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UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

INSTITUTO DE INVESTIGACIONES BIOMÉDICAS

"EVALUACIÓN DE LA RESPUESTA INMUNE EN PACIENTES CON NEUROCISTICERCOSIS POR *Taenia solium*"

TESIS

QUE PARA OBTENER EL GRADO DE

DOCTORA EN CIENCIAS

PRESENTA

MÉDICA CIRUJANA ANAHÍ CHAVARRÍA KRAUSER

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

Ciudad Universitaria, México, D.F. **Octubre, 2004**

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pdcb/grad/167**Jur**/2004.

ING. LEOPOLDO SILVA GUTIERREZ
Director General de la
Administración Escolar
P r e s e n t e .

Por medio de la presente me permito informar a usted que en la reunión del Comité Académico del Programa de Doctorado en Ciencias Biomédicas que tuvo lugar el 28 de junio del presente año, se acordó designar el siguiente jurado para examen de Doctorado en Ciencias Biomédicas de la Méd. Cir. **ANAHÍ CHAVARRÍA KRAUSER** con no. de cuenta 94516775 con la tesis titulada: "Evaluación de la Respuesta Inmune en Pacientes con Neurocisticercosis por *Taenia solium*", dirigida por la Dra. Edda Sciutto Conde.

Presidente:	Dr. Ruy Pérez Tamayo
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Suplente:	Dra. Ingeborg Becker Fauser

Sin otro particular por el momento, aprovecho la ocasión para enviarle un cordial saludo.

A t e n t a m e n t e
"Por mi raza hablará el espíritu"
Cd. Universitaria, D.F., 1°. de julio de 2004.

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Responsable en el Instituto de
Investigaciones Biomédicas

Dr. J. Javier Espinosa Aguirre
Coordinador del Doctorado
en Ciencias Biomédicas

c.c.p. - Secretaría de Asuntos Escolares

El presente proyecto se realizó en el Departamento de Inmunología del Instituto de Investigaciones Biomédicas de la Universidad Nacional Autónoma de México bajo la dirección de la Dra. Edda Sciutto.

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Octavio Yáñez (Hospital Parres, SSA, Cuernavaca, Morelos)

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1. RESUMEN

La neurocisticercosis (NC) es una enfermedad clínicamente pleomórfica. Se ha sugerido que esta heterogeneidad se asocia a factores relacionados con la exposición, el parásito y/o el hospedero. Entre los factores del hospedero la edad, el género y los factores inmuno-inflamatorios podrían estar participando en la presentación clínica. En este trabajo se describe la respuesta inmune asociada las diferentes formas de la NC considerando su heterogeneidad clínica, en la población adulta de ambos sexos, bajo el supuesto de que esta heterogeneidad podría ser la consecuencia de los diferentes perfiles inmunológicos asociados. Así, se analizaron las características de la respuesta inmunológica asociada a la magnitud de la exposición, la infección y la sintomatología. Las personas de un área de alta exposición mostraron un perfil distintivo de los individuos de baja exposición presentando niveles significativamente superiores de proliferación celular y de anticuerpos específicos. Así mismo, las personas expuestas en las que no se detectaron cisticercos en el sistema nervioso central (SNC) presentaron un perfil mixto TH1/TH2, mientras que aquellas infectadas y asintomáticas mostraron un perfil preferencialmente TH2 (IL4, IL5, IL12, IL13 e IgG4). Por otro lado, el perfil inmunológico periférico de las personas con NC sintomática se caracterizó por presentar una baja respuesta proliferativa celular específica y altos niveles de inmunoglobulinas específicas. En contraste con esta actividad periférica deprimida, en el líquido cefalorraquídeo de los pacientes con NC sintomática se detectaron niveles aumentados de IL5, IL6, IL10 y anticuerpos específicos que se asociaron con la localización del parásito en el espacio subaracnoideo de la base e intraventricular así como con la mayor severidad clínica. Los resultados de este trabajo describen los perfiles inmunológicos asociados a las diferentes formas de NC, evidencias de un

dimorfismo sexual inmunológico y de una compartimentalización de la respuesta inmunológica en el SNC.

2. ABSTRACT

Neurocysticercosis (NC) is a clinically pleomorphic disease. It has been suggested that this heterogeneity relates to exposure, parasite and/or host factors. Of the host factors gender, age and the immune-inflammatory response may be participating in the clinical presentation of NC. In this work a description of the immune response related to different forms of NC according to its clinical heterogeneity in the adult population of both sexes was made. This study was carried out under the assumption that clinical heterogeneity in NC could be the consequence of different immune profiles associated. The characteristics of the associated immune response to the magnitude of exposure, infection and symptomatology were analyzed. Persons belonging to an area of high exposure showed a distinctive immune profile with higher levels of specific cell proliferation and specific antibodies than individuals of a low exposure area. Although persons exposed but not infected in the central nervous system (CNS) displayed a mixed TH1/TH2 profile, those infected and asymptomatic inclined to a TH2 profile (IL4, IL5, IL12, IL13 and IgG4). On the other hand, the immune profile of symptomatic NC patients was characterized by a low specific cell proliferation and high levels of specific antibodies. In contrast, this group also showed increased levels of IL5, IL6, IL10 and specific antibodies in the cerebral spinal fluid. This increased response was mainly associated with parasite location and clinical severity. The results of this work describe the existence of immune profiles associated with the different forms from NC, a related immunological sexual dimorphism and a possible compartmentalized immune response within the CNS.

3. INTRODUCCIÓN

Ciclo de vida y morfología del parásito

(Revisado en Flisser et al., 1982)

La cisticercosis es una enfermedad causada por el establecimiento de la larva (cisticerco) de *Taenia solium* en el tejido de los hospederos, el humano o el cerdo (Figura 1). La forma adulta del parásito, la cual es hemafrodita, se aloja en el intestino del humano, el hospedero definitivo, causando la teniosis. La tenia adulta puede medir de 2 a 8 metros, está formada por el escólex, el cuello y el estróbilo. El escólex mide aproximadamente 1 mm, tiene 4 ventosas, un rostelo con una doble corona de ganchos grandes (0.13 a 0.16 mm) que se alternan con pequeños (0.10 a 0.12 mm). El cuello es corto y delgado. El estróbilo está formado por una cadena de proglótidos, éstos se encuentran en diversos estadios de maduración. Los más inmaduros se hallan proximales al cuello, los maduros hasta grávidos en la región más distal. Los proglótidos maduros pueden llegar a contener de 80-100 000 huevos. Cada semana de 2 a 5 proglótidos se desprenden del estróbilo.

Los huevos son liberados al ambiente al romperse un proglótido y pueden sobrevivir en el ambiente durante meses. Los huevos miden aproximadamente de 20 a 50 µm y contienen un embrión (oncosfera) con tres pares de ganchos que se hallan cubiertos por una cápsula gruesa (embrióforo). Los huevos después de ser ingeridos por el cerdo, el hospedero intermediario, o accidentalmente por el humano, continúan su trayecto por el tubo digestivo donde la oncosfera, por el efecto de las secreciones gástricas y pancreáticas, eclosiona. Una vez eclosionada puede atravesar la pared intestinal y alcanzar la circulación sanguínea. La oncosfera se puede diseminar y establecer en distintos órganos y tejidos como son el tejido subcutáneo, el músculo

esquelético, el hígado, la lengua, el diafragma, los pulmones, el corazón, el ojo, y el sistema nervioso central (SNC). La oncosfera establecida crece y se transforma en el metacéstodo o cisticerco.

El ciclo de vida del parásito se completa cuando el humano ingiere carne de cerdo infectada y mal cocida. En el intestino el escólex evagina, las ventosas se adhieren a la mucosa duodenal y se induce la protrusión de los ganchos. En un lapso de 5 a 12 semanas se transforma en el parásito adulto con capacidad de producir proglótidos grávidos.

Neurocisticercosis

La neurocisticercosis (NC) representa un problema de salud muy frecuente en los países en vías de desarrollo y actualmente es re-emergente en países desarrollados como consecuencia de la migración poblacional (Schantz et al., 1998, Shandera et al., 1994). En México, la prevalencia de la NC es de tal magnitud que se traduce en el 11% de las consultas neurológicas en instituciones especializadas (Vázquez et al., 1992), el 25% de las craneotomías (Sotelo et al., 1985, Sotelo et al., 2000), es diagnosticada en 2- 4% de las necropsias realizadas en distintas instituciones hospitalarias (Villagrán et al., 1988) y es la primera causa de epilepsia de inicio tardío (Medina et al., 1990, Del Brutto et al., 1994).

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 et al., 1988, Fleury et al., 2003). Por otro lado la NC puede asociarse con síntomas y signos inespecíficos como son la cefalea, el mareo, la epilepsia, los déficits neurológicos focales, los síntomas psiquiátricos, y/o la hipertensión intracraneal (White, 2000, Sotelo et al., 2000).

La epilepsia es la presentación clínica más frecuente, se asocia a la localización del parásito en el espacio subaracnoideo de los surcos o en el parénquima (Sotelo et al., 1985, Shandera et al. 1994). La hipertensión intracraneal se caracteriza por cefalea incontrolable, vómitos, alteración del estado de alerta y papiledema. Este síndrome se debe a la obstrucción del flujo o alteraciones de la reabsorción del líquido cefalorraquídeo (LCR) relacionadas con la presencia de uno o varios quistes vesiculares en las cavidades ventriculares y con la inflamación del espacio subaracnoideo o del parénquima cerebral (Sotelo et al., 1985). Los síntomas psiquiátricos asociados a la NC pueden ser la depresión, los episodios sicóticos, la confusión, la demencia y la apatía (Forlenza et al., 1997). Finalmente los déficits neurológicos focales se asocian a la NC parenquimatosa, y su manifestación clínica está relacionada con la localización, el tamaño y el número de parásitos (Sotelo et al 1985, Fleury et al., 2004).

El desarrollo del cisticerco después de entrar a SNC es variable, puede evolucionar a diferentes estadios visibles mediante estudios radiológicos e histopatológico. En el estadio vesicular el parásito es viable, tiene una membrana transparente con fluido vesicular claro, un escólex invaginado. En esta fase el tejido circundante muestra un infiltrado inflamatorio escaso caracterizado por la presencia de linfocitos, células plasmáticas y escasos eosinófilos (Aluja et al., 1988, Rabiela et al., 1989). Algunos pacientes presentan el estadio coloidal en el cual el fluido vesicular es viscoso y turbio, y el escólex muestra signos de degeneración hialina. En este estadio hay una intensa reacción inflamatoria, una intensa gliosis reactiva de tipo astrocitaria, con proliferación de la microglia, edema difuso y cambios degenerativos neuronales en el parénquima cerebral (Escobar et al., 1983, Restrepo et al., 2001). En la mayoría de

las personas infectadas con cisticercos el parásito involuciona sin tratamiento o sin mostrar síntomas clínicos (Fleury et al., 2003). En estas circunstancias la pared del quiste se engruesa y el escólex se transforma en un gránulo mineralizado (i.e., calcificación), el cual es visible en los estudios radiológicos (Sotelo et al, 2000). En algunas personas después del manejo farmacológico el parásito involuciona mostrando las características ya descritas. En el mismo hospedero pueden existir cisticercos en diversos estadios.

El diagnóstico de la NC se hace preferentemente mediante el empleo de técnicas imagenológicas como la tomografía axial computarizada (TAC) y la resonancia magnética (RM). Además estas herramientas se utilizan en el seguimiento de la respuesta del parásito al tratamiento, ya que permiten visualizar su número, localización y estadio de desarrollo. La eficacia diagnóstica de cada método depende del estadio y ubicación anatómica del parásito. Así, la TAC y la RM tienen la misma sensibilidad para la detección de la mayoría de los cisticercos parenquimatosos y calcificaciones, mientras que la RM 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., 1998).

La punción lumbar es un procedimiento auxiliar en el diagnóstico de NC y 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 caracterización precisa de la enfermedad, considerando el cuadro clínico, el estadio, el número y la localización de los parásitos, es fundamental con el objeto de aplicar el tratamiento más adecuado de acuerdo a cada paciente. El manejo quirúrgico se utiliza en casos de hipertensión intracraneal donde la colocación de una válvula de derivación ventrículo-peritoneal alivia la sintomatología (White, 2000, Sotelo et al., 2001). La exéresis de los parásitos ya casi no se practica dada la eficacia de los medicamentos cisticidas, el albendazol y el praziquantel. El albendazol se utiliza más frecuentemente que el praziquantel por tener una mejor distribución en el espacio subaracnoideo, ser más eficaz en la destrucción de los parásitos (70 al 90%) y tener un costo inferior. En caso de no haber respuesta al manejo con albendazol se utiliza el praziquantel (Del Brutto et al., 1999, Sotelo et al., 1988). 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, su uso posterior dependerá de la persistencia de la respuesta inflamatoria del paciente (Del Brutto et al., 1999). Además los pacientes reciben manejo terapéutico sintomático como puede ser el uso de analgésicos, anticonvulsivos, antidepresivos, etc.

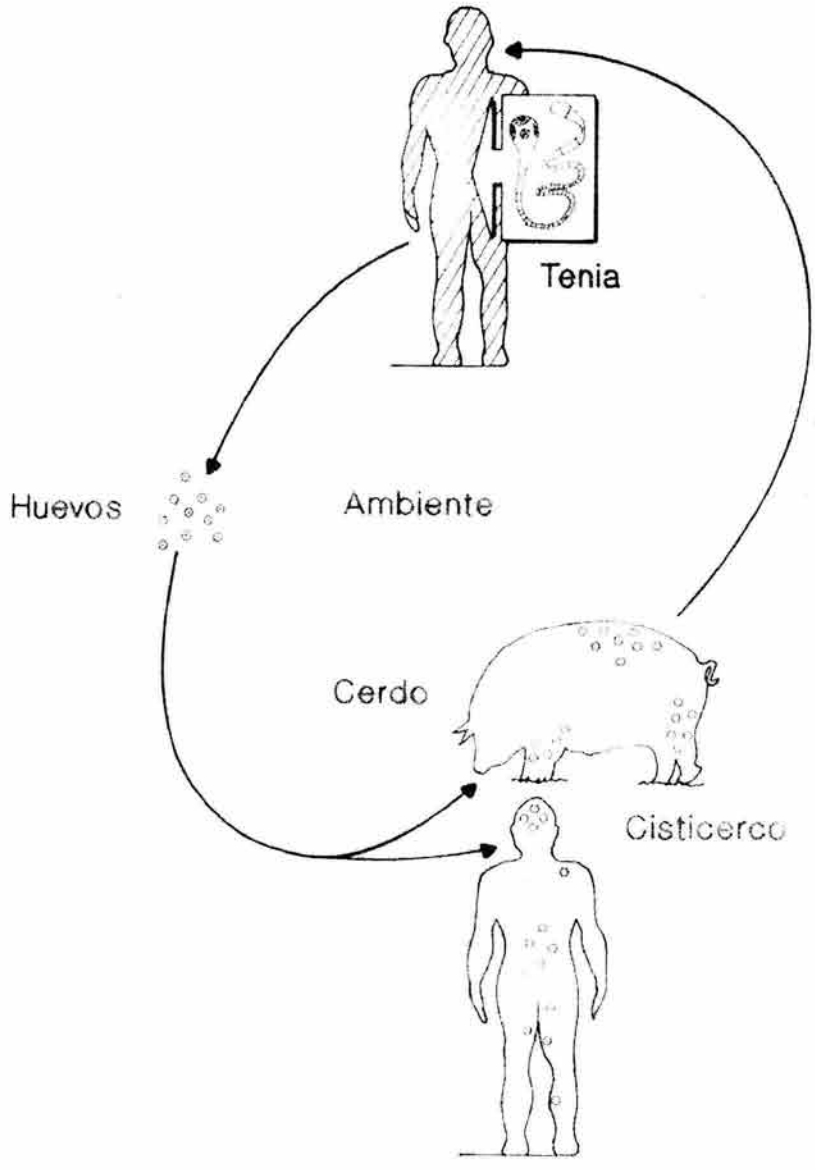


Figura 1. Ciclo de vida de *Taenia solium.*

4. PLANTEAMIENTO DEL PROBLEMA

La NC es una enfermedad clínicamente pleomórfica. Al respecto se sugiere que esta heterogeneidad se asocia con factores relacionados con la exposición (Fleury et al., 2003, Fleury sometido para su publicación), el parásito (Vega et al., 2003) y/o el hospedero (Figura 2). Con relación a los factores del hospedero, la edad y el género se asocian a distintas formas de la NC. Por ejemplo, los pacientes pediátricos presentan más frecuentemente las formas parenquimatosas únicas mientras que los pacientes adultos las formas subaracnoideas e intraventriculares (Saenz, 2004). Además, las mujeres presentan más inflamación en las formas parenquimatosas múltiples y más inflamación en el LCR que los hombres (Fleury et al., 2004, Del Brutto et al., 1988). No obstante estas observaciones, muy pocos estudios han explorado la posible relevancia y la participación de los factores inmuno-inflamatorios como determinantes de la presentación clínica de la NC (ver tabla II en Sciutto et al., 2003, en el apéndice). Sin embargo, estudios en otras enfermedades parasitarias sugieren que el tipo de respuesta inmunológica montada se asocia a diferentes formas de la enfermedad. Por ejemplo, la fibrosis periportal por *Schistosoma mansoni* se asocia a altos niveles de TNFα mientras que formas menos severas de la enfermedad se asocian a niveles altos de IFNγ (Booth et al., 2004, Henri et al., 2002). Lo cual sugeriría la posible implicación de la respuesta inmunológica en el desarrollo de las diferentes formas de NC.

En este trabajo se realizó una descripción de la respuesta inmune asociada a las diferentes formas de la NC considerando su heterogeneidad clínica en la población adulta de ambos sexos. Este estudio se realizó bajo el supuesto de que la

heterogeneidad clínica en la NC podría ser la consecuencia de los diferentes perfiles inmunológicos asociados.

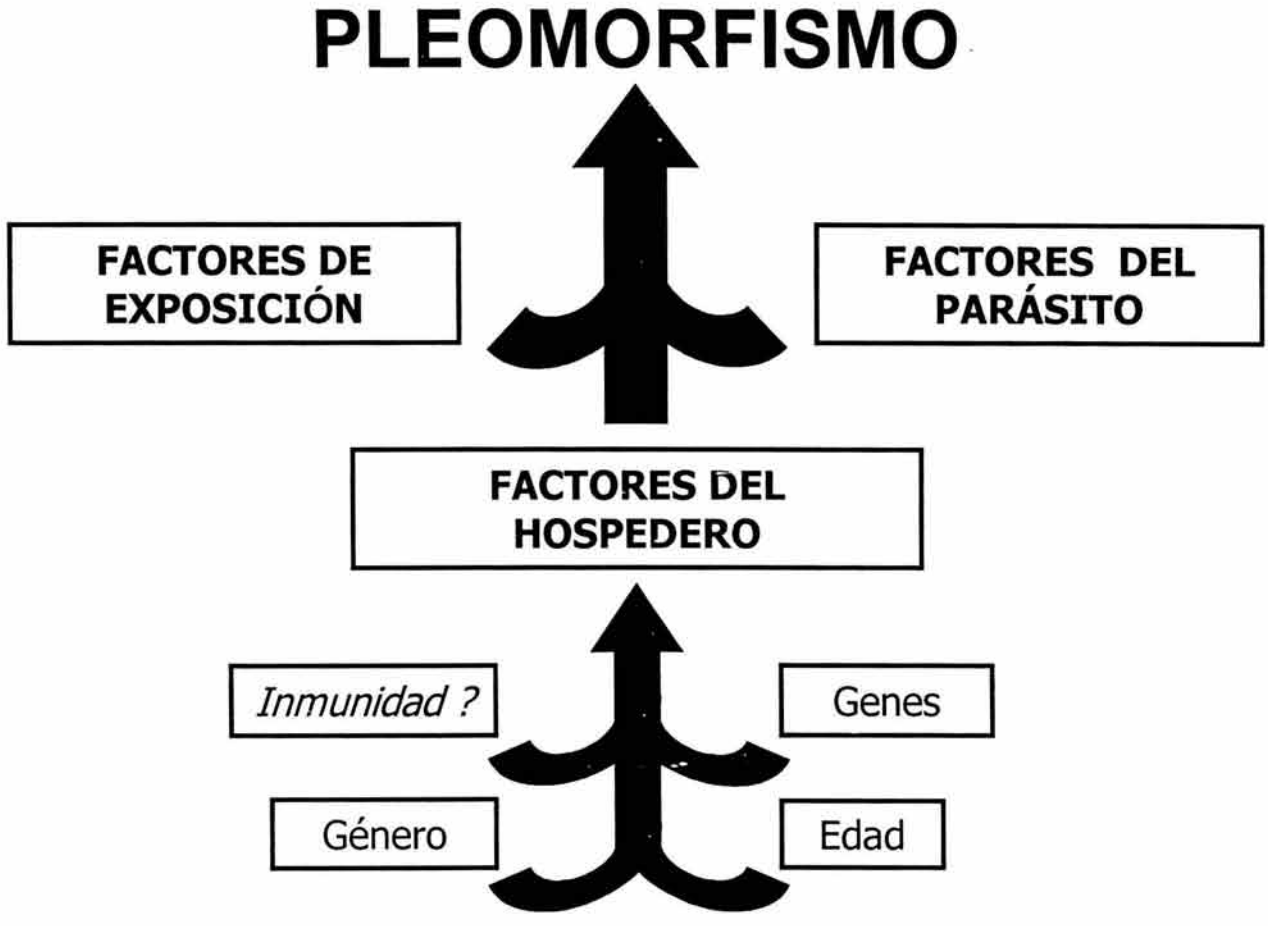


Figura 2. Factores que pudieran influir sobre la heterogeneidad clínica de la Neurocisticercosis. La participación de los factores inmuno-inflamatorios no ha sido esclarecida.

5. HIPÓTESIS

1. Los individuos expuestos a *Taenia solium* presentan un perfil inmunológico diferente de los no expuestos.
2. Los individuos infectados con *Taenia solium* se distinguen por presentar un perfil inmunológico diferente a los individuos expuestos pero no infectados en el SNC.
3. Distintos perfiles inmunológicos se relacionan con las diferentes formas clínicas de neurocisticercosis.

6. OBJETIVOS

A) Objetivos Generales:

1. Describir los perfiles inmunológicos asociados a la exposición y a la infección asintomática y sintomática por *Taenia solium*.
2. Describir los perfiles inmunológicos asociados a las distintas formas clínicas de la neurocisticercosis.

B) Objetivos Específicos:

1. Caracterizar la respuesta inmune humoral y celular en individuos expuestos a *Taenia solium* y en individuos no expuestos.
2. Caracterizar la respuesta inmune humoral y celular en individuos infectados en el SNC por *Taenia solium* y en individuos expuestos no infectados.
3. Caracterizar la respuesta inmune humoral y celular en individuos con distintas formas clínicas de neurocisticercosis.

7. MATERIAL Y MÉTODOS

Ver la descripción detallada en los artículos presentados en la sección de los resultados.

8. DISEÑO EXPERIMENTAL

Definición de casos

El diagnóstico de NC se realizó con base en los resultados de la TAC y/o del RM. En los estudios realizados en población abierta, tanto en Tepezenzintla como en Cuentepec (ver abajo), sólo se practicaron TAC sin aplicación de medio de contraste y en caso de duda se realizó RM. De los pacientes hospitalarios se tuvo acceso al acervo radiológico del Instituto Nacional de Neurología y Neurocirugía (INNN) donde se revisaron las imágenes correspondientes. Las imágenes fueron revisadas por un radiólogo del Hospital General de Puebla (Tepezezintla), un radiólogo del Hospital General Parres de Cuernavaca (Cuentepec), un neurorradiólogo del INNN y dos neurólogos del INNN.

Se consideraron casos con NC aquellos que mostraron imágenes cerebrales quísticas y/o calcificaciones. Aquellos con sintomatología y/o formas vesiculares del parásito recibieron manejo terapéutico y seguimiento médico.

Perfil inmunológico

Se midieron los siguientes parámetros para definir los perfiles inmunológicos relacionados a las distintas formas de la NC:

1. Niveles de anticuerpos específicos contra antígenos del fluido vesicular cisticercos de *T. solium*: subclases de IgG (IgG1, IgG2, IgG3, IgG4) e IgE.

2. Proliferación celular específica con antígenos de cisticerco de *T. solium*.

3. Niveles de citocinas en LCR y en sobrenadantes de células estimuladas específicamente in vitro con antígenos de cisticerco de *T. solium*: TH1 (IL12, IFNγ), TH2 (IL4, IL5, IL10, IL13) e inflamatorias (IL1β, IL6, TNFα).

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 INNN y del Instituto de Investigaciones Biomédicas.

Este trabajo de tesis se desarrolló en tres fases.

Primera fase

Con el objetivo de estudiar los perfiles inmunológicos asociados a la exposición y la infección se estudió la respuesta inmune en muestras de sangre periférica de individuos provenientes de una comunidad rural altamente expuesta a *Taenia solium* y de una comunidad de baja exposición.

