individuals of an alternate prey item. *Hydra* sp. ate fewer cercariae in these trials. Our findings suggest the need for field and laboratory studies to further explore the effects of predators on transmission of *R. ondatrae* to amphibian larvae. In addition, the results suggest that conservation of the biodiversity and numbers of aquatic predators may limit adverse impacts of trematode infections in vertebrate hosts.

Coinciding with declines in North American amphibian populations over the past decade is an increase in the frequency of limb deformities, with 50% or more deformities occurring in many populations (Johnson et al., 2002, 2003). Although it has yet to be determined if some of the deformities observed in wild populations can be attributed to other teratogenic factors, such as UV radiation and pesticide contamination, laboratory and field-based investigations have pointed to infection by trematode cercariae of the species *R. ondatrae* as the most likely cause, at least of supernumerary and branched limbs (Johnson et al., 1999, 2002; Blaustein and Johnson, 2003; Taylor et al., 2006).

Species of *Ribeiroia* have complex life cycles in which snails become infected by miracidia and release free-swimming cercariae in large numbers (A. Schotthoefer, pers. obs.). The cercariae go on to infect tadpoles or fish, encysting as metacercariae. When these latter infected intermediate hosts are ingested by avian or mammalian definitive hosts, *Ribeiroia* spp. develop to their adult stage and may engage in sexual reproduction (Johnson et al., 2004). Whether tadpoles develop deformities is a function of the timing and intensity of infections acquired during the early period of limb development (Johnson et al., 1999; Schotthoefer, Koehler et al., 2003). Identifying the conditions in aquatic habitats that influence the likelihood of transmission of *R. ondatrae* cercariae and tadpoles during this sensitive period is critical for understanding the emergence of this infectious disease.

The density of infective *R. ondatrae* cercariae in the environment is likely an important determinant of tadpole infection. Though the number of infected snails and the number of cercariae released by each infected snail will be central to determining the density of cercariae in the environment, factors that facilitate or hinder the survival or ability of cercariae to locate and infect tadpoles once released by snails will also be of critical significance. It has been proposed that cercariae are lost from the infective stage pool through predation by small aquatic predators (Anderson et al., 1978; Lafferty et al., 2006). Microcrustaceans and fish, for example, have been reported to eat cercariae of *Schistosoma mansoni* (Rowan, 1958; Knight et al., 1970; Christensen, 1979). However, few studies have demonstrated cercariophagic activity of predators. Therefore, it is important to begin to examine the possibility that predation represents a significant source of mortality that naturally limits cercaria transmission. In this study, we explored the cercariophagic potential of several invertebrate aquatic organisms, including members of the phyla Arthropoda (Odonata, Coleoptera, Anomopoda, Notostraca, Cyclopoida) and Cnidaria (Hydroida).

Preliminary observations of the potential cercariophagic activity were made with *Hydra* sp. (Hydroida), *Daphnia pulex* (Anomopoda), *Triops* sp. (Notostraca), copepods (Cyclopoida), damselfly larvae (Odonata,

Neurocysticercosis

Clinical, Radiologic, and Inflammatory Differences Between Children and Adults

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Background: Human neurocysticercosis (NC) is caused by *Taenia solium* larvae lodged in the central nervous system. NC is clinically heterogeneous, ranging from asymptomatic infection to severely incapacitating and even fatal presentations. Although NC affects adults and children, age-related factors have not been thoroughly studied.

Methods: We describe and compare the clinical, radiologic, and inflammatory features of pediatric and adult Mexican NC cases. Two hundred six NC cases (92 pediatric and 114 adult) diagnosed by computed tomography or magnetic resonance imaging were included.

Results: Seizures were more frequent in children (80.4% versus 56.1%), and intracranial hypertension and headaches were more frequent in adults (27.2% versus 15.2% and 35.1% versus 21.7%, respectively). Different causes underlie the different distribution of seizures and intracranial hypertension in the 2 patient groups. In pediatric NC patients, single colloidal parenchymal cysts were the most common radiologic findings compared with adults in whom multiple viable parasites in the basal subarachnoidal cisterns or in the ventricles were seen. Cerebrospinal fluid inflammation was greater in adults than in children ($P = 0.02$).

Conclusions: This study documents significant age-related radiologic, clinical, and inflammatory differences in Mexican NC patients. Possible causes and relevance of these age-associated findings are discussed.