Población de alta exposición

La comunidad de Tepetzezintla en el estado de Puebla, cuenta con 1782 habitantes y fue seleccionada por presentar las condiciones higiénico-sanitarias que favorecen el ciclo de vida de *Taenia solium* (90% de los cerdos son criados en libertad,

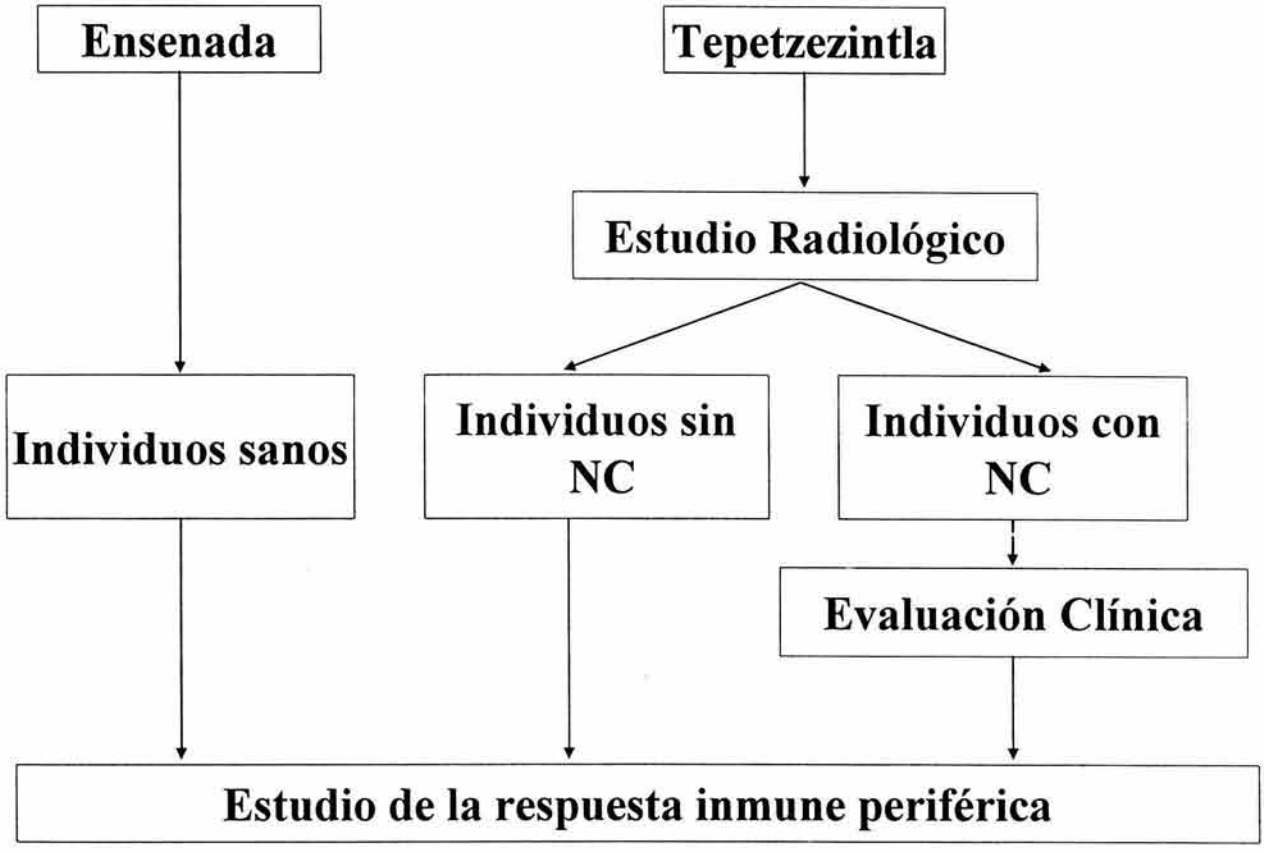
80% de los habitantes practican fecalismo al aire libre, 70% de las casas cuentan con agua potable, 100% de las casas carecen de drenaje). La existencia de transmisión activa de cisticercosis en esta comunidad fue confirmada previa al estudio, determinando la prevalencia de cisticercosis porcina por medio de la inspección visual y la palpación de lengua en un conjunto de 80 cerdos (14%). En un estudio epidemiológico previo se encontró una prevalencia del 9.1% de NC calcificada asintomática y una seropositividad del 6.47%. Mediante una encuesta epidemiológica se determinó que la población está homogéneamente expuesta al parásito y no se encontró correlación entre tener NC y mayor exposición considerando los diferentes factores de exposición evaluados (Fleury et al, 2003).

Para la evaluación inmunológica se incluyeron en el estudio aquellos individuos originarios y/o residentes de la comunidad (personas que habían residido en la comunidad más del 70% del tiempo de su vida), que aceptaron participar en el estudio a través de la firma de un consentimiento informado para la realización de una TAC sin contraste y la toma de una muestra de sangre. Todos los participantes fueron agrupados en NC o no-NC con base en los resultados de las TAC examinadas por dos neurólogos y radiólogos. Ninguno de los participantes recibió tratamiento alguno durante el estudio.

Población de baja exposición

Se seleccionó el municipio de Ensenada en el Estado de Baja California como zona de baja exposición a *Taenia solium* con base en los datos recopilados de seroprevalencia (0.31%; Larralde et al., 1992) y de la probabilidad de exposición determinada por una encuesta previa.

Se incluyeron en el estudio aquellos individuos originarios y residentes del municipio, sin antecedentes de haber viajado fuera del Estado de Baja California y que aceptaron participar en el estudio a través de la firma de una carta de consentimiento informado para la toma de una muestra de sangre. Ninguno de los participantes recibió tratamiento alguno durante el estudio.



Segunda fase del estudio

Con el objetivo de estudiar los perfiles inmunológicos asociados a la infección asintomática y sintomática se estudió la respuesta inmune en muestras de sangre periférica de individuos provenientes de una comunidad rural con casos de NC, en su mayoría asintomáticos, y casos sintomáticos del INNN.

Tipificación de los individuos con NC

Los individuos incluidos en esta fase del estudio fueron clasificados en distintos grupos según su sintomatología:

1) asintomáticos (provenientes de la comunidad rural)

2) sintomatología leve (cefalea)

3) sintomatología moderada (epilepsia, déficits focales, manifestaciones psiquiátricas)

4) sintomatología severa (hipertensión intracraneal además de los síntomas descritos en los incisos 2 y 3).

Según los estudios imagenológicos:

1) número de lesiones (únicas o múltiples)

2) estadio de los cisticercos (vesicular, coloidal, calcificado o formas mixtas)

3) localización del o los parásitos (parénquima, subaracnoideo de los surcos, subaracnoideo de la base, intraventricular o mixto).

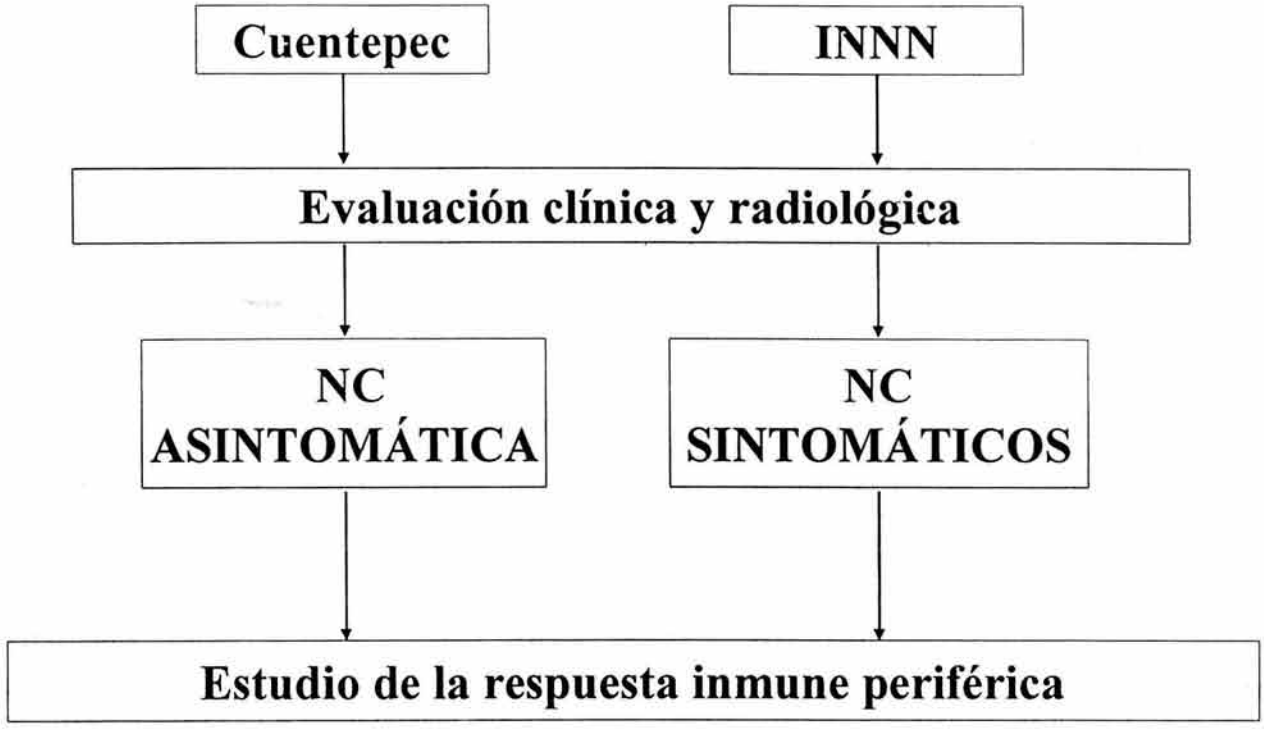
Comunidad Rural

Cuentepec en el estado de Morelos, cuenta con aproximadamente 3000 habitantes y fue seleccionada por presentar las condiciones higiénico-sanitarias que favorecen el ciclo de vida de *Taenia solium* ya mencionados previamente. La existencia de transmisión activa de cisticercosis en esta comunidad fue confirmada previa al estudio, determinando la prevalencia de cisticercosis porcina por medio de la inspección visual y palpación de lengua en un conjunto de 1087 cerdos (32.8%). En un estudio epidemiológico realizado previamente se encontró una frecuencia de NC del 9.1% y una seropositividad del 43.8%. Mediante una encuesta epidemiológica se determinó que la población está expuesta al parásito y no se encontró correlación entre tener NC y una mayor exposición, considerando los diferentes factores de exposición evaluados (Fleury et al., sometido para su publicación).

Se incluyeron en el estudio aquellos individuos residentes de la comunidad nacidos en la misma y que aceptaron participar en el estudio a través de la firma de una carta de consentimiento informado para la realización de una TAC sin contraste y la toma de una muestra de sangre. Todos los individuos identificados como NC fueron revisados por un neurólogo. En dos personas se diagnosticó NC vesicular por lo que recibieron manejo terapéutico y seguimiento médico. Sus muestras de sangre se obtuvieron previas al tratamiento. Ninguno de los demás participantes recibió tratamiento antihelmíntico ni esteroideo durante el estudio.

INNN

Los pacientes que son atendidos en el INNN provienen de todo el país, aunque la mayor parte reside en la Ciudad de México o en los estados vecinos (Morelos, Puebla, Estado de México, Tlaxcala). El INNN sólo recibe a pacientes mayores de 15 años. De cada paciente con NC se revisó el expediente clínico y radiológico. De los expedientes clínicos se obtuvo la siguiente información: edad al diagnóstico, género, cuadro clínico, antecedentes personales y familiares de NC y de teniosis, resultados de laboratorio y datos de la punción lumbar al momento del diagnóstico. Se incluyeron en el estudio aquellos individuos que aceptaron participar en el estudio a través de la firma de una carta de consentimiento informado para la toma de una muestra de sangre. Aquellos pacientes que recibieron esteroides no fueron incluidos en el estudio.



Tercera fase del estudio

Con el objetivo de estudiar la respuesta inmune local asociada a las diferentes formas de la enfermedad en el SNC se estudió el perfil inmunológico en muestras de LCR de pacientes sintomáticos sin tratamiento del INNN.

La punción lumbar fue indicada por el neurólogo tratante como parte del manejo y diagnóstico integral de la NC. Las muestras de LCR se obtuvieron del banco del INNN.

De cada paciente se recabaron los datos generales y clínicos previamente mencionados. Además se obtuvieron los siguientes datos de laboratorio de la punción lumbar: niveles de glucosa, de proteínas, la celularidad y la presencia de eosinófilos. Se consideró el LCR como inflamatorio si el número de células excedía a 5 por ml en punciones no traumáticas. Aquellos pacientes que recibieron tratamiento fueron excluidos del estudio.

INTEGRACIÓN BASICO- CLÍNICA

9. RESULTADOS

Resumen de los resultados de las tres fases

La NC es una enfermedad clínicamente pleomórfica. Se ha sugerido que esta heterogeneidad se asocia a factores relacionados con la exposición, el parásito y/o el hospedero. Entre los factores del hospedero la edad, el género y los factores inmuno-inflamatorios podrían estar participando en la presentación clínica. En este trabajo se describe la respuesta inmune asociada las diferentes formas de la NC considerando su heterogeneidad clínica, en la población adulta de ambos sexos, bajo el supuesto de que esta heterogeneidad podría ser la consecuencia de los diferentes perfiles inmunológicos asociados.

En la primera parte del estudio 10 de 132 habitantes de una comunidad rural de México (Tepetzezintla) presentaron una lesión única calcificada compatible con NC y todos eran asintomáticos. Su perfil inmunológico fue comparado con las otras 122 personas sin NC de la misma comunidad rural. La NC se asoció a una respuesta inmunológica predominantemente TH2 (IgG4, IL-4, IL-5, IL-13). Las personas de la zona de alta exposición al parásito de Tepetzezintla presentaron niveles más elevados de anticuerpos específicos (IgG1, IgG2, IgG4, IgE) y proliferación celular específica que las personas provenientes de un área de baja exposición (Ensenada). Estos datos sugieren que los individuos de Tepetzezintla han estado expuestos pero no se infectaron en el SNC (Chavarria et al., 2003, ver resultados de la primera fase).

En la segunda fase del estudio se describió la respuesta inmunológica asociada a las diferentes formas de NC. El perfil inmunológico de 26 personas con NC asintomática fue comparado con el de 26 pacientes con diferentes formas de NC sintomática. La NC asintomática mostró principalmente cisticercos únicos calcificados

en el parénquima o en el espacio subaracnoideo de los surcos y pertenecían principalmente a un área rural de alta exposición. La mayoría de los pacientes con NC sintomática provenían de áreas urbanas, presentaron múltiples cisticercos principalmente en estadio vesicular o con formas mixtas. La NC asintomática se asoció a una respuesta predominantemente TH2 (IL4, IL5, IL12 and IL13) con bajos niveles de anticuerpos específicos. En contraste, los pacientes con NC sintomática mostraron una respuesta celular específica deprimida con altos niveles de las cuatro subclases de IgG. Estos resultados describen la existencia de perfiles inmunológicos asociados a las diferentes formas de NC y confirman el perfil descrito asociado a la NC asintomática (Chavarria et al., en preparación, ver resultados de la segunda fase).

En la tercera fase del estudio se estudió la respuesta inmunológica asociada a las diferentes formas de NC sintomática en el LCR de 45 pacientes caracterizados clinica- e imagenológicamente. La severidad clínica se asoció a una inflamación en el LCR y se caracterizó por presentar altos niveles de las cuatro subclases de IgG específicas, IL6, IL5, IL10, proteínas y eosinófilos. Pacientes con múltiples lesiones mostraron niveles más elevados de IL5, IL6 e IL10 que aquellos pacientes con lesiones únicas. No se encontraron diferencias clínicas ni imagenológicas entre hombres y mujeres, sin embargo las mujeres mostraron niveles más altos de IL5, IL6 e IL10, lo cual sugiere un dimorfismo sexual de la respuesta inmunológica. La forma clínica más severa se asoció a la presencia de los cisticercos intraventricular o en el espacio subaracnoideo de la base. Estas localizaciones mostraron niveles elevados de las subclases de IgG específicas, IL5, IL6 e IL10. Estos resultados sugieren de qué manera la respuesta inmunológica en el SNC pudiera estar influyendo en el desarrollo de las

diferentes formas clínicas de la NC (Chavarria et al., en revisión en el Microbes and Infection, ver resultados de la tercera fase).

Los resultados de este trabajo describen los perfiles inmunológicos asociados a las diferentes formas de NC, evidencias de un dimorfismo sexual inmunológico y de una compartimentalización de la respuesta inmunológica en el SNC.

A) Resultados de la primera fase

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Original article

TH2 profile in asymptomatic *Taenia solium* human neurocysticercosis

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Abstract

Neurocysticercosis (NC), a parasitic disease caused by *Taenia solium*, may be either asymptomatic or have mild to severe symptoms due to several factors. In this study, the immunological factors that underlie NC pleomorphism were studied. Ten of the 132 inhabitants of a rural community in Mexico (Tepez) had a computerized tomography (CT) scan compatible with calcified NC, and all were asymptomatic. Their immunological profiles were compared with those of 122 CT scan negative (non-NC) subjects from the same village. NC was associated with a TH2 response (IgG4, IL-4, IL-5, IL-13). Subjects from Tepez had higher levels of specific antibodies (IgG1, IgG2, IgG4, IgE) and specific cell proliferation than subjects from an area with low exposure (Ensenada). This suggests that non-NC subjects from Tepez had been exposed to *T. solium* and resisted infection in the brain. Distinct immunological profiles in equally exposed individuals differing in outcome of infection support the hypothesis of host-related factors in resistance to and pathogenesis of NC. This is the first study reporting the immunological profile associated with the asymptomatic form of NC.

Keywords: *Taenia solium*; Neurocysticercosis; Asymptomatic; Immunological profiles; TH2 response

1. Introduction

Neurocysticercosis (NC) is a disease caused by the establishment of *Taenia solium* larvae (cysticercus) in the central nervous system (CNS) of the host, be it man or pig. NC is a major human health problem in developing or poor areas of Latin America, Asia and Africa, and it is spreading worldwide due to increased migration of NC cases and of tapeworm carriers [1–3], putting new populations at risk and causing significant public health costs. In Mexico, NC is the first cause of adult epilepsy onset [4,5]; it is also the cause of 11% of the neurological consultations [6], of 25% of the craniotomies [7] and is found in 2–3% of large necropsy series [8]. It is also notable that many cases of NC (approximately 50%) are asymptomatic or clinically silent [8]. In addition, there is wide variation in the clinical and pathological pictures of symptomatic NC, as well as in the parasite's macro- and microscopic appearance, some being alive, whilst others are observed at different levels of disintegration or are even totally substituted by calcium deposits [9]. The course of the symptomatic disease, the immunological and inflammatory responses of the host to the parasite, as well as the effectiveness of treatment, are also quite variable [10].

We propose that the extreme diversity of NC presentation is due not only to exposure and parasite factors [11] but also to host factors, where immunity, genes and gender might be involved [12]. The clinical variants of NC would constitute the susceptible phenotype, which has been sub-classified into silent-NC, symptomatic, mild NC and symptomatic, severe

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NC, according to symptoms and state of the host–parasite relationship. The resistant phenotype is proposed to be composed of those individuals exposed but with no CNS infection. Should the hypothesis be correct, each phenotype and its subtypes should show distinct immunological profiles, as defined by *T. solium* antigen-stimulated expression of TH1 and TH2 immunological components with known inflammatory activity. The density of each phenotype is expected to vary according to the sampled population: the symptomatic phenotypes will be found primarily in patients attending neurological institutions, whereas the silent-NC phenotypes and the resistant phenotype will have to be searched for in apparently healthy people living in areas of high exposure, distinguished from each other by computerized tomography (CT) scan and/or resonance imaging. Here we report on the immunological profiles of the 10 silent-NC cases found in a previous CT scan epidemiological survey [11] of people living in a highly exposed village in Mexico—Tepetzezintla (Tepez) state of Puebla, of the 122 non-NC individuals also living in Tepez, and of 28 individuals from an area with low exposure (Ensenada, Baja California). There are significant differences among their immunological profiles, a finding that strengthens the notion of the involvement of significant host-related immunological factors in susceptibility to and pathogenesis of human NC.

2. Materials and methods

2.1. Study subjects

This study was performed in subjects from the community of Tepetzezintla (Tepez), state of Puebla. This community was selected because of the inadequate sanitary and socioeconomic conditions, which promote [11] a high transmission of *T. solium* cysticercosis [13]. A previous report determined an NC prevalence of 9.1% by head CT scan; all cases were asymptomatic and had calcified lesions. There was no correlation between NC and any of the measured exposure factors (i.e. characteristics of living quarters, hygienic, socioeconomic and dietary data). A head CT scan without contrast was applied to a sample of 132 residents randomly selected in the Hospital General de Puebla. All participants volunteered to enter the study, donated a blood sample and gave informed consent. None of the participants received antihelminthic treatment, steroids or anticonvulsive therapy during the study, when not medically indicated. The study lasted from August 2000 to July 2001.

In addition, to determine the immunological profile of people living in the low-exposure area, blood samples from 11 women and 17 men (12–62 years old), resident in Ensenada, Baja California, were collected and assayed as for the Tepez group. The Ensenada group was considered unexposed, based on a questionnaire concerning exposure factors [11], on the absence of rustic pig breeding and on the low positive serology for cysticercosis [14]. Informed consent for this study was obtained from all participants or their guardians. The study was approved by the ethical committee of the Instituto de Investigaciones Biomédicas of the Universidad Nacional Autónoma de México.

2.2. Antigen preparation

Whole *T. solium* cysticerci were obtained from skeletal muscle of one infected pork from central Mexico, washed with phosphate-buffered saline solution, homogenized, and centrifuged at 25 000 × *g* for 45 min at 4 °C. The 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 × *g* for a further 40 min at 4 °C. The supernatant was recovered and filtered with 0.22 mm under sterile conditions, quantified with the method of Lowry, and frozen at –20 °C until used as a whole antigen fraction (*Ts*Ag).

2.3. Immunological profile

The following set of features was selected as a first approach to define the immunological profile (antibody levels, in vitro cell proliferation in response to antigen stimulation) and with TH1 (INF-γ, TNF-α) and TH2 (IL-4, IL-5, IL-10, IL-13) cytokines.

2.3.1. Lymphocyte proliferation

Ten to twenty milliliters of peripheral venous blood from each participant was drawn into a tube containing EDTA. The blood was then diluted 1:2 with RPMI medium 1640 (Gibco BRL, Grand Island, NY) and layered over Ficoll-Hypaque (Amersham Life Science, Little Chalfont, UK). The peripheral blood mononuclear cells (PBMCs) were collected after 30 min of 400 × *g* centrifugation at room temperature. Diluted plasma was recovered and frozen at –80 °C until used. The PBMCs were washed three times with RPMI, suspended in RPMI-1640 (Gibco BRL) supplemented with 10% human serum AB (donated from the blood bank of Centro Médico Siglo XXI, México), 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin, 1% nonessential amino acids and 1% pyruvate (Gibco BRL). The PBMCs were stimulated with concanavalin A (0.5 μg per well; Sigma, St. Louis, MO), *Ts*Ag (10 μg per well), incubated at 37 °C and 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 μl of final volume per well. After 6 days, the cells were pulsed with 1 μCi of methyl-[^{3}H]-thymidine (Amersham Life Science) for a further 18 h, and PBMCs were harvested onto glass filter papers. The amount of incorporated label was measured by counting in a 1205-β spectrometer (Wallac).

2.3.2. Cytokine production

PBMCs were plated at a cell concentration of 2.5×10^6 cells per ml per well in 12-well cluster plates (Costar), incubated at 37 °C and 5% CO_2 humidified atmosphere. Cells were stimulated with *Ts*Ag (10 μg/ml). After 48 (IL-10,

TNF-α) and 120 h (for the other cytokines) of incubation, the culture supernatants were harvested and stored at –80 °C for cytokine quantification.

2.3.3. Cytokine titration

Sandwich ELISAs were performed with 96-well, flat-bottom microtiter plates (Nunc-Immuno Plate Maxisorp, Rosekilde, Denmark). Microplates were coated for 18 h at 4 °C with the capture antibody (BD Pharmingen, San Diego, CA for IL-4, IL-5, IL-10, IL-13, INF-γ, R&D Duo-Set, Abingdon, UK, for TNF-α), washed three times with PBS–Tween-20 (0.05%), blocked for 30 min at room temperature with PBS–BSA 2%, and washed three times. Plates were incubated for a further 18 h with the antibody standards and supernatants diluted 1:2 with PBS–Tween-20 (0.05%)–BSA 0.5% at 4 °C, washed three times, and incubated with the detection antibody (BD Pharmingen for IL-4, IL-5, IL-10, IL-13, INF-γ, R&D Duo-Set for TNF-α) for 2 h at room temperature. Bound detection antibodies were detected using 1:10 000 diluted streptavidine–phosphatase conjugate (BD Pharmingen) and *p*-nitrophenylphosphate (Sigma) as substrate. Optical density (OD) reading was performed at 30 and 60 min of incubation at 405 nm. Assay sensitivity was 4.69 pg/ml for IL-4, 9.38 pg/ml for IL-5, 3.91 pg/ml for IL-10, 6.25 pg/ml for IL-13, 9.38 pg/ml for INF-γ and 15.63 pg/ml for TNF-α.

2.3.4. Total IgG antibody detection by ELISA

Total IgG (IgGt) titers were measured in plasma by indirect ELISA using *T. solium* cyst fluid as described before [15]. Plates were incubated overnight at 4 °C with *T. solium* cyst fluid (1 μg per well) at a final volume of 100 μl per well. Then, wells were washed, incubated with the 1:50 diluted plasma for 1 h at 37 °C, washed, incubated with rabbit anti-human IgG alkaline phosphatase conjugate (Zymed Laboratories, San Francisco, CA) for 1 h at 37 °C, washed and incubated with 100 μl of substrate (*p*-nitrophenylphosphate, Sigma) for 10 min at 37 °C. Plates were read at 405 nm. All assays were performed in duplicate.

2.3.5. IgG subclasses and IgE antibody detection by ELISA

Plasma antibody titers were measured by indirect ELISA. *T. solium* cyst fluid (1 μg per well) at a final volume of 100 μl per well was incubated overnight at 4 °C. The wells were washed, incubated with the 1:50 diluted plasma for 1 h at 37 °C. Bound immunoglobulins were developed using rabbit anti-human biotin-labeled IgG1, IgG2, IgG3, IgG4 or IgE (Zymed Laboratories) and streptavidin alkaline phosphatase (Zymed Laboratories). *p*-Nitrophenylphosphate (Sigma) was used as substrate. Plates were read at 405 nm. All assays were performed in duplicate.

2.4. Statistical analysis

Data were processed in Excel 7.0 (Microsoft) and Spss 10.0 for Windows. The Mann–Whitney non-parametric *U*-test was used to identify the differences in the immunological response between groups. The silent-NC phenotype can depend on several co-variables, some of these co-variables can be confounded with the effect of others, and their effect on the phenotype must be tested simultaneously (multivariate analysis). Logistic regression was performed considering age, gender, cytokines and immunoglobulins tested. Descending stepwise-regression analysis was performed on the risk of developing silent-NC with the following variables: IL-4, IL-5, and IFN-γ were treated as qualitative variables with two classes defined by the median cytokine level value. This was necessary, since a number of cytokine titration values were below detection levels. IL-13 and IgG4 levels were treated as quantitative variables. Variables IL-10, TNF-α, IgG1, IgG2, IgG3, IgE, IgGt that yielded *P* values >0.2 in the univariate analysis were not incorporated to the regression analysis.

3. Results

3.1. CT scan results

Ninety-two females and 40 males, with ages ranging from 3 to 79 years, participated in the CT scan study. Individuals were classified as silent-NC or non-NC according to whether their CT scan images showed lesions compatible with NC. Ten cases of silent-NC (one man and nine women) were found with a single calcified granuloma (Fig. 1). The remaining 122 subjects showed CT scans negative for NC (four had anatomical abnormalities and 118 had normal brain images). The non-NC group probably included subjects who were exposed and did not develop an established infection, others with an asymptomatic infection now resolved without calcification, and some who were not exposed. All individuals with silent-NC were examined by two neurologists and found to be apparently healthy and neurologically asymptomatic.

Calcifications found in the CT scan were considered to be of cysticercal origin based on the high prevalence of neurocysticercosis in Mexico [16], the disproportionately high prevalence of neurocysticercosis relative to tuberculous granulomas in large necropsy series of medical institutions [8,9], the characteristic image of calcified cysticerci (approximately 1 cm diameter, round, uniformly hyperdense, sharply bordered lesion, with small or no sign of surrounding inflammation, that does not resemble cerebral or meningeal tuberculoma at all [17], and the extremely low prevalence of cerebral hydatid disease of humans in Mexico [8].

3.2. Immunological profiles

The levels of PBMC proliferation after *Ts*Ag stimulation and cytokine (IL-4, IL-5, IL-10, IL-13, INF-γ and TNF-α) production in the supernatants, as well as specific antibodies (IgGt, IgG1, IgG2, IgG3, IgG4 and IgE) are shown in Table 1. The immunological features most frequently found

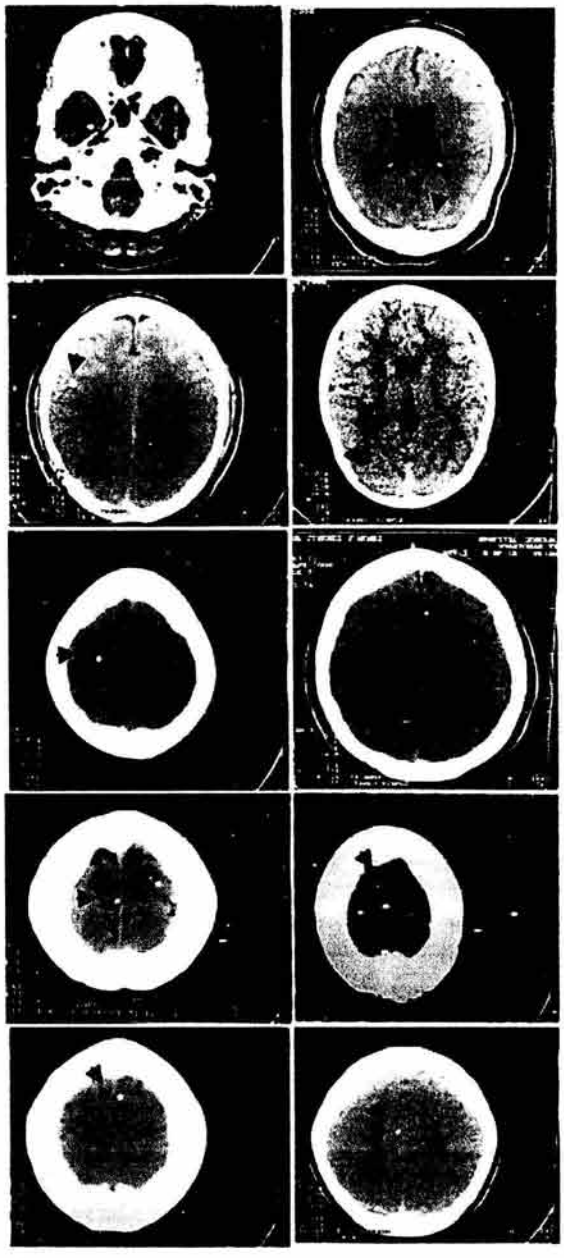

Fig. 1. CT scans of the 10 Tepetzezintla NC cases showing a single calcified granuloma (arrow heads point to the calcified cysts).

in excess in the silent-NC group were IgG4 ($P < 0.001$, Mann–Whitney U-test), IL-4 ($P = 0.001$), IL-5 ($P = 0.05$) and IL-13 ($P \leq 0.02$) as shown in Table 1. It should be mentioned that there were no significant differences between the silent-NC and non-NC cases in the remaining immunological features tested. As for the immunological profile of individuals living in a low-exposure area (Ensenada), it was clearly lower in SI ($P < 0.001$), IgGt ($P \leq 0.003$), IgG1 ($P < 0.001$), IgG2 ($P < 0.001$), IgG4 ($P \leq 0.02$), IgE ($P \leq 0.05$).

The upper 75% percentiles from Table 1 denote that the silent-NC group includes a higher proportion of strong responders in IL-4, IL-5, IL-13 and IgG4 than the non-NC cases. Subjects from Ensenada differ extremely from those of Tepez in that most subjects have much lower values of the tested features. The NC cases from Tepez were classified as immunologically positive or negative according to their response to a given variable exceeding the 95% confidence interval of the values of the non-NC population (Fig. 2).

To determine which immunological features could be markers for silent-NC, regression analysis was conducted including cytokine and immunoglobulin levels as explanatory variables for the risk of developing silent-NC versus exposed but non-NC. The best models included IgG4 ($P < 0.001$) and IL-4 ($P = 0.01$) or IgG4 ($P < 0.001$ and IL-13 ($P = 0.03$). The risk of silent-NC was enhanced when IgG4, IL-4 and IL-13 levels increased.

4. Discussion

The present results show significant differences in the immunological profiles of subjects living in areas of high (Tepez) and low exposure (Ensenada) to *T. solium*, and also in those with positive or negative CT scan for NC, living in a high-exposure situation (Tepez).

Differences in social, economic and cultural traits between Ensenada and Tepez with the consequent differences in overall risk of infection [14], and also putative differences in the antigenic environment may account for the more pronounced specific immune response of subjects from the latter village. In addition, antigenic similarities among helminths have been documented [18,19], and thus cross-reactivity may additionally explain the higher immunological reactivity of exposed individuals when tested with complex antigen mixtures that may possibly contain antigenic motifs which are similar in different parasites.

The most potent discriminating features between the high- (Tepez) and low-exposure (Ensenada) groups were IgG1, IgG2, SI, IgGt, IgG4 and IgE, in order from most to least discriminating. Any one of these immunological features, or a combination of them, may very well be used as an index of exposure to *T. solium* transmission in epidemiological studies.

There were also significant differences in immunological profile between the Tepez groups. The study shows that all the silent-NC cases responded to *T. solium* antigens, although heterogeneously, with either elevated in vitro cell proliferation, cytokine production and/or antibody levels in serum: 40% of the silent-NC cases presented significantly higher levels of IL-4 and IL-5 production than the non-NC, 30% in IL-13 levels, and 100% in IgG4 levels. However, the cellular proliferative response, IL-10, TNF-α, IFN-γ, IgGt, IgG1, IgG2 and IgG3 levels were similarly elevated in both Tepez groups. Thus, the silent-NC cases have immunological

Table 1
Immunological profiles from silent-NC and non-NC subjects from Tepetzezintla and from Ensenada

Immunological profile	Tepetzezintla		Ensenada	P1	P2
	Silent-NC	Non-NC			
SI [a]	8 [c]	5	1.52	NS [e]	<0.001
	41 [f]	*14*	*5*		
IL-4 (pg/ml)	4.9	<4.7	ND [d]	0.001	
	12	*4.7*			
IL-5 (pg/ml)	154	20	ND	0.05	
	512	*88*			
IL-10 (pg/ml)	39	56	ND	NS	
	211	*265*			
IL-13 (pg/ml)	457	113	ND	0.02	
	1029	*314*			
INF-γ (pg/ml)	93	12	ND	NS	
	446	*49*			
TNF-α (pg/ml)	1076	950	ND	NS	
	3278	*2876*			
IgGt (OD) [b]	0.325	0.249	0.171	NS	0.003
	0.425	*0.327*	*0.248*		
IgG1 (OD)	0.272	0.233	0.062	NS	<0.001
	0.437	*0.337*	*0.071*		
IgG2 (OD)	0.179	0.143	0.061	NS	<0.001
	0.695	*0.229*	*0.064*		
IgG3 (OD)	0.321	0.367	0.385	NS	NS
	0.638	*0.527*	*0.546*		
IgG4 (OD)	0.226	0.077	0.089	<0.001	0.02
	0.349	*0.086*	*0.088*		
IgE (OD)	0.137	0.135	0.125	NS	0.05
	0.152	*0.154*	*0.141*		

Immunological features from the three groups were compared by the non-parametric Mann–Whitney *U*-test. The test was performed either comparing silent-NC with non-NC, yielding *P*1 values or comparing the low-exposure group, Ensenada, with the entire high-exposure group, Tepez, yielding *P*2 values. *P*1 and *P*2 values are indicated when they are ≤0.05.

[a] SI is presented as cpm antigen-stimulated cells/cpm non-stimulated cells.

[b] OD optical density.

[c] SI, cytokine levels and antibody levels are in median values.

[d] ND, not determined.

[e] NS, not significant.

[f] The 75% upper percentile values.

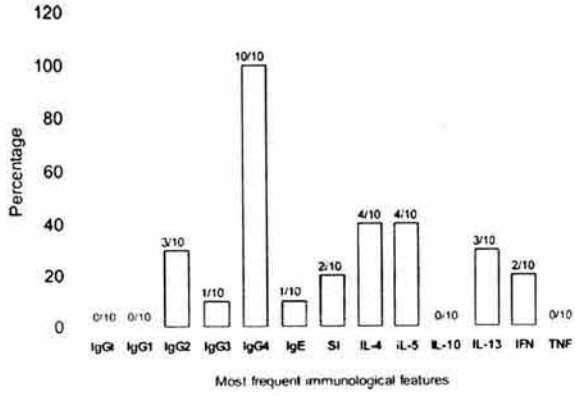

Fig. 2. Percent of silent-NC exceeding the 95% confidence interval (CI) of the values of the non-NC group in each and in all immunological features assayed. By definition, 5% of the non-NC group exceeds the CI. The most distinctive features of the immunological profile of the silent-NC cases are (IgG4) > (IL-4, IL-5) > (IL-13, IgG2) > (SI, IFN-γ) > (IgG3, IgE) > (IgGt, IgG1, IL-10, TNF-α).

profiles higher than those of non-NC subjects living in Tepez, with a predominance of TH2 clinical immunity and no associated brain damage, since they did admit parasite establishment in their CNS but managed to suppress it, perhaps by leading it to calcification. We propose that the putative resistant phenotype (exposed, but non-NC) is characterized by a prominent, balanced TH1/TH2 immunological profile, whilst the putative susceptible phenotype (silent-NC) favors the TH2 response in addition to the TH1 response.

The type of immunological profile of a patient may influence NC pathogenesis. Symptomatic NC, although not thoroughly explored yet, is frequently found to exhibit increased proteins, antibodies and inflammatory cells in cerebrospinal fluid (CSF) [7], as well as proinflammatory cytokines like IL-1β, IL-6 and TNF-α [20,21]. In contrast, silent-NC cases are seldom studied clinically but in necropsies, and they are reported to have little inflammation [22], as is the common finding in skeletal pig cysticercosis [23] and in experimental murine *Taenia crassiceps* cysticercosis [24]. The 10 cases of silent-NC had a non-inflammatory profile, it being mainly

composed of IL-5, IL-13 and IL-4 production and by the presence of IgG4 in serum.

If prominent TH2 profiles in infected subjects underlie low or no symptoms at all in NC, one could speculate that IL-4 downregulation of the inflammatory response is involved, as it is in schistosomiasis granulomas [25,26], in experimental cysticercosis by *T. crassiceps* [27], and in brain granulomas in symptomatic NC [28]. Also, IL-4 can induce the switch of IgG4 and IgE [29] and thus explain the higher level of IgG4 in the NC group. The role of IL-5 and IL-13 in parasitic disease seems to be important in the elimination of the parasite [30,31]; here we show the presence of IL-5 in cell supernatants of the silent-NC group, as shown previously in symptomatic NC in serum and CSF [30,32]. Thus, one way to eliminate the cysticercus without creating too much inflammatory response in the brain would be to induce high IL-4, IL-13 and IL-5 peaking-out of a generally more alert immune system but not particularly high in TH1 activity and inflammatory cytokines. IgG4 and IgE are both considered to be TH2 immunoglobulins in humans [29]. IgE has been associated with resistance to parasitic disease as in schistosomiasis [33,34], whilst IgG4 has been proposed as an IgE-competing immunoglobulin [34], because high levels of IgG4 in serum are associated with susceptibility, as proposed for *Schistosoma mansoni* and *haematobium* infections [33,35]. Among highly exposed individuals from Tepez, IgG4 was increased only in those with silent-NC, a suggestion of a profile of susceptibility to the benign form of the CNS infection. It is tempting to speculate that resistance to exposure may be related to their low IgG4 responsiveness. The non-NC Tepez group had levels of IgE indistinguishable from those of the silent-NC, but both differed from the low-exposure Ensenada group. This result points to IgE as an additional marker for parasite exposure.

Consequently, for NC we would propose that the immunological profile combining high TH1 with peaking TH2 features associates with a noiseless course of the infection and with its resolution in the silent-NC cases, and predict that the opposite is behind the immunoinflammatory response and clinical manifestations of the symptomatic NC cases [36,37]. The study of more NC cases, in all their forms, is necessary to formally establish their specific immunoinflammatory profiles.

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B) Resultados de la segunda fase

En preparación.

PERIPHERAL IMMUNE RESPONSE DETERMINES CLINICAL OUTCOME OF NEUROCYSTICERCOSIS

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Abstract

Human neurocysticercosis (NC), a parasitic disease caused by *Taenia solium,* may be either asymptomatic or with mild to severe symptoms due to several factors. It has been suggested that this clinical heterogeneity is related to host factors (gender, age, immune-inflammatory response). In this work a description of the immune response related to different NC forms in the adult population of both sexes was made. The immunological profiles of 26 asymptomatic NC cases were compared with 26 symptomatic NC patients. Asymptomatic NC cases presented principally single calcified cysticerci in parenchyma or subarachnoid space of the sulci and belonged to a high exposure rural area. Most of the symptomatic patients were from urban areas, had multiple cysticerci in the CNS frequently in vesicular or mixed stages. Asymptomatic NC was associated with a predominantly TH2 response (IL4, IL5, IL12 and IL13) and low levels of IgG subclasses. In contrast, symptomatic NC showed a depressed specific cellular immune response and increased levels of all four IgG subclasses. The results of this work describe the existence of immune profiles associated to the different forms from NC and add new elements to further understand the immunological mechanisms involved in NC disease as well as the relation between the immune response in the SNC and the peripheral system in NC.

Keywords: *Taenia solium* / neurocysticercosis/ CNS / inflammation / immunological profiles

1. Introduction

Human neurocysticercosis (NC), caused by *Taenia solium* larvae in the central nervous system (CNS), is a frequent parasitic disease in developing countries of Latin America, Asia and Africa, and it is re-emerging in developed countries due to increased migration of NC cases and of tapeworm carriers (White, 2000, Schantz et al., 1992 , Shandera et al., 1994). NC is a clinical pleomorphic neurological disease: it can be completely asymptomatic (Villagran et al., 1988, Fleury et al., 2003, Chavarria et al., 2003), or be manifested by a wide variety of non-specific symptoms (headache, epilepsy, dementia, depression or intracranial hypertension). Although the causes that underlie this pleomorphism are not completely understood, the relevance of host and parasite factors begins to emerge. Recent findings of genetic differences found in the cysticerci from Mexico and Madagascar suggest the possibility that parasites may vary in infectivity and pathogenicity and thus contribute to the heterogeneous clinical and immunological expression of NC (Vega et al., 2003). From host factors, the inflammatory response was found to be related to parasite number, stage and location. All these variables were associated to disease severity (Fleury et al., 2003, 2004).

To explain the extreme diversity of NC presentation, we proposed the existence of phenotypes (related to exposure, infection and symptomatology) that associate with specific immunological profiles (Chavarria et al.,2003). In a first study, inhabitants of a rural area of high exposure to *Taenia solium* were classified according to their head computerized tomography scan in not-NC (individuals exposed but not infected in the CNS, resistant to the infection) and NC (susceptible to the infection). The resistant phenotype was characterized by a prominent and balanced TH1/TH2 immunological profile. The susceptible phenotype, all of them asymptomatic and with calcified lesions,

favoured a TH2 response with high levels of IL4, IL5, IL13 and specific increased levels of IgG4 (Chavarria et al., 2003). Contrary to other helminthiasis, in which a TH1 response favours the resolution of the infection while parasite survival is favoured by a TH2 response (Rodriguez Sosa et al., 2004), a TH2 peripheral profile was associated with a noiseless course and resolution of the infection. Thus, it is possible that a different immunological profile could be related to the symptomatic patients. This possibility was began to be explored determining cytokines levels in the cerebral spinal fluid (CSF) of hospital NC patients with different grades of severity. One of the most relevant findings was increased levels of all specific IgG subclasses, IL5, IL6 and IL10 related to parasite location in the subarachnoid space of the base or intraventricular, to vesicular parasite stage and severe symptomatology (Chavarria et al., submitted). However, this information cannot be directly compared to the peripheral immune response previously described. Thus, in this study the specific peripheral immune response was explored in symptomatic NC patients, attending a neurological institution, and compared to a group of asymptomatic NC, inhabitants from a highly exposed rural community.

2. Materials and Methods

2.1. Subjects

This study was performed in subjects from the community of Cuentepec, State of Morelos, Mexico, and patients that were attended at the Instituto Nacional de Neurología y Neurocirugía (INNN) in Mexico City. None of the participants received steroid treatment at the moment of blood sampling. Age at diagnosis, gender and place of residency were collected from each patient. The study was performed between august 2000 and august 2003.

2.1.1. Hospital Subjects

Most of the patients attended at the INNN were from Mexico City and from the neighbour states like Morelos, Puebla, State of Mexico and Tlaxcala. INNN only admits patients older than 15 years of age.

2.1.2. Community Subjects

Cuentepec was selected because of its inadequate sanitary socioeconomic conditions that promote high transmission of *T. solium* cysticercosis. The cases included were considered as NC based on a head CT scan study applied during an epidemiological study performed in this community which included almost the fourth part of the inhabitants. (Fleury submitted).

2.1.3. Ethical considerations

This study fulfils the regulations on research done in human subjects considered by Mexican law as well as by international regulations. It also complies all the ethical aspects considered in the Health General Rules in Clinical Investigation. It does not contain human experimentation. All the subjects included were voluntarily invited to participate. Participants and parents or guardians (in case of minors) received an explanation of the project objectives and the specific procedures included in the study. Afterwards they gave informed consent in writing. Results were confidential. Community subjects that were diagnosed with NC and required medical attention and specific treatment were attended by neurologist of the INNN. Subjects with another neurological condition counselling and medical attention at INNN was offered.

2.2. Characterization of the NC cases

NC diagnosis was based on CT scan and magnetic resonance image (MRI) before receiving specific treatment. Parasite stage was considered as follows based on the CT and/or MRI image: 1) vesicular (parasite with transparent membrane and

vesicular fluid and scare inflammatory reaction around); 2) colloidal (degenerating parasite with turbid fluid surrounded by an inflammatory reaction); 3) calcified (dead parasite that appears as a mineralized granuloma).

From imagenological studies of each NC case, the following information was collected: number of lesions (single vs. multiple), stage of cysticerci [vesicular, colloidal, calcified or mixed forms] and CNS location [subarachnoid space of the base (SA base) or of the sulci (SA sulci), parenchymal, or intraventricular].

The clinical expression of the disease was established by clinical examination of the participants by two neurologists. Based on the symptomatology, participants were grouped in four classes: 1. Asymptomatic; 2. Mild: headache; 3. Moderate: focal deficits and/or seizures, and 4. Severe: intracranial hypertension (defined by presence of headache, nausea, vomiting and papilledema).

2.3. Antigen Preparation

Whole *Taenia solium* cysticerci were obtained from skeletal muscle of one infected pork from central Mexico, washed with phosphate-buffered saline solution, homogenized, and centrifuged at 25000 X g for 45 min at 4 °C. The soluble antigens in the supernatant were recovered; calcium was precipitated with ammonium oxalate 0.3 M and ammonium hydroxide 1:10 and centrifuged at 25000 X g for a further 40 min at 4 °C. The supernatant was recovered and filtered with 0.22 mm under sterile conditions, quantified with the method by Lowry, and frozen at –20 °C until used as a whole antigen fraction (TsAg).

2.4. Immunological profile

The following features were measured to define an immunological profile related to NC: *T. solium* specific IgG subclasses levels (IgG1, IgG2, IgG3, IgG4), in vitro specific cell proliferation, TH1 (IL12, IFNγ), TH2 (IL4, IL5, IL10, IL13) and inflammatory (IL1β, IL6, TNFα) cytokines in supernatants of specifically stimulated cells in vitro.

2.4.1. Lymphocyte Proliferation

Ten to twenty ml of peripheral venous blood from each participant were drawn into a tube containing EDTA. The blood was then diluted 1:2 with RPMI Medium 1640 (Gibco BRL, Grand Island, N.Y.) and layered over Ficoll-Hypaque (Amersham Life Science, Little Chalfont, United Kingdom), the peripheral blood mononuclear cells (PBMCs) were collected after 30 min of 400 X g centrifugation at room temperature. Diluted plasma was recovered and frozen at –80 °C until used. The PBMCs were washed three times with RPMI, suspended in RPMI Medium 1640 (Gibco BRL) supplemented with 10 % of human serum AB (donated from The Blood Bank of Centro Médico Siglo XXI, México), 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin, 1 % non-essential amino acids and 1 % pyruvate (Gibco BRL). The PBMCs were stimulated with concanavalin A (0.5 μg/well; Sigma, St. Louis MO), TsAg (10 μg/well), incubated at 37 °C and 5 % CO2 humidified atmosphere in 96-well flat-bottom culture plates (Costar, Cambridge, Mass.) 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 1 μCi of methyl-[^{3}H]-thymidine (Amersham Life Science, Little Chalfont, United Kingdom) for further 18 hours, PBMCs were harvested onto glass filter papers; the amount of incorporated label was measured by counting in a 1205-β spectrometer (Wallac).

2.4.2. Cytokine Production

PBMCs were plated at a cell concentration of 2.5 X 10^6 cells/ml per well in 12-well cluster plates (Costar, Cambridge, Mass.), incubated at 37 °C and 5 % CO2 humidified atmosphere. Cells were stimulated with TsAg (10 μg/ml). After 48 (IL-10, TNF-α) and 120 hours (for the other cytokines) of incubation, the culture supernatants were harvested and stored at –80 °C for cytokine quantification.

2.4.3. Cytokine Titration

Sandwich ELISAs were performed with 96-well, flat-bottom microtiter plates (Nunc-Immuno Plate Maxisorp, Rosekilde, Denmark). Microplates were coated for 18 hours at 4 °C with the capture antibody (BD Pharmingen, San Diego, CA for IL1β, IL4, IL5, IL6, IL10, IL12, TNFα and INFγ;R&D Duo-Set, Minneapolis, USA, for IL13), washed three times with PBS-Tween 20 (0.05 %), blocked for 30 minutes at room temperature with PBS-BSA 2 %, washed three times. Plates were incubated for a further 18 hours with the antibody standards and CSF diluted 1:2 with PBS-Tween 20 (0.05 %) -BSA 0.5 % at 4 °C, washed three times, and incubated with the detection antibody (BD Pharmingen for IL1β, IL4, IL5, IL6, IL10, IL12, TNFα and INFγ;R&D Duo-Set, Minneapolis, USA, for IL13), for 18 hours at 4°C. Bound detection antibodies were detected using 1:3000 diluted streptavidine-phosphatase conjugate (Zymed Laboratories, San Francisco CA) and p-nitrophenyl phosphate (Sigma, St Louis, Mo) as substrate. Optical density (OD) readings were performed at 30 and 60 minutes of incubation at 405 nm. Assay sensitivity was 9.4 pg/ml for all cytokines. All assays were performed in duplicate.

2.4.4. IgG subclass antibody detection by ELISA

Plasma antibody titres were measured by indirect ELISA. *T. solium* cyst fluid (1 μg/well) at a final volume of 100 μll/well was incubated overnight at 4 °C. The wells were washed, incubated with the 1:10 diluted CSF for one hour at 37 °C. Bound immunoglobulins were developed using rabbit anti-human IgG1, IgG2, IgG3 or IgG4 biotin labeled (Zymed Laboratories, San Francisco CA) and streptavidin alkaline phosphatase (Zymed Laboratories, San Francisco CA). Para-nitrophenylphosphate (Sigma, St Louis, Mo) was used as substrate. Plates were read at 405 nm. All assays were performed in duplicate.

2.5. Statistical Analysis

Data were processed in Excel 7.0 (Microsoft) and Spss 10.0 for Windows. The U Mann-Whitney non-parametric test and univariate analysis of were used to identify the differences in the immunological response between groups. $P \leq 0.05$ was considered significant.