Key Words: neurocysticercosis, *Taenia solium*, pediatric, adult, differences, Mexico

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Neurocysticercosis (NC) is caused by *Taenia solium* larvae located in the central nervous system (CNS) of humans.[1] NC exhibits a heterogeneous clinical picture: it can range from asymptomatic infection to clinically mild forms (headache, dizziness or occasional seizures) or to a severe, life-threatening neurologic syndrome with intracranial hypertension (ICH).[2] The severity of the disease is related to the parasite's location (parenchyma, subarachnoidal [SA] space or ventricles), to the number of cysticerci[3] and to the local and systemic inflammatory status.[4] Level of exposure, as well as host (age, gender and genes) and parasite factors, can account for this clinical and radiologic heterogeneity.[5–8]

With respect to the host's age, differences in prevalence and severity in Mexican NC patients have been reported. NC is less frequently found in children than in adults (0.5% versus 2%), as reported in the autopsy series from several Mexico City hospitals.[9,10] In addition, in some Mexican series of pediatric patients, children generally present with less severe disease than do adults.[11,12]

Despite these observations, to the best of our knowledge, there is no formal comparison between adult and children NC patients in any specific geographic region. Thus, the present study explores systematically the relevance of age in the clinical, radiologic, and inflammatory manifestations of NC in Mexico by a detailed description and a comparison of pediatric and adult NC patients.

MATERIALS AND METHODS

Three hospitals located in Mexico City participated in the study: Hospital Infantil de México "Federico Gómez" (HIM), Instituto Nacional de Pediatría (INP) and Instituto Nacional de Neurología y Neurocirugía (INNN). All 3 are referral hospitals where patients lacking social security from all over the country receive care. Because of the low prevalence of pediatric NC (1 to 10 cases per year according to the medical records of different institutions), all confirmed NC patients from the HIM and the INP evaluated in the period from 1993 to 2003 were included. Cases from the INNN were included in a prospective way during 2003. Patients were considered pediatric if younger than 15 years of age. Only adult and pediatric patients who had not received specific treatment of NC before radiologic studies were included in this study. NC was diagnosed based on computed axial tomography and/or magnetic resonance imaging. The following information was collected from each patient: number of lesions (single versus multiple), CNS localization (SA space, parenchymal or ventricular) and stage of cysticerci (vesicular, colloidal, calcified or mixed forms).

Vesicular cysticerci are viable parasites characterized as those showing transparent membrane, vesicular fluid, with-

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TABLE 1. Comparison of Clinical and Radiologic Findings in Adults and Children With NC

	Adults (N = 114)	Children (N = 92)	*P*
Symptoms*			
Seizure	64 (56.1)	74 (80.4)	<0.0001
Headache	40 (35.1)	20 (21.7)	0.04
Intracranial hypertension	31 (27.2)	14 (15.2)	0.04
Focal deficits	19 (16.7)	11 (12)	0.34
Number of parasites*			
Single	29 (25.4)	54 (58.7)	<0.0001
Multiple	85 (74.6)	38 (41.3)	
Stage of the parasite*			
Only vesicular	29 (25.4)	4 (4.3)	<0.0001
Only colloidal	12 (10.5)	37 (40.2)	<0.0001
Only calcified	15 (13.2)	35 (38)	<0.0001
Mixed	58 (50.9)	16 (17.4)	<0.0001
Localization of the parasites			
Parenchyma/convexity SA space	58 (50.9)	90 (97.8)	<0.0001
Basal SA cisterns	46 (40.4)	0	<0.0001
Ventricle	19 (16.7)	3 (3.3)	0.002
Cerebrospinal fluid, cells/mm^3†			
<5	28 (43.8)	23 (74.2)	0.02
5–49	21 (32.8)	4 (12.9)	
>49	15 (23.4)	4 (12.9)	
Cerebrospinal fluid, protein, mg/dL†			
<40	29 (46)	25 (86.2)	0.001
40–100	29 (46)	4 (13.8)	
>100	5 (7.9)	0	

*Values are expressed as number of patients (percentage).
†CSF analysis was made only in some patients (64 adults and 31 children for cell determination and 63 adults and 29 children for protein determination).

out surrounding inflammatory reaction. They appear in imaging studies as hypodense or hypointense lesions with slightly thicker margins. Colloidal cysticerci are degenerating parasites and appear as hypodense or isodense lesions surrounded by edema, and ring enhancement after contrast medium administration is common. Calcification occurs when the parasite is dead. At that stage, cysticerci appear on computed axial tomography as hyperdense lesions and on magnetic resonance imaging, albeit more difficult to visualize, as hypointense foci. Mixed forms are characterized by the presence in the same patient of parasites in different stages.

When a lumbar puncture was performed before treatment, cerebrospinal fluid cell count and protein concentration were recorded and considered as indicators of the CNS inflammatory status.

The clinical expression of the disease was established by a direct questionnaire (patients from INNN) or by the information in the hospital records (patients from HIM and INP). Based on the symptoms, patients were grouped into 4 classes: (1) headache; (2) focal deficit; (3) seizures and (4) ICH (defined by simultaneous presence of headache, nausea, vomiting and papilledema).

Statistical analysis was performed with Microsoft Excel and the SPSS 10 program. Univariate analysis was done with parametrical tests (*t* Student, χ^2, Fisher).

RESULTS

Two hundred six patients were included, 114 adults and 92 children. In the adult group, 58 were females (50.9%) and 56 males (49.1%), and ages varied from 15 to 64 years, without significant differences between genders ($P = 0.3$). The pediatric group consisted of 52 females (56.5%) and 40 males (43.5%), with ages varying from 11 months to 14 years, without significant differences between genders ($P = 0.06$). The female:male ratios were similar for both groups.