3. Results

3.1. General description of NC subjects

Fifty two subjects, 32 women and 20 men, participated in this study. Twenty two were patients of the INNN, 10 women (21-64 years old) and 12 men (19-68 years old). The rest (30) were residents of Cuentepec, 22 women (7-69 years old) and 8 men (16-88 years old) (Table 1).

From Cuentepec inhabitants, twenty six (86.7%) were asymptomatic, 3 (10%) presented mild and 1 (3.3%) moderate symptomatology. From hospital patients three

out of twenty three subjects had mild (13.6%), eleven moderate (50%) and 8 severe symptoms (36.4%) (Table 1).

3.1.1. Imagenological description of NC cases

3.1.1.1. Number and stage of brain cysticerci

Multiple cysticerci were found in 40.4% of the studied patients (21), while only 30 (57.7%) had single cysticerci. Vesicular cysticerci were found in 6 (11.5 %) of the 52 NC cases, while in 4 (7.7 %), cysticerci were colloidal and in 29 (55.8%) the parasites were calcified. Mixed forms were found in 12 subjects (23.1%) who presented parasites in different stages (4 vesicular and colloidal, 6 vesicular and calcified, 2 vesicular and colloidal and calcified). One patient (1.9%) at the moment of the study had no cysticerci but presented intracraneal hypertension as a sequel of NC.

3.1.1.2. Parasite location

The precise CNS location of the cysts could not be determined in 16 cases (30.8%), not even with MRI. In these cases it was impossible to distinguish between parenchymal or SA sulci location. A single parasite location was found in 27 NC cases: ventricular in 2 (3.8%), parenchymal in 7 (13.5%), in the SA sulci 16 (30.8%) and in the SA base 2 (3.8%). In contrast, 9 patients presented a mixed location: 3 (5.8%) in the SA sulci and parenchyma, 3 (5.8 %) in SA base and intraventricular, 1 (1.9%) with intraventricular and SA sulci, 1 (1.9%) with intraventricular and parenchyma and 1 (1.9%) patient with SA sulci and SA base cysticerci.

Multiple lesions were mainly located in the undetermined SA sulci or parenchymal location (6 cases of 21, 11.5%) and single lesions were mainly located in SA sulci (15 cases of 30, 22.8%).

3.2. NC exposure is related to a pronounced TH2 immune profile

In order to evaluate the relevance of exposure two kinds of groups of participants were considered according to the geographic location and sanitary conditions of their household. The first group comprised 32 individuals living in rural communities, Cuentepec or another rural community, with no sanitary conditions (i.e. absence of potable water and latrine). The second group included 17 subjects living in an urban area with all sanitary facilities. Because of the difficulty to define the exposure level, an intermediate group of 3 subjects living in suburban locations, were not considered in the analysis. The rural group presented higher levels of IL4 (P=0.003), IL5 (P=0.016), IL12 (P=0.012), IL13, IFNγ (P=0.015), TNFα (P=005) as well as increased specific proliferation response (P=0.023) and lower levels of all four IgG subclasses than the urban group (P<0.005) (Table 2).

3.3. Decreased immune response in multiple and vesicular NC cases

NC cases with multiple cysticerci presented higher levels of IgG subclasses (IgG1 P=0.018, IgG2 P=0.012, IgG3 P=0.019, IgG4 P=0.011) but lower levels of IL4 (P=0.046), IL5 (P=0.033), IL12 (P=0.015), IL13 (P=0.05), IFNγ (P=0.002) and TNFα (P=0.011), and specific cell proliferation than NC subjects with unique lesions (P=0.008) (Table 1).

Patients with vesicular and mixed forms did not present different immune responses between each other and presented lower levels of IL4 (P≤0.004), IL12 (P<0.04), IL13 (P≤0.055), TNFα (P≤0.004) and specific cell proliferation (P<0.04) than patients with calcified lesions (data not shown). Patients with colloidal stage of the parasite presented higher levels of IL1β when compared to patients with vesicular

(P=0.025), calcified (P=0.077) and mixed lesions (P=0.08) (data not shown). Patients with calcified parasites elicited a distinctive immune-inflammatory response characterized by high levels of IL4 (P<0.03) and IL13 (P≤0.055) and low IgG3 (P<0.03) when compared to patients with vesicular, colloidal and mixed forms (data not shown).

3.4. A decreasing immune-inflammatory profile related to increasing clinical severity

Symptomatology was mild in 6 patients (11.5%), moderate in 12 (23.1%) and severe in 8 (15.4%), while 26 (50%) were asymptomatic (Table 1). The asymptomatic group presented higher levels of IL4 (P=0.046), IL5 (P=0.021) and IL13 (P=0.023) when compared to the three symptomatic groups (Figure 1); also showed lower IgG subclasses levels than the symptomatic group (P<0.04) (Figure 2). Specific cell proliferation was lower in the moderate and severe group (P<0.05) (Figure 3). Patients with severe symptomatology presented significant lower levels of IL1β, IL10, IL12, IFNγ and TNFα (P=0.053, P=0.035, P=0.003, P=0.002 and P=0.008, respectively) (Figure 1 for IL12, the rest data not shown). In almost all measured immunological features no significant differences were found between the mild, moderate and severe group.

4. Discussion

In this study the peripheral immune response related to NC subjects was evaluated. Two clear different immune profiles were found, one related to asymptomatic NC and the other symptomatic NC. Most of the asymptomatic NC cases presented single calcified cysticerci in parenchyma or SA sulci and belonged to high exposure rural areas. Asymptomatic NC cases exhibited increased levels of IL4, IL5, IL13 and IL12

after specific cysticercal antigen stimulation of peripheral lymphocytes and low levels of specific IgG subclasses in the serum. In contrast, hospital symptomatic patients presented a depressed specific cellular immune response and increased levels of all four IgG subclasses. Most of the patients were from urban areas, had multiple cysticerci in the CNS frequently in vesicular or mixed stages.

The immune profiles related to asymptomatic NC cases confirmed the results previously reported in a small group of asymptomatic NC subjects from a different rural community of Mexico (Chavarría et al., 2003). This observation clearly points out that a TH2 immune profile favors a benign course of the infection. A protective role related to this immune status is not completely unexpected since several independent studies in murine models and human infections had also highlighted this protective role in different parasitic diseases. This is the case of the TH2 response (IL4 and IL13) involved in the expulsion of the gastro-intestinal nematode *Trichinella spiralis* (Akiho H, et al., 2002); the local TH2 protective response induced by vaccination against the experimental infection with *Necator americanus* (Girod et al., 2003) and in the resistance to helminth *Trichuris muris* infection (Else et al., 1994). However, if this immune profile is the cause or the consequence of the parasite evolution remains to be elucidated. In addition, it has to be considered that this protective TH2 response could be promoted by the extensive contact with the parasite. This is highly possible since almost all the asymptomatic NC cases were from rural areas with high exposure to the parasite (Morales et al., 2002, Fleury et al., submitted). These evidences are of special interest, they point to the existence of an acquired immunity against the disease and this suggests the possibility

of developing vaccines to protect inhabitants from endemic countries against the severe forms of NC.

The immune profile in symptomatic NC patients found is in agreement with previous studies in which an depressed immune response was found in NC patients (Bueno et al., 2004). However, this study differs in some critical points. As far as we know this is the first study in which a depressed specific cellular immune response was identified in non treated, clinically and imagenologically classified patients. This is of special importance considering the extreme differences between asymptomatic and seriously affected NC subjects (White, 2000 , Fleury et al., 2004) and also because the heterogeneity due to side effects of treatment. In fact, most treatment schedules include cysticidal drugs (albendazol and/or praziquantel) and corticosteroids (Marquez-Caraveo et al., 2004) with the purpose to avoid the exacerbated inflammation that results of the parasite damage due to specific treatment (Nash, 2003).

Since T cell proliferation indexes induced by mitogens like concanavalin A in patients with viable parasites were not significantly reduced by the infection, the observed immune depression could be due to the secretion of parasite products that specifically induced apoptosis to the immune cells. This is not completely unexpected, since the parasite induced apoptosis occurs during experimental cysticercosis and is apparently responsible for the persistent concomitant cellular immune suppression (O'Connell et al., 2000; López-Briones et al., 2003). Moreover, a cysteine protease from *T. solium* metacestode is apparently involved in down-regulating cell-mediated responses in infected hosts (Tato et al., 2004).

Despite the mechanisms that mediate this immune depression its biological relevance remains obscure. It has been proposed that parasites may remain in vesicular

stage in human brains as long as they are capable of suppressing the local immune response (Restrepo et al., 1998). This specific immune depression could prevent the increased local inflammatory response induced by the migration of activated lymphocyte to the CNS avoiding further neural damaged (Chavarria et al, 2004). Thus, it is possible that the exacerbated local inflammatory response with increased IL5 and IL6 levels in the CSF of severe NC cases (Rodrigues et al., 2000, Chavarría, submitted), could be the consequence of the in situ production of these cytokines. On the other hand, an obvious consequence of the observed immune depression could be the increased susceptibility to different infectious agents and diseases. However, concomitant infections in NC is only an unusual findings as it is the epidural spinal racemose neurocysticercosis case in an HIV-infected recently documented (Delobel et al., 2004). Since no undesirable consequences were found related to the immune depression induced by NC infection, one could speculate that it has an additional protective effect. This immune depression could avoid lymphocytes chromosome instability due to persistent antigen stimulation and so it could reduce the probability of promoting malignant hematological diseases. This could be of special interest considering that despite many efforts it has not been possible to demonstrate that NC is a causal agent of cancer (Del Brutto et al., 2000).

In summary, this study adds new elements to further understand immunological mechanisms that underlie the susceptibility and resistance to NC disease as well as the relation between the immune response in the CNS and the peripheral system in NC.

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Figure Legends

Figure 1. Cytokine levels in supernatants of *Taenia solium* stimulated cells of NC cases according to clinical severity. Each patient is represented according to his/her residency (rural in white, suburban in grey, urban in black) and the respective parasite stage (vesicular in diamonds, colloidal in squares, calcified in circle and mixed in triangle). Medians of each group are represented by lines. The non-parametric Mann-Whitney U test was performed, only when $P \leq 0.05$ (**) and $P \leq 0.08$ (*) are indicated.

Figure 2. *Taenia solium* specific IgG subclasses in serum of NC cases according to clinical severity. Each patient is represented according to his/her residency (rural in white, suburban in grey, urban in black) and the respective parasite stage (vesicular in diamonds, colloidal in squares, calcified in circle and mixed in triangle). Medians of each group are represented by lines. The non-parametric Mann-Whitney U test was performed, only when $P \leq 0.05$ (**) and $P \leq 0.08$ (*) are indicated. OD optical density.

Figure 3. Stimulation Index (SI) of *Taenia solium* whole antigen (TsAg) and concanavalin (ConA) stimulated cells of NC cases according to clinical severity. Each patient is represented according to his/her residency (rural in white, suburban in grey, urban in black) and the respective parasite stage (vesicular in diamonds, colloidal in squares, calcified in circle and mixed in triangle). Medians of each group are represented by lines. SI is presented as cpm-antigen-stimulated cells/cpm-not-stimulated cells. The non-parametric Mann-Whitney U test was performed, only when $P \leq 0.05$ (**) and $P \leq 0.08$ (*) are indicated.

Table 1. Clinical and radiological description of 52 NC subjects

Severity	Women/Men	Exposure Level	Single/Multiple Lesions	Parasite Location	Parasite Stage
Asymptomatic	19/7	26 rural	22/4	11 SA sulci 3 Parenchyma 12 Parenchyma or SA sulci	1 vesicular 25 calcified
Mild	3/3	2 urban 4 rural	4/2	2 SA sulci 1 Parenchyma 3 Parenchyma or SA sulci	2 vesicular 3 calcified 1 mixed
Moderate	4/8	9 urban 2 suburban 1 rural	5/7	3 SA sulci 3 Parenchyma 1 Parenchyma or SA sulci 5 mixed	3 colloidal 1 calcified 8 mixed
Severe	6/2	6 urban 1 suburban 1 rural	0/7	2 SA base 2 Intraventricular 4 Mixed 1?	3 vesicular 1 colloidal 3 mixed 1?

Table 2. Immune-inflammatory profile determined in 52 NC subjects

Immunological Profile	Exposure Rural/Urban (32/17)	Single/ Multiple Lesions (30/21)
IgG1 (O.D[a])	0.102/0.338[c, e]	**0.104/0.142[e]**
	0.114/1.4[d]	***0.122/0.447***
IgG2 (O.D)	0.09/0.142[e]	0.09/0.12 [e]
	0.108/0.433	***0.114/0.468***
IgG3 (O.D)	0.073/0.143[e]	0.08/0.11[e]
	0.091/0.198	***0.093/0.179***
IgG4 (O.D)	0.078/0.334[e]	0.082/0.122[e]
	0.089/>3.0	***0.094/2.87***
IL4 (pg/ml)	42/<9.4[e]	**40/<9.4[e]**
	56/23	***51/42***
IL5 (pg/ml)	313/<9.4[e]	299/<9.4[e]
	473/106	***464/354***
IL12 (pg/ml)	343/<9.4[e]	346/<9.4[e]
	381/226	***383/287***
IL13 (pg/ml)	183/<9.4[e]	176/<9.4[e]
	349/<9.4	***333/314***
IFNγ (pg/ml)	306/56[e]	356/64[e]
	736/247	***755/227***
TNFα (pg/ml)	670/23[e]	713/85[e]
	763/441	***766/531***
SI[b]	20.3/2.9[e]	23.7/2.8[e]
	39.9/19.9	***49.2/18.9***

Immunological features from different clinical and radiological variables were compared by the non-parametric Mann-Whitney *U* test.

[a]O.D optical density. [b]SI, stimulation index, is presented as cpm-antigen-stimulated cells/cpm-not-stimulated cells. [c]SI, cytokine levels and antibody levels are in median values. [d]The 75 % upper percentile values. [e]$P \leq 0.05$. [f]$P \leq 0.08$.

Figure 1.

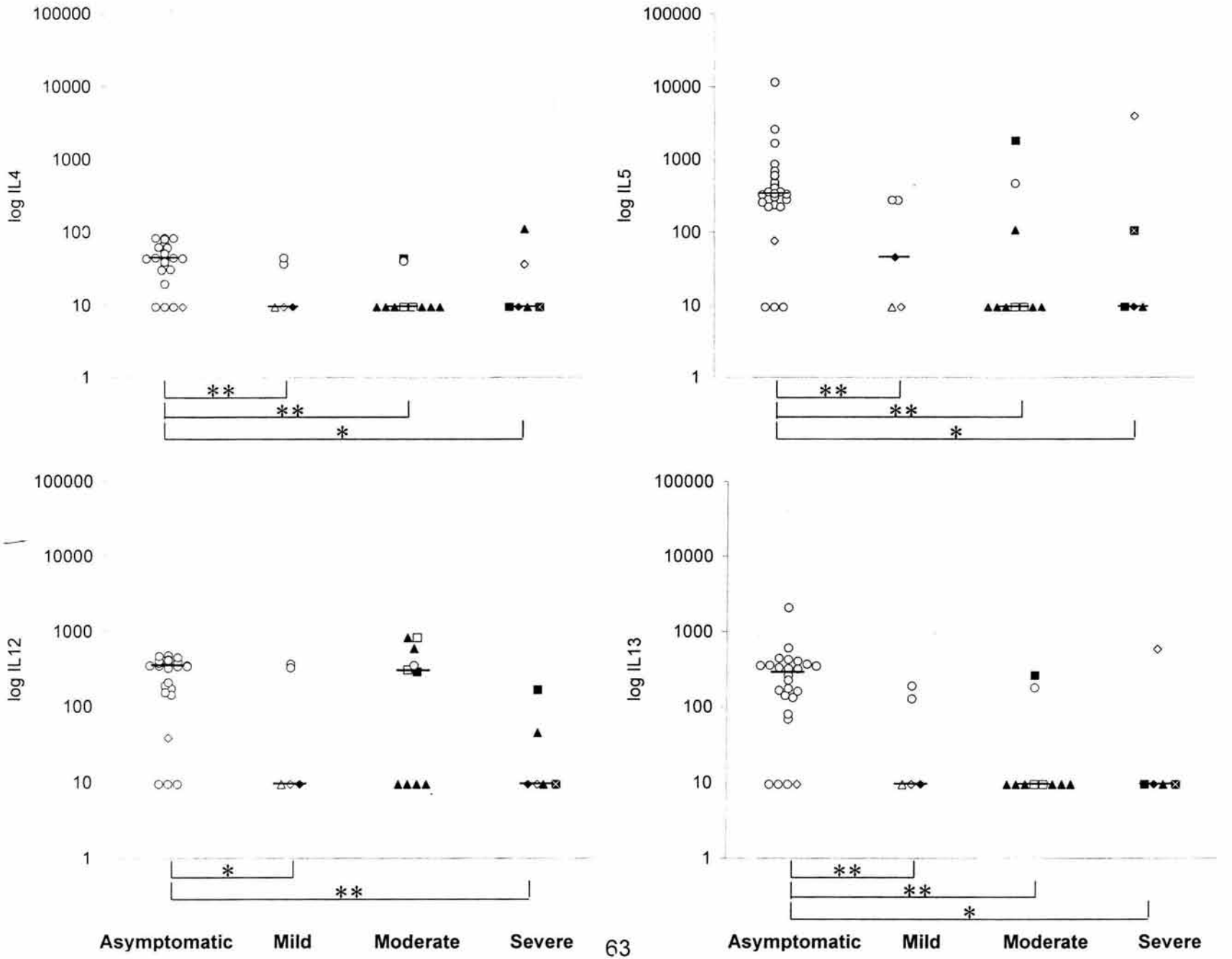
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Figure 2.

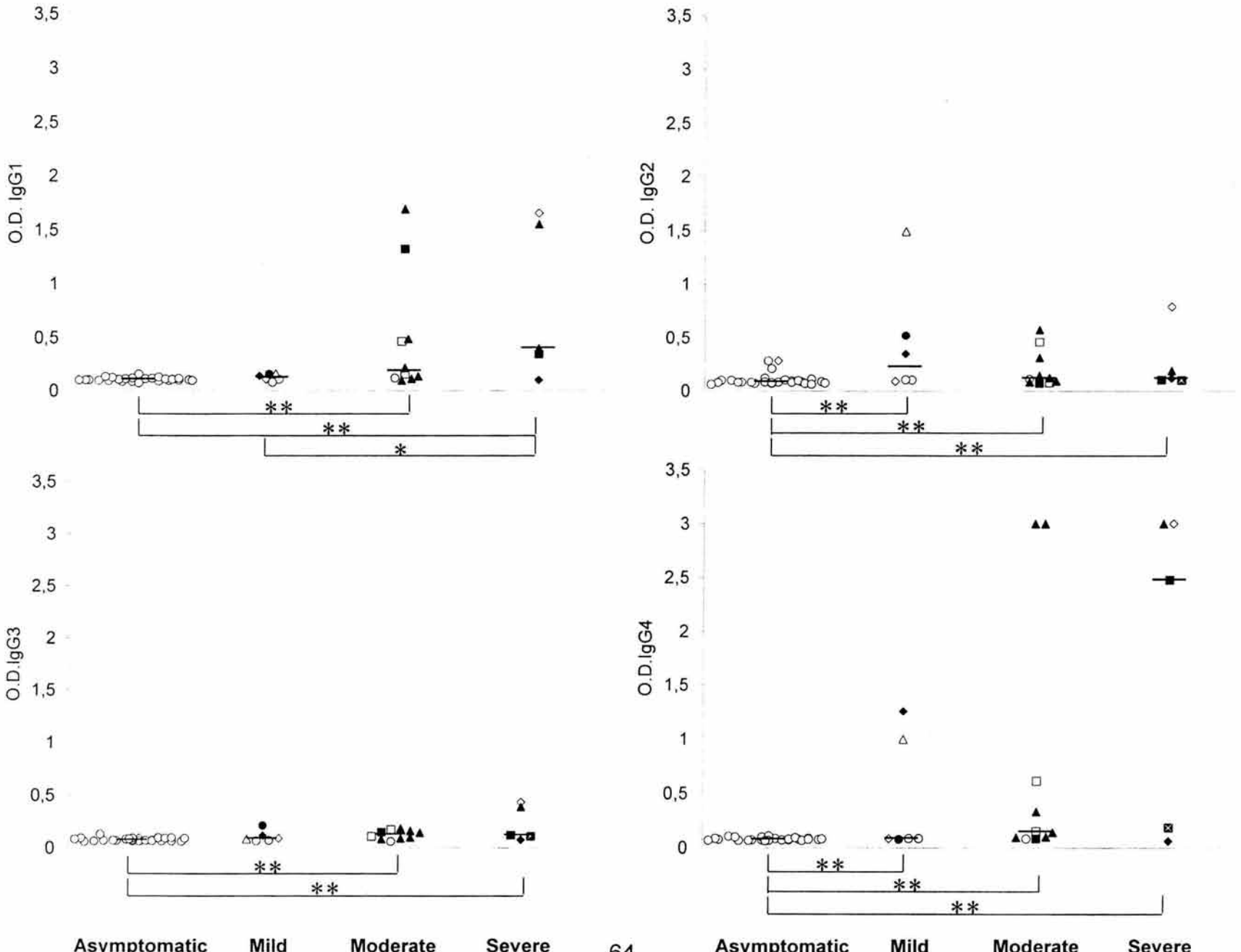
O.D. IgG1
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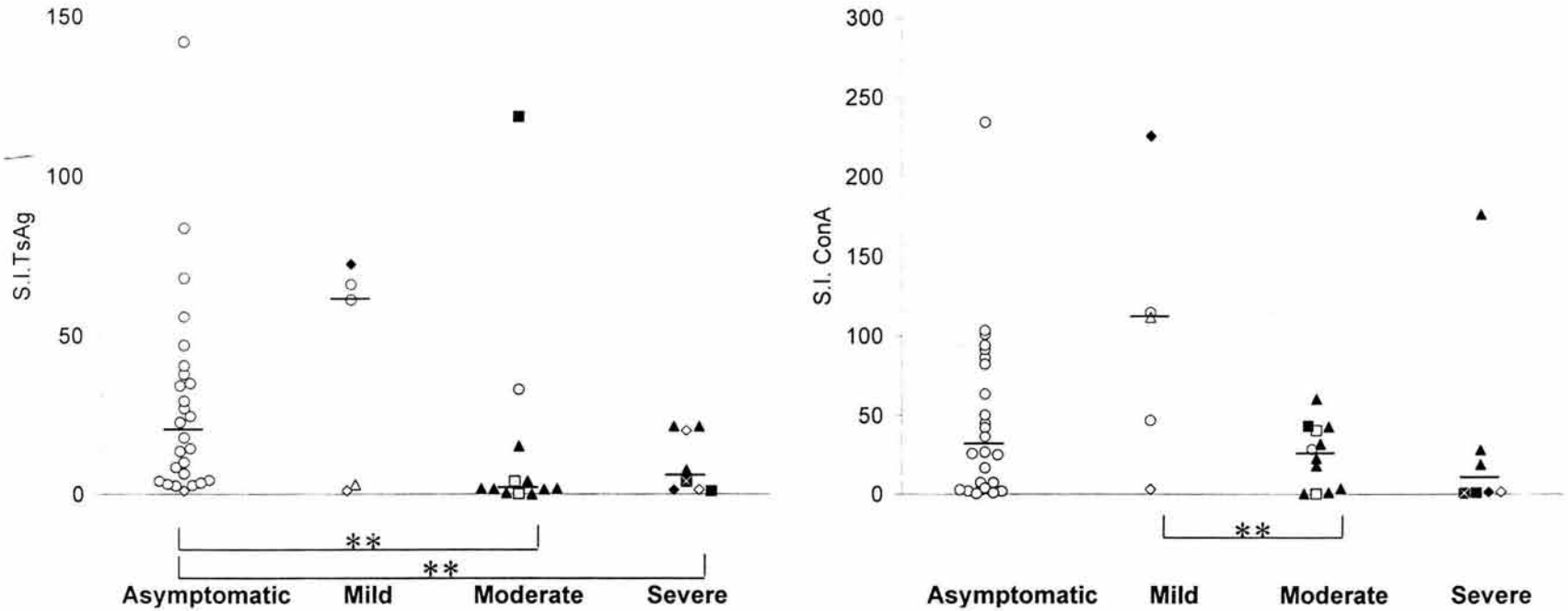
Figure 3.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Asymptomatic
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Severe

C) Resultados de la tercera fase

En revisión en la revista Microbes and Infection.

Dra.Edda Sicutto

De: <cchitnis@icgeb.res.in>; <cchitnis@icgeb.res.in>
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Enviado: Martes, 27 de Julio de 2004 12:49 a.m.
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This is to acknowledge receipt of your manuscript mic042369 "Towards the unraveling of the complexity of clinically heterogeneous neurocysticercosis: severity is related to an immune-inflammatory profile"; it has been sent to reviewers and will be carefully examined by experts in the field.

Sincerely,

Chetan Chitnis

Towards the unraveling of the complexity of clinically heterogeneous neurocysticercosis: severity is related to an immune-inflammatory profile

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Abstract

Human neurocysticercosis is a disease caused by the establishment of the Taenia solium cysticercus in the central nervous system. Neurocysticercosis may be asymptomatic or manifested by non-specific mild or severe neurological symptoms. Host factors (immunity, genes and gender) may be involved in the heterogeneous clinical picture. An immune-inflammatory profile that underlies neurocysticercosis presentation was herein determined in 45 cerebral spinal fluid, from clinical and imagenologically characterized neurocysticercosis patients measuring the levels of specific IgG subclasses and proinflammatory-immuneregulatory cytokines. Severity was related with increased inflammation in the cerebral spinal fluid characterized by increased levels of IgG subclasses, IL6, IL5, IL10, proteins and eosinophils; multiple neurocysticercosis showed higher levels of IL5, IL6 and IL10 than single neurocysticercosis. No clinical nor imagenological differences were found between women and men, although women presented increased IL5, IL6 and IL10 levels pointing out immunological differences due to gender. The most severe symptomatology was found when cysticerci were intraventricular or located in subarachnoid space of the base, inducing an exarcebated inflammatory cerebral spinal fluid with increased levels of IgG subclasses, IL5, IL6 and also the immunoregulatory cytokine IL10. These results constitute a first insight to understand the immune-inflammatory response that underlies the clinical picture of neurocysticercosis.

Keywords: *Taenia solium* / neurocysticercosis/ CNS / inflammation / immunological profiles

1. Introduction

The differential expression of cytokines and antibodies is generally associated with different disease status either of autoimmune, traumatic or infectious etiologies, in which the immune-inflammatory response is closely related to symptomatology and severity and is due to their side effects [1,2]. In the human central nervous system (CNS), the local immune response induced by infections has been poorly studied because of the difficulties to obtain biological samples from well-characterized patients. This is also the case of neurocysticercosis (NC), a frequent and serious parasitic disease of the CNS, caused by the larval stage of *Taenia solium*. In Mexico, it is the cause of 11% of neurological consultations [3], of 25 % of craniotomies [4] and it is the first cause of adult epilepsy onset [5,6]. NC is a pleomorphic neurological disease that can present a heterogeneous clinical and imagenological picture. In fact, NC can be completely asymptomatic [7-9], or be manifested by a wide variety of non-specific symptoms: headache, epilepsy, dementia, depression or intracranial hypertension in its most severe form. The causes that underlie this heterogeneity are not completely understood, and it is speculated that it could be related to host, parasite and/or exposure factors [8, 10-13].

Differences in NC severity due to host immunological factors have barely been explored. To gain understanding of the role of immune-inflammatory factors, a study design involving the main clinical categories of the disease, i.e. imagenological status and clinical severity as well as host factors, was performed. Also, both gender and age merit special consideration due to previous studies in which increased inflammatory CSF cellularity was found in women and age was related to increased number of vesicular cysts [13,14].

We previously proposed that different clinical NC phenotypes (asymptomatic or mild to severe NC) might be related to diverse immune-inflammatory profiles. In a previous study, the immunological profile related to asymptomatic NC was characterized by increased IL4, IL5 and IL13 levels after specific stimulation and increased specific IgG4 levels [9]. Thus, it seems that a Th2 profile promotes a silent resolution of the NC infection. In this study, we explored if an exacerbated immune-inflammatory profile is related with the different symptomatic presentation in well-characterized symptomatic NC patients. The relevance of gender, age, parasite stage and location and the clinical phenotype was also explored.

2. Materials and Methods

2.1. Patients

The 45 patients included in this study were attended at the Instituto Nacional de Neurología y Neurocirugía (INNN) in Mexico City and had never been treated for NC before. INNN only admits patients older than 15 years of age. Available CSF of these untreated patients was included in the study that lasted from 2001 to 2003. Age and gender data were collected from each patient.

2.2. Characterization of the disease

NC diagnosis was based on computed tomography (CT) and magnetic resonance image (MRI) before receiving specific treatment. Parasite stage was determined based on the CT and/or MRI image: 1) vesicular (the parasite is viable, a cerebrospinal fluid-like signal within a cyst is seen); 2) colloidal (the cyst fluid is turbid, there is an intense inflammatory reaction in the surrounding brain parenchyma); 3) calcified (the parasite debris appear as a mineralized granuloma).

From radiological studies of each NC case, the following information was collected: number of lesions (single vs. multiple), stage of cysticerci [vesicular, colloidal, calcified or mixed (colloidal and vesicular) forms] and CNS location [subarachnoid space of the base (SA base) or of the sulci (SA sulci), parenchymal, or intraventricular].
CSF cellularity, content of proteins and the presence of eosinophils was recorded (cellularity was considered increased when the number of cells exceeded 5 per ml).

The clinical expression of the disease was established by clinical examination of the patients by two neurologists. Based on the symptomatology, patients were grouped in three classes: 1. Mild: headache or no symptoms; 2. Moderate: focal deficits and/or seizures, and 3. Severe: intracranial hypertension (defined by presence of headache, nausea, vomiting and papilledema).

2.3. Immunological profile

The following features were measured in the CSF to define an immunological profile related to NC: *T. solium* specific IgG subclasses (IgG1, IgG2, IgG3, IgG4) and IgE antibody levels, TH1 (IL12, IFNγ), TH2 (IL4, IL5, IL10) and inflammatory (IL1β, IL6) cytokines.

2.3.1. Cytokine Titration

Sandwich ELISAs were performed with 96-well, flat-bottom microtiter plates (Nunc-Immuno Plate Maxisorp, Rosekilde, Denmark). Microplates were coated for 18 hs at 4 °C with the capture antibody (BD Pharmingen, San Diego, CA for IL1β, IL4, IL5, IL6, IL10, IL12 and INFγ), washed three times with PBS-Tween 20 (0.05 %), blocked for 30 minutes at room temperature with PBS-BSA 2 %, washed three times. Plates were incubated for a further 18 hours with the antibody standards and CSF diluted 1:3 with

PBS-Tween 20 (0.05 %) -BSA 0.5 % at 4 °C, washed three times, and incubated with the detection antibody (BD Pharmingen for IL1β, IL4, IL5, IL6, IL10, IL12 and INFγ), for 18 hours at 4°C. Bound detection antibodies were detected using 1:3000 diluted streptavidine-phosphatase conjugate (Zymed Laboratories, San Francisco CA) and p-nitrophenyl phosphate (Sigma, St Louis, Mo) as substrate. Optical density (OD) readings were performed at 30 and 60 minutes of incubation at 405 nm. Assay sensitivity was 9.4 pg/ml for all cytokines. All assays were performed in duplicate.

2.3.2. IgG subclasses and IgE antibody detection by ELISA

Plasma antibody titers were measured by indirect ELISA. *T. solium* cyst fluid (1 μg/well) at a final volume of 100 μl/well was incubated overnight at 4 °C. The wells were washed, incubated with the 1:10 diluted CSF for one hour at 37 °C. Bound immunoglobulins were developed using rabbit anti-human IgG1, IgG2, IgG3, IgG4 or IgE biotin labeled (Zymed Laboratories, San Francisco CA) and streptavidin alkaline phosphatase (Zymed Laboratories, San Francisco CA). Para-nitrophenylphosphate (Sigma, St Louis, Mo) was used as substrate. Plates were read at 405 nm. All assays were performed in duplicate.

2.4. Statistical Analysis

Data were processed in Excel 7.0 (Microsoft) and Spss 10.0 for Windows. The U Mann-Whitney non-parametric test, univariate analysis of variances and the two-tailed Fisher's exact test were used to identify the differences in the immunological response between groups.

3. Results

3.1. Imagenological description of NC patients

3.1.1. Number and stage of brain cysticerci

Multiple cysticerci were found in 71.1 % of the studied patients (32), while only 13 (28.9 %) had single cysticerci. Vesicular cysticerci were found in 24 (53.3 %) of the 45 NC patients, while in 11 (24.4 %), cysticerci were colloidal and in 6 (13.3 %) they were calcified. Mixed forms were found in 4 subjects (8.9 %) who presented parasites in different stages (colloidal and vesicular) (Table 1).

3.1.2. Parasite location

The exact CNS location of the cysts could not be determined in 4 patients (8.9 %), not even with MRI. In these cases it was impossible to distinguish between parenchymal or SA sulci location. A single parasite location was found in 35 NC patients: ventricular in 5 (11.1 %), parenchymal in 6 (13.3 %), in the subarachnoid space of the sulci 15 (33.3 %) (SA sulci) and in the subarachnoid space of the base 9 (20 %) (SA base). In contrast, six patients presented a mixed location: 3 (6.7 %) in the SA sulci and parenchyma, 2 (4.4 %) in SA base and intraventricular and 1 patient (2.2 %) with intraventricular and SA sulci cysticerci (Table 1).

Multiple lesions were mainly located in SA base cisterns (8 cases of 32, 25 %) or in the SA sulci (8 cases of 32, 25 %) and single lesions were mainly located in SA sulci (7 cases of 13, 53.8 %).

3.2. Cellular and immune-inflammatory profile determined in the CSF

3.2.1. General CSF description of NC patients

Lumbar puncture showed that CSF cellularity varied between 0 and 260 cells/ml (mean = 37.6 ± 53.6) and was greater than 5 cells/ml in 30 cases (66.7 %). Inflammation level of the CSF was considered high when cellularity was ≥ 15 cells/ml, and this was presented by 51.1 % (23) of the patients. Lymphocytes were most frequently found in

CSF. Univariate analysis of variances showed that increased CSF cellularity was associated with basal CSF cistern and intraventricular location (P = 0.009), and to the vesicular stage of the cysticerci (P = 0.023), it was not associated with parasite number. Proteins varied between 2 and 926 mg/dl (mean = 81.2 ± 167.7) and in 21 (46.7 %) patients, the value was above 40 mg/dl.

3.2.2. Severity is related to high CSF inflammation level and an immune-inflammatory response

Symptomatology was mild in 9 patients (20 %), moderate in 27 (60 %) and severe in 9 (20 %). Figure 1 presents the relationship between clinical presentation and inflammatory level of the NC patients. The percent of NC cases with high inflammation level was significantly higher in patients with severe than in those with mild symptoms (OR = 0.036; 95% CI 0.003-0.48, P = 0.015).

Patients with moderate symptomatology more frequently presented eosinophils (P = 0.07, data not shown) in the CSF compared to mild cases; in contrast, the latter showed higher levels of IL10 (P = 0.022, data not shown) and were older (P = 0.039, data not shown). Severe patients presented consistently higher values of protein content, cellularity and eosinophils than the other two kinds of patients (P < 0.04, data not shown), while IL5, IL10, IgG1, IgG2, IgG3 and IgE showed higher levels compared to the moderate cases (P < 0.05, data not shown).

3.2.3. Increased CSF cellularity is related to an immune-inflammatory response

Only features that exhibited significant differences were included in Table 2 and in the Figures. NC patients with increased CSF cellularity presented higher levels of protein content (P = 0.015), eosinophils (P = 0.005) and all specific IgG subclasses (P <

0.001) but not of the IgE class. Of all measured cytokines, only IL5 (P = 0.05), IL6 (P < 0.001) and IL10 (P = 0.002) were increased (Table 2).

High antibody and IL6 levels are related to CSF eosinophils. Twelve patients (26.7 %) presented eosinophils in the CSF as well as an increase in all four IgG subclasses (IgG1 P = 0.001, IgG2 P = 0.002, IgG3 P < 0.001, IgG4 P < 0.001), IL6 (P = 0.005), CSF proteins (P < 0.001) and cellularity (P = 0.001) (Table 2).

3.2.4. Gender elicits a dimorphic immune-inflammatory response in the CSF

The 45 analyzed CSF samples were taken from 24 women (15-70 years old) and 21 men (16-68 years old). Women elicited higher levels of IL5, IL6 and IL10 cytokines (P = 0.057, P = 0.015, P = 0.025, respectively) than men; in contrast, although the antibody response was remarkably increased in women, the difference was not statistically significant (IgG2 P = 0.053, IgG3 P =0.059) (Table 2). No significant differences were found between men and women when the clinical presentation, the number, stage and location of established cysticerci were compared.

3.3. Radiological and immune-inflammatory interactions

3.3.1. Parasite location influences the immune-inflammatory response, disease severity and parasite stage

A clear immune-inflammatory profile was shown by NC patients with cysticerci located in the SA base or intraventricularly, most of them with severe symptomatology and vesicular parasites, exhibiting increased levels of most IgG subclasses, IL5, IL6 and IL10 (P < 0.05) (Fig.2 and Fig.3), and of CSF cells (P = 0.01, data not shown) and eosinophils (P = 0.047, data not shown). IgG1 levels were higher in patients with cysticerci located in the SA base (P = 0.05), while intraventricular location showed higher levels of IL10 (P = 0.065) and IL1β (P = 0.08, data not shown). In contrast,

patients with cysticerci located in the SA sulci or parenchyma showed lower cytokine levels, while patients with parasites located in the sulci showed higher levels of IgG1 ($P = 0.008$), IgG2 ($P = 0.02$), IgG4 ($P = 0.02$) and IFNγ ($P = 0.005$, data not shown) than patients with parenchymal location (Fig.2 and Fig.3).

Mixed parasite location induced a heterogeneous immune-inflammatory response. Two patients with mixed cyst location, in the SA base and intraventricular, behaved the same as those with cysts in only the SA base or intraventricular. They presented higher levels of IL6 ($P = 0.01$, data not shown), IL10 ($P = 0.001$, data not shown), IL5 ($P = 0.07$, data not shown), IgG2 ($P = 0.07$, data not shown) and IgG4 ($P = 0.07$, data not shown) than patients with NC located only in SA sulci, and higher levels of all IgG subclasses ($P \leq 0.046$, data not shown) and IL10 ($P = 0.076$, data not shown) than patients with parenchymal NC. Mixed cyst location in the SA sulci and parenchyma in 3 patients elicited higher levels of IL4 ($P = 0.03$, data not shown), IL6 ($P = 0.003$, data not shown) and IFNγ ($P = 0.002$, data not shown) than patients with only SA sulci cysticerci, and higher levels of IgG2, IgG3 and IL6 ($P = 0.07$, data not shown) than patients with only parenchymal cysts. This mixed location behaved the same as intraventricular cysts and presented only higher IL4 levels than SA base cysts ($P = 0.7$, data not shown).

Patients with multiple parasites exhibited increased levels of IL5 and IL6 ($P = 0.03$, $P = 0.015$ respectively), while antibody levels, although increased, were not significant (Table 2)

Interestingly, arachnoiditis was found related to the presence of eosinophils in the CSF, five patients (11,1%) with radiological evidence of arachnoiditis showed elevated IgG3

(P = 0.07, data not shown), IgG4 (P = 0.056, data not shown), IL6 (P = 0.068, data not shown), CSF proteins (P = 0.065, data not shown), CSF cells (P = 0.07, data not shown) and eosinophils (P = 0.001, data not shown).

4. Discussion

This study provides new insight into the immune response that underlies the heterogeneous clinical and radiological picture exhibited by NC patients.To begin with, an important finding is the clear relation between increased CSF cellularity and clinical NC severity (Fig.1), which was also accompanied by increased levels of specific IgG subclasses, eosinophils, inflammatory IL5, IL6 and the immuneregulatory cytokine IL10. Most of these inflammatory NC cases occurred when the parasite was established in the SA base or in the ventricles, where it remained without apparent damage and predominantly in a vesicular stage (Fig.2 and Fig.3). The relevance of this reaction to damage the parasite cannot be easily explored since these patients need immediate treatment; however, the finding that in these conditions the parasite exhibits no radiologically detectable damage, points to the possible ineffectiveness of the inflammatory response. In contrast, parasites found in the SA sulci or in the parenchyma were more frequently damaged, induced low CSF inflammation and only mild or moderate symptomatology. The differences in parasite condition could be a result of the interaction between the local antigen-presenting cells (APC), immigrated lymphocytes and eosinophils and the cysticerci. When parasites were located in brain parenchyma or SA sulci a closer contact with activated immune competent cells could favor cyst death and account for the higher frequency of calcified or colloidal forms in these compartments.

IL5, IL6 and IL10 can be locally produced by APC of the CNS: e.g., microglia and perivascular macrophages among others [15,16] or by infiltrated T cells. IL5 and IL6 participate in cell and eosinophil recruitment [17-19]. Patients with parasites located in the ventricles or in the SA base or with inflammatory CSF had higher levels of IL5 and IL6, which could explain the increased CSF cellularity and the presence of eosinophils. The recruited B cells may become plasmatic cells and be the source of local antibody synthesis [1], while eosinophils could degranulate within the CNS, and damage the parasite but also the nervous tissue [20,21]. It has been previously reported that the presence of eosinophils is associated to severity and/or inflammatory CSF in NC [4]. Present data support this finding and relate it to the presence of high levels of IL6 and specific antibodies. Regarding the presence of IL10, which could be produced by CD4+ regulatory T cells [22], considering its inmune-supressive and regulating functions, this cytokine could favor neuronal survival, protecting the blood brain barrier (BBB) endothelium and inhibiting the effect of other cytokines and cells [23-25]. The increased levels of IL10 could also feed back the inflammatory effect of IL5 and IL6. On the other hand, one should not discard the possibility that, although CSF IL10 levels are high, the molecule may be not functional or present some kind of polymorphism, as reported in patients with multiple sclerosis, another inflammatory disease of the CNS [26-28].

Another point to be considered is the possible peripheral immune response of the secondary lymphoid organs promoted by the exit of parasite products from the efferent limb of the CNS. Parasites located in the ventricles or the SA base could secrete high levels of cysticercal antigens, thus more antigens could drain from the CNS and initiate an immune response in the secondary lymphoid organs, activating T and B cells that should then be able to cross the BBB and reach the parasite [29]. Antigens secreted in

the brain parenchyma probably do not exit so effectively; therefore, a consistently lower inflammation of the CSF would take place with a reduced probability of stimulating as many lymphocytes as in the other locations [30,31].

Interestingly, our data also point to a sexual dimorphism of the immune response. Although in this sample of studied patients there were no clinical or radiological differences between women and men, women produced higher levels of IL5, IL6 and IL10 revealing an increased inflammatory local response not due to differences in the location of the parasite. This profile is in agreement with previous reports in which an exacerbated inflammatory response was found in women, defined by CSF cellularity and imagenological appearance [13,14].

In addition, our data support the hypothesis of a compartmentalized immune response within the CNS. In this study, parasite location allowed us to observe different immune responses in the CNS by compartments. Brain parenchyma parasites induce a response not detectable in the CSF; parasites in the SA sulci induce a similar response but with higher CSF antibody levels, while intraventricular parasites or those in the SA base induce a more pronounced response with high levels of IL5, IL6, IL10 and specific IgG subclasses. The increased CSF inflammation could be responsible not only for the severe clinical symptoms induced by the parasite's presence in these two locations, but also for additional CNS damage as a sequel of the disease. The balance between the benefit and damage caused by inflammation, especially when this event occurs in a CNS compartment, is a research area of interest. It is also relevant to consider that inflammation in the CNS does not usually exert a repair function, rather, its tendency is to decline to damage, which would explain most of the CNS pathologies.

Understanding why in some patients, NC produces none or only mild symptomatology and in others it leads to a severe clinical picture and death, will contribute to the design of improved therapeutic management of NC, and perhaps to an early treatment of the disease before the struggles between parasite and host cause major damage to the CNS. The CNS compartmentalized immune response opens new perspectives to explore in the pathology of the NC and possibly of other neurological diseases.

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Figure Legends

Fig.1 Frequency of NC cases with high (≥15 cells/mm3, black bars) or low (<15 cells/mm3, grey bars) inflammation level according to the clinical severity (mild, moderate or severe). The percent of NC cases with high inflammation level was significantly higher in patients with severe than with mild symptomatology (Fischer´s exact test, P = 0.023, OR= 14).

Fig.2 *Taenia solium* specific IgG subclasses in CSF of NC cases according to parasite location (SA Base= Subarachnoid space of the base, Intraventricular, SA Sulci= Subarachnoid space of the sulci, Parenchyma). Each patient is represented according to his/her symptomatology (mild in white, moderate in grey, severe in black) and the respective parasite stage (vesicular, colloidal, calcified and mixed). Medians of each location are represented by lines. The non-parametric Mann-Whitney U test was performed, only when P ≤ 0.05 (**) and P ≤ 0.08 (*) are indicated. OD optical density.

Fig.3 Cytokine levels in CSF of NC cases according to parasite location (SA Base= Subarachnoid space of the base, Intraventricular, SA Sulci= Subarachnoid space of the sulci, Parenchyma). Each patient is represented according to his/her symptomatology (mild in white, moderate in grey, severe in black) and the respective parasite stage (vesicular, colloidal, calcified and mixed). Medians of each location are represented by lines. The non-parametric Mann-Whitney U test was performed, only when P ≤ 0.05 (**) and P ≤ 0.08 (*) are indicated.

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Table 1. Clinical and radiological description of 45 NC patients

Severity	Women/Men	Single/Multiple Lesions	Parasite Location	Parasite Stage
Mild	7/2	2/7	1 SA sulci 2 SA base 3 Parenchyma 1 intraventricular 1 mixed	4 vesicular 2 colloidal 3 calcified
Moderate	11/16	10/17	14 SA sulci 2 SA base 3 Parenchyma 3 Parenchyma or SA sulci 3 mixed	12 vesicular 3 colloidal 3 calcified 4 mixed
Severe	6/3	1/8	4 SA base 3 Intraventricular 2 Mixed	8 vesicular 1 colloidal

Table 2. Immune-inflammatory profile determined in CSF of 45 NC patients

Immunological Profile	With/Without Increased CSF Cellularity ≥6 cells (30/15)	Women/ Men (24/21)	Single/Multiple Lesions (13/32)	With/Without Eosinophils (12/33)
IgG1 (O.D)	2.2/0.11 [a, c]	1.5/0.21	0.35/1.01	2.7/0.21[c]
	2.7/0.21 [b]	*2.7/1.5*	*1.8/2.7*	***2.7/1.8***
IgG2 (O.D)	0.79/0.08 [c]	0.77/0.15[d]	0.18/0.25	2.3/0.15[c]
	2.6/0.14	*2.6/0.6*	*0.65/2.5*	***2.6/0.86***
IgG3 (O.D)	0.43/0.07[c]	0.28/0.10[d]	0.14/0.2	0.76/0.09[c]
	0.76/0.09	*0.79/0.4*	*0.36/0.6*	***1.3/0.45***
IgG4 (O.D)	2.3/0.13[c]	1.8/0.31	0.31/1.4	2.7/0.2[c]
	2.7/0.22	*2.7/1.7*	*1.4/2.7*	***2.7/1.7***
IL5 pg/ml)	<9.4/<9.4[c]	<9.4/<9.4[d]	<9.4/<9.4[c]	<9.4/<9.4
	36.5/<9.4	*66/<9.4*	***<9.4/37***	*58.3/18*
IL6 pg/ml)	24.5/<9.4[c]	20/<9.4[c]	<9.4/20.2[c]	38.5/<9.4[c]
	129.5/<9.4	***227/23***	***10.5/65***	***1015.3/22***
IL10 pg/ml)	12.5/<9.4 [c]	10/<9.4[c]	<9.4/<9.4	12/<9.4
	48.3/<9.4	***43/<9.4***	*9.8/43*	*208/15*
CSF Proteins	53/31 [c]	37.5/39	43/ 35.5	68.5/33[c]
	77/43	*81.8/55.5*	*55/66.8*	***131.3/45.5***
CSF Cells		23/6	6/15	53/6[c]
		66/41.5	*69.5/59.8*	***123.3/37***
~~CSF~~	0/0 [c]	0/0	0/0	
Eosinophils	***1/0***	*1/0*	*0.5/1*	

Immunological features from different clinical variables were compared by the non-parametric Mann-Whitney *U* test.

[a] Cytokine levels and antibody levels are in median values. [b] The 75 % upper percentile values. [c] $P \leq 0.05$. [d] $P \leq 0.08$.