Table 1 shows the most frequent symptoms, which were, in order of frequency, seizure, headache, ICH, and focal deficits in both pediatric and adult NC patients. Seizures were more frequent in children ($P < 0.0001$), and headache and ICH were more frequent in adults ($P = 0.04$). The origin of ICH also differed between the 2 groups (Table 2). In children, ICH was manifested by increased inflammatory response surrounding parenchymal or convexity SA space colloidal parasites, whereas in adults it resulted from obstruction of CSF circulation by the presence of cysticerci located in the basal SA cisterns or in the ventricles. In those with seizures, single parasites were generally involved in children.

TABLE 2. Relation Between Radiologic and Clinical Presentations in Adult and Pediatric NC Patients

	Headache Only			Seizure			Intracranial Hypertension		
	Adults	Children	*P*	Adults	Children	*P*	Adults	Children	*P*
Stage of the parasite									
Vesicular only	4 (23.5)*	1 (14.3)	NS	14 (22)	2 (2.7)	<0.0001	10 (32.3)	1 (7.1)	NS
Colloidal only	2 (11.8)	1 (14.3)	NS	8 (12.5)	32 (43.2)	<0.0001	3 (9.7)	5 (35.7)	0.08
Calcified only	2 (11.8)	3 (43)	NS	12 (18.8)	32 (43.2)	0.002	0	0	NS
Mixed†	9 (53)	2 (29)	NS	30 (47)	8 (10.8)	<0.0001	18 (58.1)	8 (57.1)	NS
Total number of parasites									
Single	4 (23.5)	4 (57)	NS	22 (35)	42 (65)	<0.0001	3 (9.7)	1 (7)	NS
Multiple	13 (76.5)	3 (43)		48 (65)	26 (35)		28 (90.3)	13 (93)	
Localization									
Parenchyma – convexity SA space	8 (47.1)	7 (100)	0.02	46 (72)	74 (100)	<0.0001	2 (6.5)	13 (93)	<0.0001
Ventricle	2 (11.8)	0	NS	8 (12.5)	0	0.002	14 (45.2)	2 (14.3)	0.09
Basal SA cisterns	7 (41.2)	0	0.06	15 (23.4)	0	<0.0001	22 (71)	0	<0.0001

NS indicates not significant.
*Number (percentage) of each radiologic status in each clinical characteristic.
†Presence of parasites in different stages in the same patient.

while in adults, multiple parasites were more frequent (Table 2). The number, location, and stage of parasites differed significantly between the 2 age populations: a single colloidal or calcified parenchymal parasite was the most frequent form in children and multiple vesicular parasites in the basal SA cisterns or in the ventricles were most common in adult patients (Table 1). The CSF inflammatory responses (cells and proteins) were significantly greater in adults than in children ($P = 0.02$ and $P = 0.0001$, respectively, Table 1).

DISCUSSION

This study documents important differences in the radiologic and clinical presentations, as well as in CSF inflammatory intensity, between pediatric and adult Mexican NC patients. Some methodologic features have to be mentioned. As pediatric NC is relatively uncommon, we had to adopt a mixed design for this study: pediatric cases were reviewed retrospectively, whereas adult NC cases were enrolled prospectively. We realize that it is not ideal, but it was the only possible way for us to perform the study. Despite these methodologic problems, we think that our results substantiate age-related differences in NC presentations.

Our results differ somewhat from those published from other geographic regions, such as India, where a single enhancing lesion is the most frequent presentation in children and adults.[13,14] These differences emphasize the pertinence of performing age-related studies in different geographic areas, as their comparison could provide information about the mechanisms of the disease.[15]

In Mexico, children more frequently suffer from a single degenerating parasite located in the parenchyma, while multiple viable parasites located in the basal SA cisterns are more common in NC adult patients. The differences in parasite localization found in this study are clear, although probably an overrepresentation of parenchymal cysts in children exists because of their higher susceptibility to develop seizures.[16,17] However, results of autopsies, in which symptomatic and asymptomatic cases are recorded,[10] confirm that in children parasite location is mainly parenchymal and strengthen the hypothesis of participation of age-related factors. Colloidal cysticerci, seen in almost all pediatric NC cases, could be related to the more effective destruction of the parasite in the parenchyma than in the basal SA cisterns, as reported in some hospital studies.[2]

The differences in CSF inflammatory responses between pediatric and adult patients could also be related to the different locations of the parasite. Parasites located in basal SA cisterns or in the ventricles (directly in contact with CSF) are accompanied by higher CSF inflammation than parenchymal parasites.[4] However, the participation of other age-related factors, such as immunologic or hormonal, cannot be discarded.

Age-related differences in the number of parasites embedded in the CNS might be the consequence of particularities of this parasitosis (eg, prolonged incubation period of the disease,[18] prolonged and cumulative exposure to infection in adults); a higher resistance to infection might also pertain.

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