Figure 1

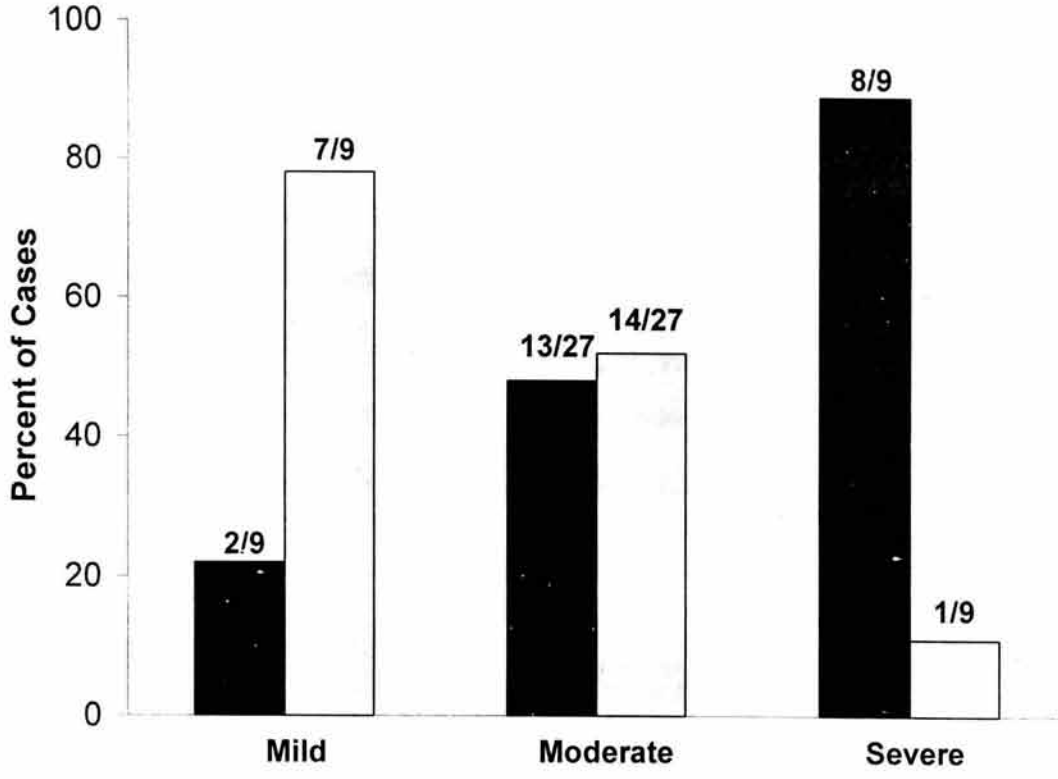
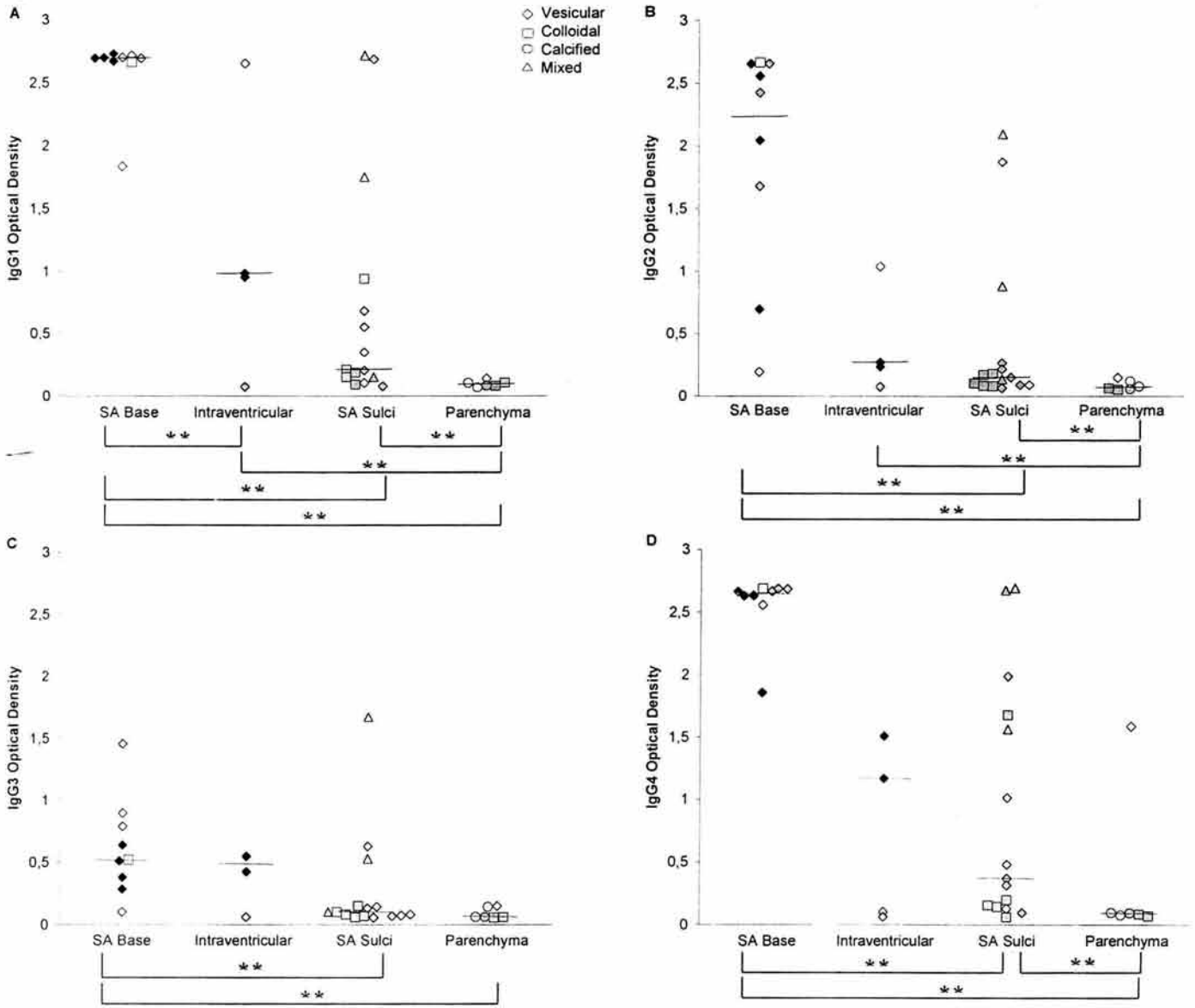

100
80
60
40
20
0
Percent of Cases
2/9
7/9
13/27
14/27
8/9
1/9
Mild
Moderate
Severe

Figure 2


A
B
C
D
◇ Vesicular
□ Colloidal
⬡ Calcified
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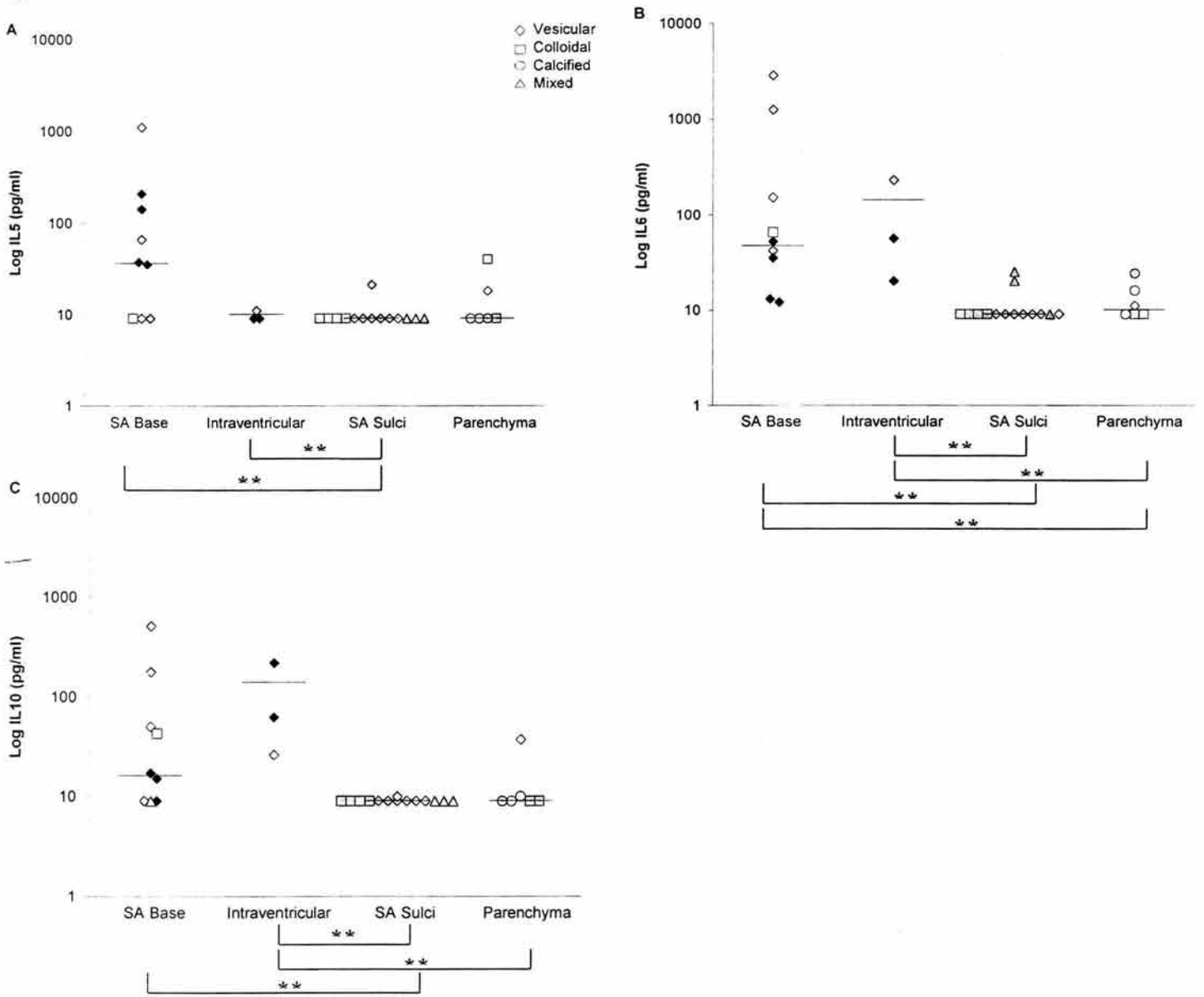
IgG1 Optical Density
IgG2 Optical Density
IgG3 Optical Density
IgG4 Optical Density
0
0,5
1
1,5
2
2,5
3
SA Base
Intraventricular
SA Sulci
Parenchyma
**

Figure 3


A
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B
Log IL6 (pg/ml)
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Intraventricular
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Parenchyma
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C
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Parenchyma
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10. DISCUSIÓN

En este trabajo se evaluó la respuesta inmunológica en poblaciones expuestas y no expuestas a *Taenia solium* así como en individuos infectados en el SNC asintomáticos y sintomáticos. La población altamente expuesta mostró una respuesta inmunológica específica caracterizada por niveles significativamente superiores de IgG total, IgG1, IgG2, IgG4, IgE específicos y proliferación celular específica con respecto al grupo de individuos residentes de áreas de baja exposición. Dentro del grupo de individuos altamente expuestos se describieron dos perfiles inmunológicos. Los individuos no infectados en el SNC mostraron un perfil inmunológico prominente pero balanceado tanto con componentes TH1 como TH2. En contraste, el perfil de los individuos con NC asintomática se caracterizó por presentar una respuesta predominantemente de tipo TH2 (IL4, IL5, IL12, IL13 e IgG4), lo que sugiere que este perfil favorece un curso silencioso y la resolución de la NC (lesiones únicas calcificadas y sin sintomatología). Finalmente, los grupos sintomáticos mostraron principalmente un perfil inmunológico con los niveles elevados de las cuatro subclases de IgG específicas y una muy baja proliferación celular específica con baja producción de citocinas. Con base a los resultados descritos podemos proponer que existen diferentes perfiles inmunológicos asociados a la magnitud de la exposición, a la infección y a la sintomatología (Figura 3). Considerando las claras diferencias entre el perfil observado en la respuesta periférica en individuos sintomáticos y asintomáticos cabe considerar que una respuesta preferencialmente TH2 se asocia a una evolución benigna de la NC y probablemente sea resultado de la alta exposición. Estudios adicionales, como por ejemplo evaluaciones longitudinales de individuos provenientes de medios con diferentes niveles de exposición, podrían contribuir a confirmar esta posibilidad.

Una de las características de la NC es su heterogeneidad clínica (respecto a la sintomatología así como su presentación imagenológica). Si bien se han comenzado a explorar, las causas de ésta heterogeneidad aún se están dilucidando. En este trabajo se exploró la posibilidad de que la heterogeneidad de la respuesta inmunológica pudiera estar participando en el pleomorfismo de la NC. Los resultados observados apoyan esta posibilidad. Del conjunto de personas altamente expuestas al parásito, según criterios epidemiológicos e inmunológicos, sólo algunos individuos se infectaron en el SNC. Estas personas con NC asintomática presentaron en su mayoría lesiones únicas con el parásito calcificado localizado en el parénquima y/o en el espacio subaracnoideo de los surcos. En estos individuos se observó una respuesta inmunológica periférica con IL4, IL5, IL12, IL13 y bajos niveles de las subclases de IgG específica. En contraste, el grupo con NC sintomática presentó predominantemente lesiones múltiples y el parásito en estado coloidal, vesicular o formas mixtas localizados en el espacio subaracnoideo de la base, intraventricular o localizaciones mixtas. Este grupo mostró una respuesta inmune periférica con poca proliferación celular específica, bajos niveles de citocinas y altos niveles de inmunoglobulinas G específicas. En conjunto estas observaciones sugieren que en aquellos individuos con formas sintomáticas de la NC, la respuesta celular mediada por linfocitos T a nivel periférico está deprimida siendo insuficiente para controlar la infección. Paradójicamente, la respuesta inmunológica en el SNC de pacientes sintomáticos está exacerbada. En apoyo a esto, los pacientes con sintomatología severa tenían el parásito frecuentemente en estado vesicular y alojado en el espacio subaracnoideo de la base o intraventricular, con una respuesta exacerbada de IL5, IL6, IL10 e incrementados los niveles de las cuatro subclases de IgG específicas en el LCR. En contraste, los

pacientes sintomáticos leves y moderados presentaron los parásitos principalmente en estado coloidal o calcificado en el parénquima y/o espacio subaracnoideo de los surcos, con más bajos niveles de inmunoglobulinas específicas y citocinas en el LCR. Independientemente de las causas que las median, las diferencias entre las respuestas inmunológicas periférica y central en individuos sintomáticos con NC sugieren que pudieran existir dos mecanismos de control, regulados independientemente (Abel et al. 1997). Adicionalmente, las diferencias en la intensidad de la respuesta inmunológica asociada a la localización diferencial de los cisticercos señalan la posible compartimentalización de la respuesta inmunológica en el SNC. Al parecer existen regiones en el SNC (espacio subaracnoideo de la base y ventrículos) que son capaces de montar una respuesta inmunológica más intensa que otras (parénquima y espacio subaracnoideo de los surcos). Las causas de estas diferencias se desconocen, si bien pudieran resultar de la distribución diferencial de células presentadoras de antígeno, presencia o ausencia de células inmunológicas y de diferencias en la distribución y densidad de la capilarización y rutas de drenaje de los antígenos (Streilein et al., 1997). Finalmente, la idea de que las diferencias en la sintomatología y la evolución de los parásitos en la NC son resultados de las diferencias cuali- y cuantitativas de la respuesta inmunológica podría extenderse y explicar la heterogeneidad clínica en otras patologías del SNC (e.g., enfermedades neurodegenerativas, infecciosas o neoplasias) dentro de una población dada (Kalkers et al., 2000, Duffau et al., 2004, Huijbregts et al., 2004, Koh et al., 2004, Taylor et al., 2004).

Además de la relevancia de la localización del parásito existen evidencias que señalan la participación de otros factores en la heterogeneidad de la NC. Entre ellos destacan la magnitud de la exposición, la edad y el sexo. En este trabajo no se

encontraron diferencias en las características de la respuesta inmunológica con respecto a la exposición ni a la edad, aunque el diseño experimental no resultó el más adecuado para valorar estos factores. Sin embargo sí se observaron resultados que sustentan el dimorfismo sexual en la respuesta inmunológica en la NC. En pacientes con NC sintomática las mujeres mostraron niveles más elevados de IL6 e IL10 en el LCR y presentaron una tendencia a tener niveles más altos de IL5, IgG2 e IgG3 específica que los hombres. Estas diferencias fueron independientes del cuadro sintomático e imagenológico. Estas observaciones no resultan sorprendentes ya que las respuestas dimórficas sexuales de la respuesta inmunológica han sido documentadas en diferentes mamíferos (Morales et al, 2004). Además el dimorfismo sexual anatómico y funcional ha sido descrito en diversos órganos que constituyen el sistema endocrino, nervioso y digestivo (Gorski, 1984). Independientemente de estas consideraciones, es importante enfatizar que el dimorfismo sexual debe de ser considerado en el manejo terapéutico de los pacientes con NC sintomática.

Como colorario, este trabajo representa un esfuerzo por dilucidar la participación de la respuesta inmune en la comprensión de porqué en algunas personas la NC cursa de manera asintomática o con sintomatología leve mientras que en otras lleva a cuadros clínicos severos. Esta pregunta resulta válida para la mayoría de las enfermedades incluyendo aquellas que son de origen infeccioso, donde se ha establecido el paradigma de que el contacto con un agente infeccioso lleva a la enfermedad, lo cual claramente hoy se visualiza como una simplificación. La información obtenida en esta tesis podría además resultar de interés para optimizar el diseño y manejo de medidas preventivas y terapéuticas dirigidas a evitar que las interacciones entre el parásito y el huésped causen daño al SNC.

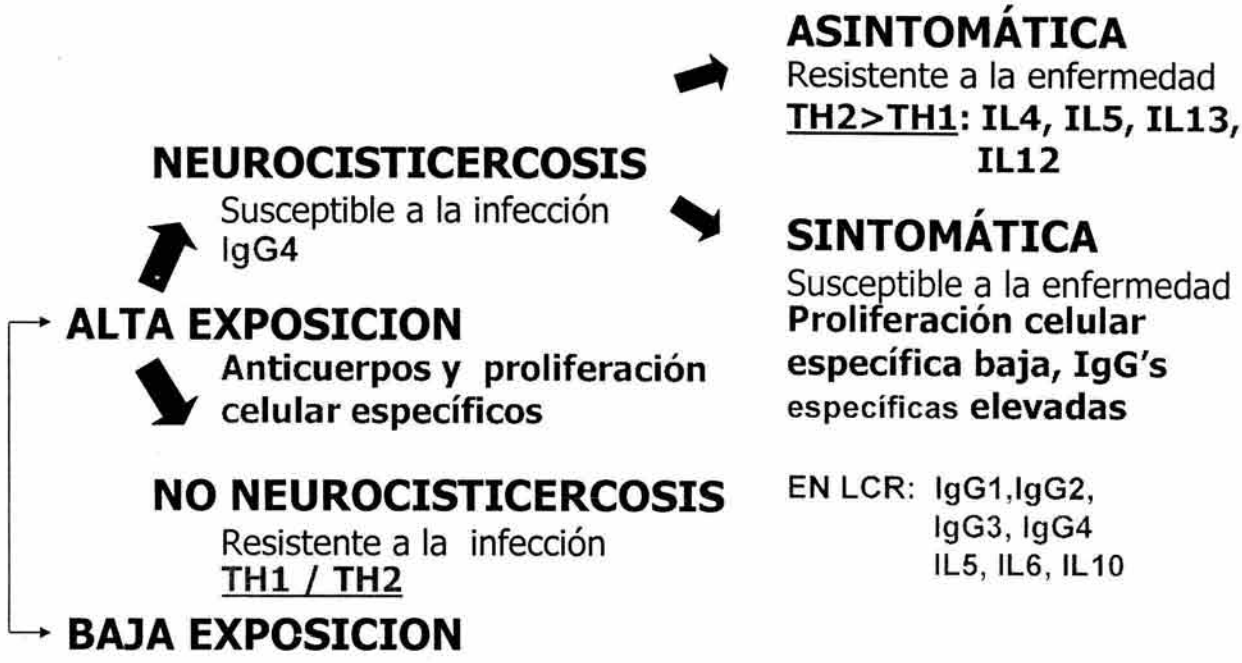

Figura 3. Perfiles inmunológicos relacionados a los fenotipos de la exposición y la infección asintomática y sintomática.

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11. APÉNDICES

RIC

RINCÓN DEL RESIDENTE

La red de comunicación neuroinmunoendocrina y la regulación de la homeostasis: el uso de hormonas y neurohormonas como inmunoterapia

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Immunoneuroendocrine communication network and the regulation of the homeostasis: the use of hormones and neurohormones as immunotherapy

ABSTRACT

The complex communication between the immune and neuroendocrine systems is bi-directional, and involves sharing different ligands and receptors in tissues and organs in vertebrate in general, and mammals in particular. It have been demonstrated that many hormones, neurohormones and neurotransmitters have profound effects on the immune system, and in turn, cytokines produced by cells of the immune system cause important changes in the neuroendocrine function. It has been shown in different animal models with induced and spontaneously occurring autoimmune diseases that alterations of the immunoendocrine interactions are involved in the breakdown of self tolerance, and by extend, the homeostasis. This review discusses the role of the neuroimmunoendocrine interactions in maintaining the homeostasis of mammals, and their role in the development of some autoimmune diseases. The finding that could exist a defective immunoneuroendocrine communication may lead to more specific therapy of several autoimmune human diseases.

Key words. *Immunoendocrine. Homeostasis. Interactions. Regulation.*

RESUMEN

La comunicación entre el sistema inmunológico y el neuroendocrino es bidireccional, ya que ambos sistemas comparten ligandos y receptores en diferentes tejidos y órganos en los vertebrados en general, y muy particularmente en los mamíferos. Se ha demostrado que muchas hormonas, neurohormonas y neurotransmisores afectan el funcionamiento del sistema inmunológico, y que las citocinas producidas por varias células del sistema inmunológico causan cambios importantes en la función del sistema neuroendocrino. También se ha observado, en varios modelos animales con enfermedades autoinmunes espontáneas e inducidas, que las interacciones neuroinmunoendocrinas que ocurren determinan el rompimiento de la autotolerancia y, por lo tanto, de la homeostasis. Esta revisión discute el papel de las interacciones neuroinmunoendocrinas y el mantenimiento de la homeostasis. El hallazgo de que podría existir una comunicación neuroinmunoendocrina deficiente puede dirigirnos a la búsqueda de terapias más específicas en varias enfermedades del ser humano.

Palabras clave. Inmunoendocrino. Homeostasis. Interacciones. Regulación.

INTRODUCCIÓN

La interacción entre los sistemas nervioso, endocrino e inmunitario es uno de los elementos clave que intervienen para mantener la homeostasis de los vertebrados, siendo particularmente imprescindible en los mamíferos.[1] La capacidad del sistema inmunológico para discriminar entre lo propio y lo no propio está

basada en un amplio espectro de especificidad expresada por las células inmunológicas.[2] Esta característica del sistema inmunológico implica que puede percibir una imagen interna de los componentes del organismo y reaccionar a las distorsiones particulares de esta imagen (como son las células propias transformadas).[2] La respuesta inmunológica, como una respuesta homeostática bajo control fisiológico, contribuye al mantenimiento de la integridad de las células corporales y de los tejidos.[3] Las hormonas y neurotransmisores que están presentes en el micro-ambiente de las células inmunológicas pueden restringir su autonomía, probablemente por su acción sobre los receptores de estos factores neuroendocrinos.[3] La comunicación eficiente de estos tres sistemas implica la existencia de vías aferentes y eferentes que constituyen un sistema complejo de retroalimentación (Figura 1). Cuando se producen alteraciones en esta red, se desencadenan patologías que involucran a los diferentes componentes de la misma.

En los últimos años se ha avanzado en forma notable en el conocimiento de las múltiples funciones del sistema inmunológico, una de ellas ha sido la adaptación biológica, a través de la eliminación de patógenos y células extrañas del organismo.[4] Estas funciones requieren a su vez de sistemas de control delicados que permitan la adaptación a las diferentes situaciones fisiológicas y patológicas por las que puede atravesar todo ser biológico durante su vida, siendo necesaria la interacción con los otros sistemas del organismo.[4] Esta interacción es constante y hace posible el funcionamiento armónico de estos tres sistemas, esto implica la existencia de mensajeros y receptores comunes que participan al mismo tiempo en un sistema muy complejo de retroalimentación. La alteración de la comunicación entre estos sistemas conduce al desarrollo de patologías diferentes. Tal es el caso de los trastornos neuropsiquiátricos que causan inmunosupresión como es la depresión,[5] los trastornos inmunitarios que causan

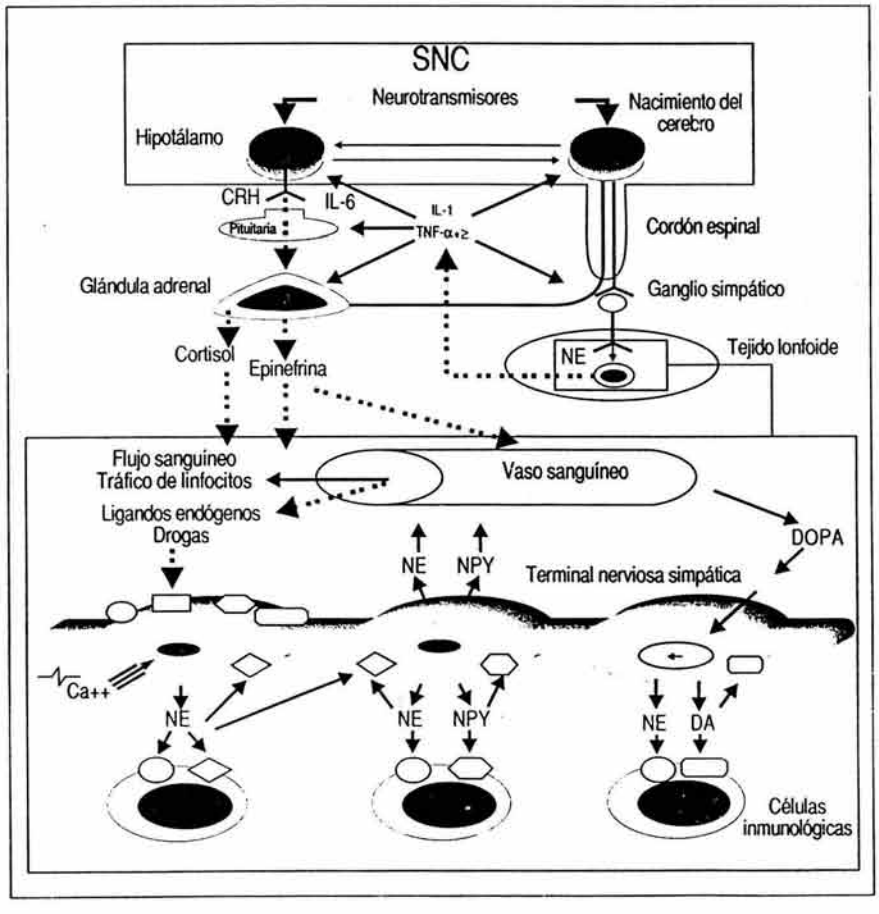

Figura 1. Esquema simplificado de la comunicación bidireccional neuroinmunológica: la activación del sistema nervioso simpático y del eje HPA por citocinas, tales como TNF-α, IL-1β e IL-6 es un sistema de retroalimentación por parte del sistema inmunológico.

problemas endocrinos (como la tiroiditis de Hashimoto y la diabetes mellitus tipo 1), que ejemplifican la interacción funcional entre el sistema inmunológico y el neuroendocrino.[6]

Numerosos datos experimentales demuestran que, al igual que otras células corporales, las células del sistema inmunológico se ven influidas por el sistema neuroendocrino, ya que existen diversos niveles de control, tanto en el metabolismo como en la división celular, reguladas por las hormonas y los neurotransmisores.[7] La respuesta inmunológica es, tal vez, el único fenómeno fisiológico, en el cual la amplificación de su respuesta está basada en la proliferación celular y la transformación específica de sus componentes. Este proceso requiere cambios metabólicos y factores de crecimiento que hacen a esta respuesta dependiente del control neuroendocrino.[8]

Por ejemplo, se conoce que la fase de activación de los linfocitos T no diferenciados de fenotipo $CD4^+$ está determinada por el reconocimiento específico de determinantes antigénicos que se presentan en el contexto de moléculas del complejo mayor de histocompatibilidad clase II (MHC II, por sus siglas en inglés), que se expresan sobre las células presentadoras de antígeno profesionales, tales como macrófagos, linfocitos B o células dendríticas.[2] La especificidad de la respuesta inmunitaria determinada por estos linfocitos T $CD4^+$ está modulada por la expansión selectiva de las clonas que son capaces de reconocer dichos determinantes antigénicos y, por lo tanto, de diferenciarse a células efectoras (del inglés "helper" Th1 o Th2) que contribuyen a la protección del organismo contra enfermedades infecciosas.[2,9] La clasificación de Th1 y Th2 se hizo basados en el patrón específico de citocinas que producen: los linfocitos Th1 se caracterizan por la producción de citocinas como la interleucina 2 (IL-2), el interferón gamma (INF-γ) y el factor de necrosis tumoral beta (TNF-β) y están principalmente involucrados en la regulación de la inmunidad de tipo celular activando macrófagos, y también en la hipersensibilidad de tipo retardada.[9] Mientras que los linfocitos Th2

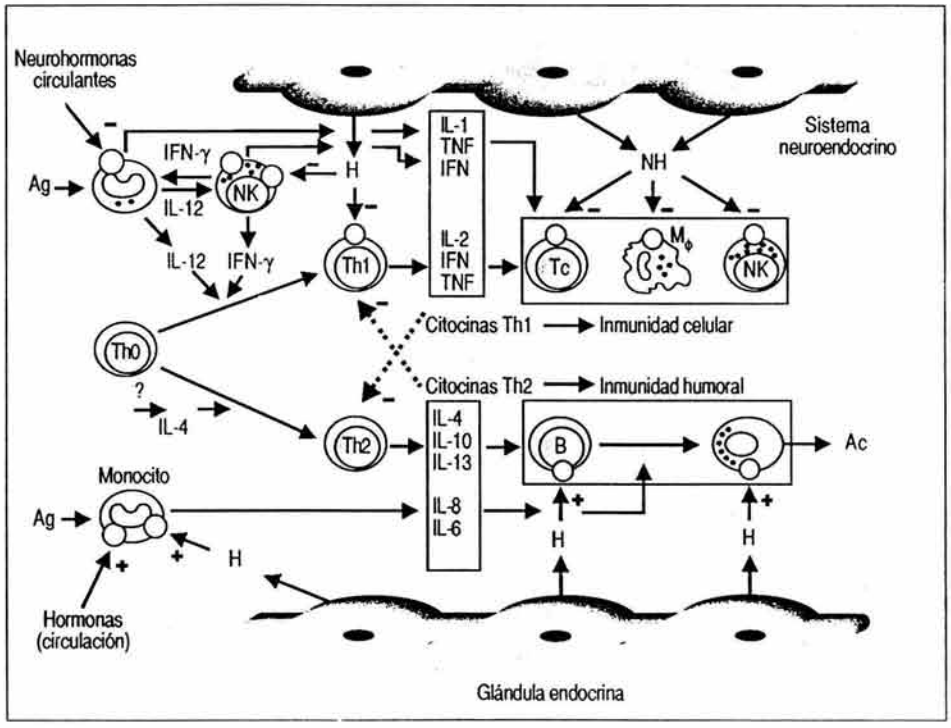

Figura 2. *Papel de las células Th1 y Th2, y citocinas tipo 1 (proinflamatorias) y tipo 2 (antiinflamatorias) en la regulación de la inmunidad celular y humoral. La inmunidad celular es estimulada por citocinas tipo Th1, secretadas por las células presentadoras de antígeno (APC's) y células Th1, mientras que la inmunidad humoral es estimulada por citocinas tipo Th2, secretadas por las APC's y las células Th2. La fuente celular de IL-4 que estimula la diferenciación de Th0 a Th2 son los macrófagos. La inmunidad celular provee protección contra bacterias intracelulares, protozoarios, hongos y varios virus, mientras que la inmunidad humoral provee protección contra parásitos multicelulares, bacterias extracelulares, algunos virus, toxinas solubles y alergenos. De manera que los efectos sistémicos controlados por Th2 pueden ser controlados por la liberación de la NE de los nervios simpáticos postganglionares en vasos sanguíneos y órganos linfoides, y el efecto de la epinefrina, secretada desde la médulas adrenal, sobre la producción de citocinas reguladoras clave tipo Th1 o Th2, sobre la función de células Th1 o Th2, y, respectivamente, componentes de la inmunidad celular y humoral.*
Líneas sólidas denotan estimulación, líneas punteadas denotan inhibición, Ag = antígeno, T = células T, B = células B, PL = célula plasmática, Mø = macrófagos, NE = norepinefrina, NPY = neuropéptido Y, DOPA = dopamina, NH = Neurohormonas.

producen las interleucinas IL-4, IL-5, IL-6, IL-10 e IL-13, regulando la respuesta inmune humoral a través de la proliferación de linfocitos B y el cambio del isotipo específico de anticuerpos, además de promover la diferenciación de eosinófilos y mastocitos.[2,9] Sin embargo, tanto las hormonas como los neurotransmisores ejercen influencias sobre las células inmunológicas, afectando la producción de varias citocinas, y que varios productos de la respuesta inmunológica tanto tipo Th1, como la respuesta tipo Th2, ejercen un efecto regulador sobre el sistema neuroendocrino[9] (Figura 2).

Comunicación neuroinmunológica

Se ha observado que los órganos linfoides están inervados por el sistema nervioso simpático y que la denervación de estos órganos conlleva a respuestas inmunes exacerbadas. Otros estudios demuestran que después de la administración *in vivo* e *in vitro* de σ-agonistas (como metoxamina) se produce la inhibición de la respuesta inmunológica. La conclusión a la que se ha llegado es que la inervación simpática media un mecanismo de restricción sobre la actividad de las células inmunológicas.[10] En otros estudios se ha evaluado la actividad simpática en el microambiente de los órganos linfoides midiendo los niveles de noradrenalina (NA) durante la respuesta inmunológica, y los resultados demuestran que estos niveles están disminuidos en ratas inmunizadas. Estos datos constituyen dos evidencias de que un cambio en el sistema simpático produce un efecto sobre la función inmunitaria.[11]

Comunicación inmunoendocrina

La hipófisis controla directa o indirectamente la actividad de casi todas las glándulas endocrinas y a su vez esta glándula es regulada por el hipotálamo. La hipófisis es directamente influenciada por la interleucina 1 (IL-1) y la interleucina 6 (IL-6), que ejercen su efecto principalmente sobre la secreción de la hormona adrenocorticotrófica (ACTH).[12] Ésta suprime la respuesta de anticuerpos a antígenos dependientes (eritrocitos de carnero) e independientes (dinitrofenol, DNP) de células B.[13] La ACTH también inhibe la producción de interferón-γ por linfocitos T en cultivo[14] y bloquea la capacidad tumoricida de los macrófagos.[15] Otras hormonas producidas en la hipófisis con efectos sobre el sistema inmunológico son: la hormona de crecimiento (GH), la prolactina (PRL), la hormona luteinizante (LH), la hormona foliculoestimulante (FSH), entre otras. En el cuadro 1 se resumen los efectos que las diferentes hormonas tienen sobre el sistema inmunológico.

Comunicación inmunoneuroendocrina a través de ligandos y receptores

Como ya se mencionó, las interacciones neuroinmunoendocrinas son muy complejas, e involucran un asombroso grado de evolución y comunicación bioquímica. Hormonas, neuropéptidos, citocinas y quimiocinas figuran de manera prominente en estas interacciones. De forma que estos sistemas están interrelacionados por una red de comunicación en la cual varias hormonas y neuropéptidos modulan la función inmunológica, y en cambio, la respuesta inmunológica se refleja en cambios neuroendocrinos.[16] Podemos clasificar estas interacciones de manera general en la siguiente forma: 1) Moléculas del sistema inmunológico, endocrino y nervioso coexisten en tejido linfoide, endocrino y neural, 2) Mediadores endocrinos y neurales modulan la actividad del sistema inmunológico y viceversa, y 3) Células de los sistemas nervioso, endocrino e inmunológico expresan receptores para citocinas, hormonas, neuropéptidos y neurotransmisores.[16] De manera que, al compartir ligandos y receptores comunes, el organismo tiene varias maneras distintas de regular la misma función, y se protege a sí mismo de reacciones exacerbadas que implican una alteración de la homeostasis.[16] Por ejemplo, una manera de ejemplificar esta comunicación bioquímica se produce con la respuesta al estrés, en la que el organismo controla la respuesta inflamatoria no sólo vía la activación por citocinas (IL-1, IL-6, MIF) del eje hipotálamo-hipófisis-suprarrenal, sino que también se incrementa la sensibilidad a los esteroides suprarrenales por un incremento en el número de receptores a esteroides en las células inmunológicas, por el aumento de la unión a los elementos de respuesta a hormonas en el DNA, y la transcripción de genes blanco de hormonas esteroides (como por ejemplo TNF-α, IL-2, IL-6). Así, estos mecanismos contribuyen a aumentar la actividad antiinflamatoria o inmunoestimulatoria de las hormonas esteroides y regulan de manera importante su efectividad y control.[17]

En relación con las hormonas esteroides sexuales, se ha sugerido en general que: 1) Las hormonas sexuales juegan un papel importante en la respuesta inmunológica regulando la proliferación y/o activación linfocitaria a través de receptores nucleares a las mismas, 2) Un microambiente de "hembra" es conductivo a la expansión de células secretoras de auto-anticuerpos por medio del incremento en el cambio de clase de inmunoglobulina, inducida por la unión de estradiol a su receptor, y su translocación al DNA y 3) La actividad de las células inmunológicas depende del microambiente endocrino, ya que

Cuadro 1. Efecto específico de varias hormonas sobre el sistema inmunológico.

Hormona	Fuente	Célula inmunológica blanco	Efecto principal
17β-Estradiol	Ovario Testículos Glándulas Suprarrenales Neuronas	Células B Mastocitos Células Th1 yTh2 Mø Células NK Eosinófilos	Activador policlonal de células B, promueve la diferenciación a células plasmáticas, ↓ la masa del timo y médula ósea, ↑ las células secretoras de IL-10 e IL-6, ↓ la producción de IFN-γ e IL-2, regula negativamente la actividad de NK's, ↑ la fagocitosis por Mø, ↑ la liberación de serotonina e histamina.
Testosterona	Testículos Ovario Neuronas	Células B Células T Mø	↓ la respuesta de B a mitógenos, ↓ la secreción de serotonina e histamina por mastocitos, ↓ la producción de IL-1, IL-6 y TNF-α.
Progesterona	Testículos Ovario Neuronas	Células T Células B Células NK	↓ la actividad citotóxica de NKs, ↑ la secreción de TNF-α, ↓ la secreción de citocinas y la producción de ON por Mø.
Cortisol	Glándula suprarrenal Adipositos	Células T Células B	↓ la producción de leucotrienos y PGEs, modula la maduración de células T y B, afecta el tráfico y activación de células proinflamatorias, ↓ la producción de IL-1, IL-2, IL-6, IL-8, IL-10, IL-12, y TNF-α.
DHEA	Glándula Suprarrenal Neuronas	Células T Monocitos Mø	↑ la producción de IL-2, ↑ la producción de IFN-γ ↓ la secreción de IL-6 y TNF-α, protege contra daño neurooxidativo, ↑ la inmunidad de células T, ↑ la DTH.
CRH	Hipotálamo	Mø Leucocitos Linfocitos B	↑ la producción de IL-1 e IL-6, ↑ la quimiotaxis y producción de metabolitos reactivos del O^2, ↑ la proliferación de células B y la expresión de los receptores a IL-2 en células T.
ACTH	Hipófisis	Células T Células B	↑ la producción de Ac, la secreción de citocinas y la proliferación de T.
Prolactina	Hipófisis	Células T Células B Mø	↑ la proliferación de linfocitos en respuesta a antígenos y mitógenos, ↑ la secreción de IFN-γ e IL-2, ↓ los mecanismos de muerte celular en células inmunes, induce la diferenciación de NKs a células asesinas activadas por prolactina (PAKs).
VIP	Plexos Mesentéricos	Mø Células T	↓ la producción de agentes proinflamatorios, ↑ la producción de citocinas antiinflamatorias (ambas funciones en Mø activados), ↑ la diferenciación a células Th2
GH	Hipófisis	Timocitos	↑ la adhesión de timocitos a células tímicas epiteliales, ↑ la liberación de los timocitos desde la médula, ↑ el tráfico de intratímico.
Hormonas Tiroideas	Tiroides Hipófisis	Médula ósea	↑ La proliferación de células B inmaduras.
Vasopresina y oxitocina	Hipófisis	Células T	↑ la proliferación de células T y B.
Encefalinas	Hipófisis	Células B Células T	Bajas dosis: ↑ activa células B y T. Altas dosis: inmunosupresión.
Endorfinas	Hipófisis	Células B Células T	↓ la proliferación de células B y la producción de Ac.
hCG	Placenta	Células T Células NK	↓ la proliferación de T y NK y la inducción de T supresores.
Melatonina	Glándula Pineal	Células T Timocitos	Afecta la maduración y diferenciación de los timocitos

Símbolos y abreviaciones: ↑ = incrementa, ↓ = suprime, Ac = anticuerpos, DTH = hipersensibilidad de tipo retardado, CRH = corticotropina, ACTH = adrenocorticotropina, DHEA = dehidroepiandrosterona, GH = hormona de crecimiento, VIP = péptido intestinal vasoactivo, hCG = gonadotropina coriónica humana, IL = interleucina, ON = óxido nítrico, PGEs = prostaglandinas, O_2 = oxígeno, Mø = macrófagos, NK = natural Killer.

este ambiente determina el tipo, el número de receptores expresados y la estabilidad de su unión al ligando.[16,17]

El uso de las hormonas como inmunoestimuladores

Cuando existe una desregulación del sistema inmunológico, el organismo es incapaz de responder adecuadamente. Esta respuesta inapropiada puede asociarse a un desbalance hormonal específico, en particular en aquellas hormonas que pudieran actuar como hormonas inmunorreguladoras (HIRs), como son la dehidroepiandrosterona (DHEA), el cortisol y los esteroides sexuales.[16,17] Estas hormonas parecen actuar a nivel genómico, regulando la producción de un mensajero químico conocido, como son las citocinas.[17] La desregulación puede ser aguda o crónica, y está asociada a enfermedades que dan como resultado la muerte en schistosomosis, o una calidad de vida gravemente comprometida para millones de personas cada año en el paludismo o la enfermedad de Chagas. El sistema inmunológico normalmente se encuentra regulado por hormonas y citocinas íntimamente relacionadas, generando así un control apropiado en la respuesta inmunológica.[18] En el ser humano existe una gran variedad de agentes que originan fallas en la regulación del sistema inmunológico. Estos agentes incluyen a virus (como el virus de la inmunodeficiencia humana [VIH], el virus de la hepatitis B [VHB] y c [VHC]), algunos parásitos como *Plasmodium falciparum* y células tumorales.[19] El sistema inmunológico puede estar desregulado por una sobreproducción de las citocinas Th2 y una disminución en la producción de las citocinas Th1, o viceversa; estas condiciones dan como resultado una deficiente capacidad de control de algunas enfermedades o la eliminación de patógenos en las infecciones que de otra forma estarían controlados.[20]

Un desbalance en las citocinas Th1/Th2 no sólo se observa en las enfermedades infecciosas, sino también en enfermedades autoinmunes, procesos inflamatorios y en algunas condiciones asociadas a la edad (como la enfermedad de Alzheimer).[21] De manera que el uso de hormonas como inmunorreguladores para corregir la desregulación inmunitaria puede potencialmente ser útil en el tratamiento de una gran variedad de enfermedades.[22] Sin embargo, diferentes eventos endocrinos pueden interferir en el funcionamiento del sistema inmunológico, teniendo como resultado consecuencias adversas. Así, se sabe que el estrés incrementa la producción de cortisol, el cual a su vez actúa como un inmunosupresor, favoreciendo la respuesta de tipo Th2,[23] por lo que las personas que sufren de un episodio de estrés agudo son más susceptibles a las infecciones.[24] Por otra parte, diversos agentes infecciosos como virus, parásitos y otros patógenos pueden ser capaces de sobrevivir en el organismo por una interrupción de la regulación de las citocinas Th1/Th2.[25] Estos patógenos han encontrado la forma de desviar la respuesta inmunológica tipo Th1 hacia una de tipo Th2, de manera que las células infectadas escapan al control inmunológico.[26]

La edad también es importante en el funcionamiento de nuestro sistema inmunológico, ya que se ha observado una marcada deficiencia en la respuesta inmunológica de tipo Th1, tanto en niños como en ancianos. En la vejez, la respuesta inmunológica de tipo Th1 se pierde progresivamente, de manera que a medida que envejecemos nos volvemos susceptibles a las infecciones virales (como la influenza) que un individuo joven normalmente resuelve con facilidad.[27] Además, la pérdida de la respuesta inmunológica afecta la capacidad del anciano a responder apropiadamente a la vacunación.[28] Se sabe que las citocinas Th2 tienden a presentarse en los primeros años del desarrollo, y que al paso de los años éstas cambian a las citocinas Th1, y que nuevamente regresan a las citocinas Th2 en la vejez.[29] Esta reducción en la respuesta inmunológica de tipo Th1 está fuertemente asociada a enfermedades como el asma y las alergias en edades tempranas.[30] En muchos casos, el asma o las alergias se hacen nuevamente patentes a medida que el individuo envejece y su respuesta de tipo Th1 disminuye. La falta en la respuesta de tipo Th1 también está implicada en la incapacidad del organismo para eliminar las células cancerígenas.[31] Estos datos sugieren que una terapia hormonal pudiera restablecer apropiadamente el balance de las citocinas Th1/Th2 en la gente que sufre enfermedades causadas por la desregulación inmunitaria, como son enfermedades infecciosas, cáncer y otras.[32]

De esta forma, las hormonas podrían actuar manteniendo el balance Th1/Th2, necesario para defenderse ante cualquier reto antigénico (Figura 2). De manera que las HIRs actúan a través del sistema endocrino, y regulan de manera natural el balance en una respuesta inmunológica. Estas HIRs pueden ser metabolitos o análogos de las hormonas circulantes en los organismos.[33,34] Los niveles de estas hormonas, como, por ejemplo, la DHEA, el cortisol y los esteroides sexuales, normalmente se incrementan después del nacimiento, tienen un pico entre los 20 y 30 años, y empiezan progresivamente a decaer, de forma que durante el envejecimiento sólo representan una

pequeña fracción de lo encontrado en jóvenes adultos sanos.[35] El incremento y la disminución de estas hormonas es paralelo al desbalance Th1/Th2 a medida que envejecemos. Este fenómeno sugiere que las terapias hormonales pueden ser una forma apropiada de restaurar el balance inmunitario.[36]

La forma en que las HIRs actúan podría ser a nivel celular, a través de receptores nucleares que estimulan la transcripción de citocinas. Esta regulación ocurre en un contexto dependiente de la enfermedad. Esto significa que el efecto de las HIRs sobre la producción de citocinas puede variar según la naturaleza del desbalance inmunitario.[37] Es decir, un desbalance en la producción de citocinas Th1 podría generar una respuesta reguladora diferente que la producida por un desbalance por Th2. A diferencia de la administración sistémica de una sola citocina que puede inclinar la respuesta inmunológica hacia una sola dirección, las HIRs pueden tener la capacidad de restaurar apropiadamente el balance inmunitario a través de la producción natural endógena de citocinas. Como resultado, esta nueva terapia se espera que reduzca la propensión a la citotoxicidad. Por otro lado, varias HIRs han sido estudiadas y se ha encontrado que influyen en la producción de múltiples citocinas del balance Th1/Th2 en los casos donde existe desregulación inmunitaria.[38] También se ha encontrado que estas HIRs son capaces de corregir el mal funcionamiento de las células dendríticas,[39] que son células presentadoras de antígenos, claves en el sistema inmunológico, que ayudan a dirigir la respuesta Th1/Th2. Estudios preclínicos han demostrado que la terapia con las HIRs puede corregir la desregulación Th1/Th2 que se presenta en células dendríticas como resultado de una infección y/o enfermedad.[40] También se ha visto que la administración de hormonas produce beneficios funcionales en diversas enfermedades, incluyendo el SIDA, diversos tipos de cáncer, varias enfermedades autoinmunes y enfermedades relacionadas con el envejecimiento.[41]

CONCLUSIONES

Hasta hace unos años el sistema inmunológico ha sido visto como un sistema aislado de los otros sistemas corporales. Es evidente en esta revisión que el sistema inmunológico y neuroendocrino comparten numerosos ligandos y receptores, lo que resulta en una constante e importante comunicación bidireccional. De hecho, se ha postulado que una nueva e importante función del sistema inmunológico sería la de servir como un órgano sensorial para los estímulos no cognoscitivos que para el sistema nervioso central pasan inadvertidos, como pueden ser los agentes infecciosos. Lo que en la actualidad estamos proponiendo es la reintegración de un sistema importante en el contexto fisiológico de todo el organismo. Esto, indudablemente nos llevará a una mejor comprensión básica de la fisiología y a generar cambios en la práctica de la Medicina moderna. Para entender aún más el proceso de la comunicación bidireccional del sistema inmunológico y el neuroendocrino será necesario continuar con la búsqueda de ligandos y receptores comunes de los dos sistemas, así como ahondar en las similitudes y diferencias en su regulación funcional. Eventualmente se encontrarán nuevas funciones del sistema inmunológico sobre los neuropéptidos o las neurohormonas, así como otras propiedades endocrinas de las citocinas. Además, será un reto para los fisiólogos integrar esta información en el contexto del organismo como un todo. Por otro lado, los avances en el conocimiento básico de la interacción inmunoendocrina debe llevarnos al diseño de nuevas terapias para el tratamiento y diagnóstico de enfermedades en humanos, tanto de aparente origen inmunitario como endocrino. Dos recientes descubrimientos ilustran fehacientemente las posibilidades futuras del uso de este conocimiento. El primero es la observación de que la corticotropina es un agente proinflamatorio,[42] y el segundo es la disminución del rechazo a un injerto renal en ratas tratadas con el antagonista opiáceo, naltindrolona.[43] Hace algunos años hubiera resultado difícil de imaginar que se pudiera tratar una inflamación periférica con un antagonista de una hormona liberadora hipotalámica o el usar un antagonista de un opiáceo para facilitar un trasplante de tejido.

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High Prevalence of Calcified Silent Neurocysticercosis in a Rural Village of Mexico

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Key Words
Neurocysticercosis · Epidemiology · CT scan · *Taenia solium* · Cysticercosis

Abstract
Human neurocysticercosis (NC) is a parasitic disease caused by *Taenia solium* when its larvae lodge in the central nervous system. NC prevalence estimates are obscured by the variable and often asymptomatic clinical picture. While infection depends on exposure, severity is possibly related with various host factors (immunity, genes and gender). This epidemiological study of cranial CT scans in an endemic rural community found that 9.1% of apparently healthy subjects had calcified lesions and were completely asymptomatic. Silent NC cases did not correlate with the exposure factors tested but showed family aggregation and higher rates of positive serology. Thus, NC prevalence may be higher than currently considered and host-related factors appear to be involved in infection and pathogenesis.

Introduction

Neurocysticercosis (NC) is a frequent disease of the human central nervous system (CNS) in developing countries of Latin America, Asia and Africa, where conditions favoring transmission persist. NC has recently reemerged in affluent societies due to immigration [1]. In endemic countries, it is the main cause of late-onset epilepsy (30–50%) [2, 3]. it represents the third most common cause of admissions to neurological hospitals in Mexico, and was found in 1.3–3.1% of the autopsies performed at the General Hospital in Mexico City [4]. The clinical pleomorphism of NC is thought to result from parasite factors (location, size, and number) and host factors (degree of immune and inflammatory reactions). The clinical course of NC is variable: some individuals remain asymptomatic and the parasite dies without treatment, while provoking severe neurological disorders in others.

The diagnosis of NC is difficult on clinical grounds alone and requires the use of neuroimaging techniques (computed tomography and magnetic resonance imaging); these tests have restricted availability and disproportionate costs in endemic countries. This factor accounts, at least partially, for the lack of reliable epidemiological

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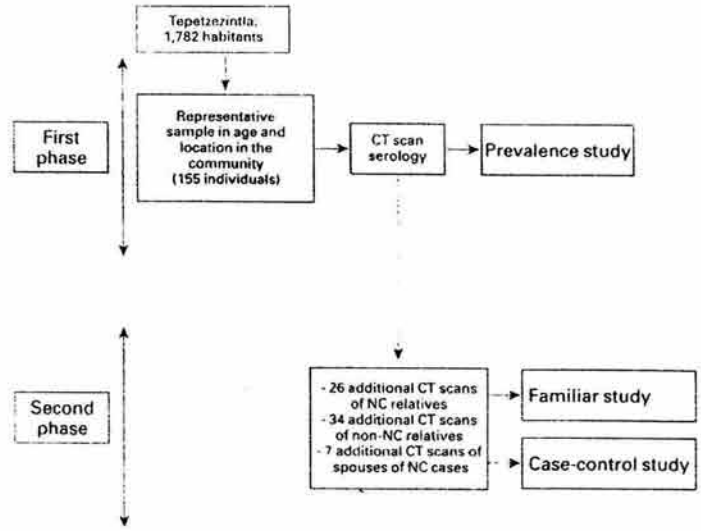

Fig. 1. Study design

data about the real prevalence of NC. Several immunological tests have been used to estimate parasite seroprevalence in rural communities. Seroprevalence varies between 1 and 11% [5–9] depending on the study design and sample size. Although available immunological methods are suboptimal, these data indicate that contact with the parasite is frequent. However, despite widespread exposure, only few individuals develop the disease, which may be mild or severe, suggesting variability in host susceptibility. In other human parasitic diseases, it has been demonstrated that gender [10], age [11] and genetic background [12] modulate host susceptibility, although, in most cases, the mechanisms remain to be elucidated. Clear evidence of the relevance of the nonclassic MHC antigen (Q9) and of hormonal factors [13–15] has been obtained in experimental murine cysticercosis caused by *Taenia crassiceps*. Few studies are available on host factors implicated in the susceptibility to *T. solium* infection. Some evidence has been found in experimental swine cysticercosis [16], also pig castration and pregnancy were associated with higher prevalences in natural disease. In humans, one study claims an association between HLA DQw2 antigen and resistance [17]. In addition, hospital studies show a higher prevalence in middle-aged adults and an inflammatory multicystic form has been reported more frequently in women [18].

The present report is an epidemiological survey of NC based on cranial CT scans in a rural community in Mexico pointing to the relevance of biological host factors in the risk of NC in a highly endemic situation.

Materials and Methods

Study Community

This survey was conducted in Tepetzezintla, a rural community of 1,782 inhabitants, located in the state of Puebla in central Mexico. This community was selected because of its inadequate sanitary and socioeconomic conditions that promote the life cycle of the parasite (open-air defecation, rustic pig-rearing methods, consumption of pig meat without inspection and poor hygienic and dietary habits). In addition, a prevalence of 14% of pig cysticercosis in a sample of 80 pigs inspected at the beginning of the study confirmed active transmission in the village.

Study Design

The survey was conducted in two phases (fig. 1). NC prevalence was estimated by CT scan in a random representative sample of 155 residents. Sample size was calculated considering an expected prevalence of 4% and a precision of 3%. After the subjects had given their informed consent, cranial CT scans were obtained of all included subjects at a general hospital in Puebla (a 3-hour ride from the community). In a second phase, additional CT scans were obtained of relatives of NC and non-NC individuals to study the relation of NC with exposure and genetic factors. The genealogies of all participants were analyzed to assess the family links amongst them.

Definition of NC Case and Neurological Test

Subjects were considered NC cases if they presented cerebral lesions compatible with NC [cysts and/or rounded hyperdense lesion(s) compatible with nodular brain cysticercus calcification] in the CT scan. Nonconclusive CT scans were excluded from the analysis (2 cases). A neurologist clinically examined all subjects and searched for present or past neurological symptoms.

Exposure Factors

A standardized questionnaire to collect demographic, socioeconomic, hygienic, dietary and epidemiological data was applied in each household by a rural doctor and two technicians who had lived in the community for 2 years. The characteristics of the living quarters (e.g. type of floor, roof, or WC) were collected by direct visual inspection of all households involved in the study.

Specimen Collection

After informed consent had been given, blood samples were taken to determine antibody levels against *T. solium* cysticerci by ELISA as previously reported [19]. A serum sample was considered to be positive in ELISA when its optical density reading exceeded the mean value of subjects from Tepetzezintla with negative cranial CT scan, plus two standard deviations (optical density ≥0.4). Three samples of feces from individuals were also collected on different days, fixed in 10% formalin and examined microscopically using conventional coproparasitoscopic Faust and Graham techniques.

Swine Cysticercosis

Pigs bred in the community were examined for the presence of *T. solium* cysticerci by visual inspection and palpation of the tongue surface by two veterinarians experts in this form of diagnosis.

Statistical Analysis

Data were processed using Excel 7.0 (Microsoft) and Statistica for Windows (Statsoft). Distribution of independent variables was compared by χ^2 tests with Yates correction, two-tailed Fisher's exact tests or t test, using 95% confidence intervals (CI). Odd ratios (OR), with corresponding 95% CI and p values, were calculated to identify the risk factors associated with cysticercosis and seropositivity. Logistic regression, using a forward stepwise analysis, was also used to assess the association between the different variables and the results of the CT scans and serological studies. The variables included in this analysis were those with a $p \leq 0.3$ in univariate analysis.

Results

Prevalence Study

We estimated NC prevalence in a sample of 154 individuals living in 70 households. Males were underrepresented (28.4%) due to migration. The main characteristics of the sample were as follows: 28% were illiterate, 54% were living in houses without cement roof, 26% in houses with earth floor, 61% in houses without latrines or toilets, 12% had a car, 49% declared washing fruits and vegetables, 69% practiced rustic pig rearing, 6% claimed antecedents of passing tapeworms and 7% declared to have consumed infected pork meat.

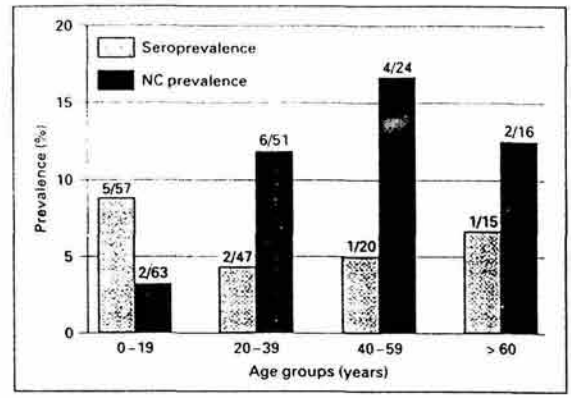

Fig. 2. Age-specific NC prevalence and seroprevalence. Seroprevalence does not differ at different ages (p = 0.58). NC prevalence weakly increased with age (p = 0.06).

Cranial CT scans showed lesions compatible with NC in 14 individuals (3 males and 11 females), amounting to a prevalence of 9.1%. All lesions were calcified: single lesions were found in 12 individuals and multiple lesions in 2. Neither neurological symptoms nor signs were present in any of the NC cases. Only 1 subject with normal CT scan presented seizures. Calcifications were considered of cysticercal origin as in Mexico the prevalence of other brain granulomatous diseases is much lower than NC and because the calcifications had a size and shape typical of cysticercosis. NC prevalence was higher in women (10 vs. 6.8%) but the difference was not statistically significant (OR = 1.52; 95% CI 0.4–21.3, p = 0.39). Ages varied from 8 to 66 years. Subject age was weakly associated with NC-compatible lesions (p = 0.06, χ^2 test for linear trend; fig. 2). Multiple lesions were more frequent in males (p = 0.046) and in subjects over 60 years old (p = 0.0007).

Serology was performed on 139 individuals (90.3%), and seroprevalence was 6.47% (9/139). For NC diagnosis, the serology sensitivity was 15.4% (2/13) and specificity 95.5% (120/126); 25% (2/8) of seropositive subjects and 8.4% (11/131) of seronegative individuals had a cranial CT scan image characteristic of NC. Seropositivity was more frequent in females (6.4 vs. 4.5%) although the difference was not statistically significant (p = 0.5), and did not significantly vary with age (p = 0.58, χ^2 test for linear trend; fig. 2).

Table 1. Main characteristics of the 18 subjects with brain lesions compatible with NC

Case No.	Gender	Age years	Calcifications at CT scan	Localization	ELISA	Coproparasitoscopic test	Neurological symptomatology	Teniasis in family members	Swine cysticercosis	Genealogies
1	F	50	1	right frontal	+	normal	no	no	yes	cousin of case 9
2	F	39	1	left occipital	-	normal	no	no	no	
3	F	43	1	left parietal	-	normal	no	no	no	
4	F	24	1	left frontal	+	ND	no	no	no	cousin of case 18
5[a]	M	17	1	left frontal	+	normal	no	yes	no	brother of cases 6 and 7
6[a]	M	15	1	left frontal	-	*Taenia* spp. + *Escherichia histolytica*	headache	no	no	brother of cases 5 and 7
7	F	10	1	right occipital	-	*E. coli* + *Giardia lamblia*	no	yes	no	brother of cases 5 and 6
8	F	34	1	right frontal	-	normal	no	no	no	
9	F	47	1	right frontal	-	*E. coli*	no	no	no	cousin of case 1
10	F	8	1	right frontal	-	ND	no	no	no	
11[a]	M	34	1	right frontal	ND	normal	headache	no	no	
12	M	66	M	left temporal left parietal	ND	*E. coli*	no	no	no	
13	F	28	1	right frontal	-	normal	no	no	no	
14	M	24	1	right frontal	-	*E. coli*	no	no	no	
15	F	43	1	left frontal	-	ND	no	no	no	aunt of case 16
16[a]	F	22	1	right frontal	+	*E. coli* + *Enterobius vermicularis*	no	no	no	niece of case 15
17	M	61	M	right temporal left frontal	+	normal	no	no	no	
18	F	31	1	right temporal	+	*Giardia lamblia*	no	no	no	cousin of case 4 aunt of cases 5, 6 and 7

[a] Cases diagnosed in the second phase of the study.

Coproparasitoscopic tests of stools were made in 123 individuals (79%). *Taenia* spp. eggs were found only in 2 subjects (prevalence of 1.6%); however, taeniosis was not confirmed by expulsion of the parasite after an appropriate taenicidal treatment. These 2 subjects, as well as their relatives were negative for NC on CT scan.

Family Study

To evaluate the relevance of exposure and host-related factors in NC, 67 additional CT scans were made: 26 of relatives of CT scan positive individuals, 34 of relatives of CT scan negative individuals and 7 of spouses of NC cases. Among all, only 1 individual presented seizures albeit having a normal CT scan. Of the total, only 4 individuals (3 men and 1 woman) presented brain calcifications compatible with NC, 2 of which reported headache but did not present abnormalities in neurological examination. *Taenia* spp. eggs were found in stool tests of 1 relative of a positive CT scan case but the diagnosis of taeniosis could not be confirmed by an appropriate treatment. The main characteristics of the overall 18 NC cases are shown in table 1. It should be noted that most of the brain lesions (13/20) were localized in frontal lobes without evidence of lateralization.

Household Clustering

Analysis was performed to search for clusters within the 77 participants' households. Fifteen of these households, housing in all 55 people (15/77 = 19.5%), had 1 NC case in each household, but only 1 household, housing 2 people, (1/77 = 1.3%) had 3 NC cases and 61 households, housing in all 156 people (77.2%) had no NC cases. Prevalence of NC among the household members living with at least 1 NC case [4.16% (2/48)] was lower than the overall prevalence in this study [9.1% (14/154)], indicating no significant household clustering of the cases.

Family Aggregation

Of the 26 CT scans obtained of relatives of NC individuals, 3 had calcified lesions compatible with NC (2 brothers of an NC case and 1 niece). Prevalence of NC cases in relatives of NC cases was 11.5% (3/26) and 0% (0/34) in relatives of non-NC individuals. Additionally, 50% of the cases were related to each other (9/18).

No significant association of NC in marital couples was found in the 27 couples that participated (p = 0.924): in 20, no member was affected, in 7, 1 member was affected and in none were the 2 members affected.

Case-Control Studies

CT Scan-Positives versus CT Scan-Negatives. Exposure and socioeconomic factors were compared between the 18 individuals with positive CT scan and 206 individuals with normal CT scan. No association was found between NC cases and the exposure variables, with the exception of the following ones that were, however, not statistically significant: detection of *Taenia* spp. eggs in the stool test (OR = 4.8; 95% CI 0.002–11590), open-air fecalism (OR = 2.8, 95% CI 0.82–10.4), antecedents of rearing pigs (OR = 1.94; 95% CI 0.25–588), pig rearing at the moment of the survey (OR = 1.85; 95% CI 0.63–5.5), antecedents of eating infected pork meat (OR = 1.85; 95% CI 0.03–9.8), consumption of pork meat more than once per week (OR = 1.66; 95% CI 0.1–6.8), illiteracy or primary school level (OR = 1.65; 95% CI 0.34–33.3), lack of refrigerator (OR = 1.5; 95% CI 0.47–5), presence of swine cysticercosis at the moment of the survey (OR = 1.25; 95% CI 0.4–10.8), neurological symptoms (OR = 1.2; 95% CI 0.2–6) and with washing of fruits and vegetables (OR = 1.06; 95% CI 0.37–3). Using multiple regression analysis, no significant association was found between the different exposure variables and CT scan results.

Seropositives versus Seronegatives. The relation between serology and socioeconomic factors was compared in the 15 seropositive and 184 seronegative individuals. Only the absence of television was statistically associated with seropositivity (OR = 4.02; 95% CI 1.1–14.4; p = 0.02). No significant associations were found between seropositivity and the detection of *Taenia* spp. eggs in the stool test (OR = 18; 95% CI 0.23–1378), the absence of toilets (OR = 3.5; 95% CI 0.5–49), living with an individual that claimed antecedents of teniasis (OR = 2.1; 95% CI 0.06–11.4), precedents of rearing pigs (OR = 1.57; 95% CI 0.2–3), earth floor (OR = 1.53; 95% CI 0.4–5.6), precedents of swine cysticercosis (OR = 1.3; 95% CI 0.4–4.5), rearing pigs (OR = 1.3; 95% CI 0.41–4.2), washing of fruits and vegetables (OR = 1.3; 95% CI 0.4–4.2), rustic pig rearing (OR = 1.1; 95% CI 0.3–4.3), or full-time living in Tepetzezintla (OR = 1.05; 95% CI 0.2–1.4). However, using multiple logistic regression analysis, the best fit model with a positive ELISA result was the presence of *Taenia* spp. eggs in the stool test (OR = 65; 95% CI 2.7–1543; p = 0.009) and living with an individual who claimed having passed a tapeworm (OR = 26; 95% CI 2–328; p = 0.01).

Discussion

This epidemiological survey contrasts the relevance of exposure and biological factors (gender, age and genetic background) in NC infection. It was performed in a highly endemic rural community to allow the identification of environmental, behavioral and biological factors that could influence infection and disease development.

A CT scan-based NC prevalence of 9.1% was found, three times higher than the average of NC reported in several autopsy studies [20–22]. This result confirms the magnitude of the NC problem in Mexico and stresses the need to promote control measures. All the factors that favor the life cycle of *T. solium* persist in this endemic community. However, being in contact with the parasite does not necessarily imply NC infection, as most of the exposure factors were not associated with it. This leads us to propose that the success of infection in those living in highly endemic areas does not only depend on exposure to the parasite but is related to other host or parasite factors, not yet elucidated.

All diagnosed NC cases had calcified lesions and were asymptomatic. Two of 18 NC cases were positive for a history of headache (11% of the cases). A similar percentage of controls also had a history of headache. Considering that this symptom is nonspecific and similarly affects cases and controls, it was assumed that headache is not related to the presence of calcified cysticerci. Only 2 subjects presented seizures but both had normal CT scans. These 2 subjects were under 20 years of age and did not have antecedents of cranial traumatism. These data contrast with the results of hospitalary studies that find a high prevalence of seizures in NC patients and strengthen the notion that in rural areas, the main etiological factor for pediatric seizures is still perinatal damage. In other series of NC patients studied by CT scan, the calcified form has also been the most frequent ones [23, 24]. In a CT scan-based epidemiological study performed in Honduras, 80.6% of the 31 NC patients diagnosed had calcified lesions [25]. These data confirm that the NC disease

is relatively benign in most cases, and suggests that the parasite may die without specific treatment. They also suggest that the severity of NC does not correlate with exposure, but that additional factors may be involved in the development of the severe clinical forms of the disease. For instance, immunity acquired as a consequence of living in a highly endemic area could protect against severe forms, or else, specific host factors may modulate the relationship with the parasite. These possibilities should be explored and may be of importance in considering human vaccination to prevent the disease.

Although all cases found in this study were calcified clinically silent lesions, inflammation due to the presence of the parasite in the CNS could promote pathogenic states. Calcified lesions in the CNS have recently been found to be associated with perilesional edema and morbidity [26]. This possibility has to be addressed in a follow-up study of the apparently silent cases.

Regarding parasite localization in the brain, most cases (66.7%) had calcifications localized in the right and left frontal lobes partially explaining the absence of symptoms. In 3 cases, the lesions localized in temporal lobes, but all were asymptomatic.

Seroprevalence in ELISA was 6.47%, almost five times higher than the overall seroprevalence found in the National Seroepidemiologic Survey made in Mexico in 1988 [9]. The low observed sensitivity (15.4%) is in agreement with the results obtained in different hospital-based studies [19, 27] in which low or negative levels of antibodies were found, especially in patients with single and calcified lesions. These data show that seropositivity does not imply NC and that surveys based on serology only indicate contact with the parasite and must be examined with prudence.

We found no gender-related differences in prevalence. In contrast, in symptomatic hospital cases, evolution of the infection significantly differs between males and females [18]. Concerning age, a slight association was found between NC prevalence and increased age ($p = 0.06$); however, the data must be considered with caution, as prevalence is cumulative in time. The highest seroprevalence and the lowest NC prevalence in children are of interest (fig. 2), as it could indicate higher resistance to infection in early life. The age data agree with the low prevalence of NC (0.16–0.54%) found in autopsies performed in Mexican children's hospitals [28, 29]. The significant association between age and multiplicity of lesions is perhaps related to higher probabilities of reinfection although an increased susceptibility due to ageing cannot be discarded.

We found possible familial aggregation of NC cases. However, an individual in one family was a tapeworm carrier, a fact that increases exposure to cysticercosis [30, 31]. This does not explain the other 15 familial NC cases living in other less exposed households. The absence of household clustering and the lack of association of NC in marital couples also point to the possibility that genetic factors may be involved in the risk and pathogenesis of the infection and to other mechanisms of transmission of wider spread rather than sharing households with tapeworm carriers (i.e. eating contaminated food).

In conclusion, we found a high prevalence (approximately 9%) of clinically silent NC among seemingly healthy subjects in an endemic rural area of Mexico. In contrast, patients with severe NC, who are usually reported in hospital studies, represent the 'tip of the iceberg' of this complex disease. Tests for higher mental and emotional performance may be required to recognize less evident clinical manifestations in subjects with silent NC.

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Is damage in central nervous system due to inflammation?

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Abstract

The aim of this work was to review the inflammatory factors involved in central nervous system (CNS) inflammation and the damage associated to their participation in an inflammatory disease of CNS, multiple sclerosis in humans and experimental allergic encephalomyelitis in the murine model. Inflammation has an important repairing function, nevertheless frequently in the CNS inflammation is the cause of damage and it does not fulfill this repairing function as it happens in other compartments of the body. The inflammatory response in the CNS involves the participation of different cellular types of the immune system (macrophages, mast cells, T and B lymphocytes, dendritic cells) and resident cells of the CNS (microglia, astrocytes, neurons), adhesion molecules, cytokines and chemokines among other proteic components. During neuroinflammation chemotaxis is an important event in the recruitment of cells to the CNS. The lymphocyte recruitment implies the presence of chemokines and chemokine receptors, the expression of adhesion molecules, the interaction between lymphocytes and the bloodbrain barrier (BBB) endothelium, and finally their passage through the BBB to arrive at the site of inflammation. If this process is not controlled, is prolonged, inflammation loses its repairing function and can be the cause of damage. Usually neuroinflammation has the tendency to decline to damage, which would explain most of the CNS pathologies.

Keywords: Neuroinflammation; Damage; Multiple sclerosis

1. Introduction

Inflammation is a complex response that involves cells, plasma components and cellular products, which has the aim of repairing the produced damage. This response may exist in any vascularized compartment of the body, including the central nervous system (CNS). The produced damage may have different causes like infection, traumatism, ischemia, necrosis, hemorrhage, among others. Usually, it is accompanied by the cardinal points described by Celsus: pain, tumor, rubor and heat. These cardinal points are the result of the vascular response as it is the increase of the sanguineous flow to the site of inflammation, an increase of the capillar permeability by retraction of

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the endothelium, allowing the exit of molecules and soluble mediators, and the lymphocyte migration to the site of the inflammation.

The inflammatory response includes the participation of different cellular types such as neutrophils, macrophages, mast cells, lymphocytes, platelets, dendritic cells, endothelial cells, fibroblasts, to mention the main ones. During the inflammatory process chemotaxis is an important event in the recruitment of cells to the site of inflammation. The first cells in arriving are neutrophils and the macrophages. The recruitment of leukocytes implies the presence of chemotactic factors as chemokines, the expression of their receptors in leukocytes, the expression of adhesion molecules in leukocytes and vascular endothelium, the narrow interaction between leukocytes and endothelium, and finally their passage through endothelium to arrive at the site of inflammation.

In addition to the chemokines, other molecules contribute to the process of recruitment of leukocytes and the inflammatory process: complement system, kinin system, fibrinolytic system, leukotriene and prostaglandin production, neuropeptides and cytokines (summarized in Fig. 1, network of inflammation).

Inflammation is part of a physiological process that has the aim of repairing the damage, howev-

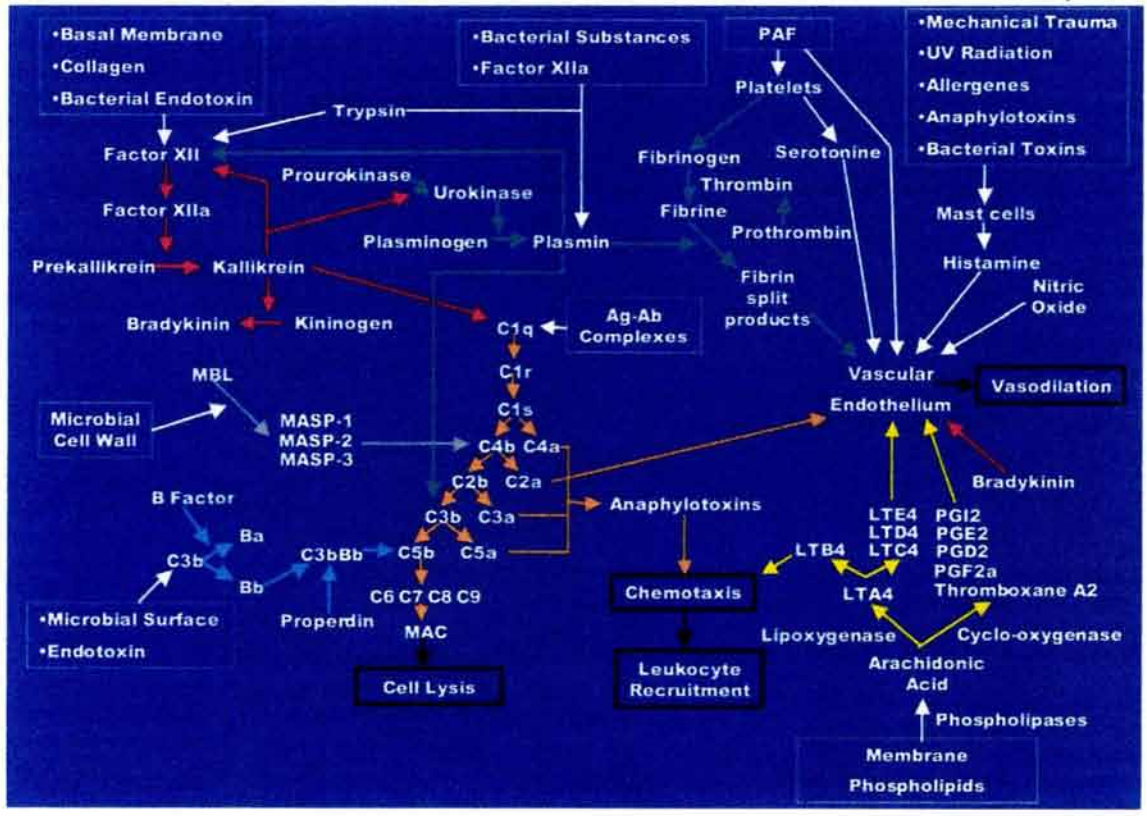

Fig. 1. Network including most of the components of the inflammatory response. Initiating factors of inflammation are represented in the white squares, effector functions in the black ones. The kinin system is represented in red, the fibrinolytic system in green, the classical pathway of complement in orange, the alternative pathway of complement in turquoise, the lectin pathway of complement in grey, the arachidonic acid products in yellow, in white others like histamine, serotonin, nitric oxide, etc. PAF, platelet-activating factor; Ag, antigen; Ab, antibody; LTA4, leukotriene A4; LTB4, leukotriene B4; LTC4, leukotriene C4; LTD4, leukotriene D4; LTE4, leukotriene E4; PGD2, prostaglandin D2; PGE2, prostaglandin E2; PGF2, prostaglandin F2; PGI2, prostaglandin I2; MBL, mannan-binding lectin; MASP, mannan-binding lectin-associated serum protease; MAC, membrane-attack complex.

er, when this process is not controlled, is extended, the inflammation loses its repairing function and can be the cause of damage [1]. Several diseases involve inflammation and it could be the cause of damage: multiple sclerosis (MS), Alzheimer disease, rheumatoid arthritis, systemic lupus erythematosus, Hashimoto's thyroiditis, to mention some.

Frequently in the CNS, inflammation is the cause of damage and it does not fulfill the repairing function as it happens in other compartments of the body. The objective of this revision is to present information that sustains the inflammation as a cause of damage in the CNS, focusing in an inflammatory disease of CNS, MS in humans and experimental allergic encephalomyelitis (EAE) in the murine model, approaching two main aspects of the inflammation: the participation of cellular components and their protein products.

2. Multiple sclerosis: an inflammatory disease of the CNS

MS is characterized by a rupture of the bloodbrain barrier (BBB), an important mononuclear cell infiltration of the white substance and its subsequent demielinization. A similar autoimmune disease can be induced in susceptible strains of rodents injecting different components of myelin like myelin basic protein, proteolipid protein and myelin oligodendrocyte glycoprotein.

MS can display several clinical forms, whose course can be very different. In one form of the disease, inflammation is episodic and is associated to discreet attacks of neurological disfunction followed by recovery, which can leave some residual neurological damage. Another form of the disease is progressive, in which the inflammation is less important but the neurological damage is secondary to the degenerative process initiated by the inflammation.

The inflammatory process associated to MS and EAE involves cells of the immune system and the CNS, molecules and inflammatory mediators produced by these, as chemokines, cytokines, adhesion molecules in activated endothelial cells and matrix metalloproteinase [2].

3. Cells of the immune system that participate in neuroinflammation

EAE as an experimental model has turned out to be very useful in the study of leukocytes migration to CNS and their participation in the pathology and inflammation of the disease.

Since Paterson's [3] work it is known that lymphocytes have a fundamental role in the development of EAE, which studies their migration to the CNS. This migration happens in two phases: in the first only lymphocytes do participate, they must be activated, independently of their specificity and compatibility of the MHC of the host, to be able to pass the intact BBB [4,5]. In the second phase that is accompanied by the rupture of the BBB there is an intense infiltration of more cellular types [4].

It is probable that after the first entrance specific activated T cells recognize their antigen and this triggers an inflammatory process with the subsequent production of cytokines that could activate and/or damage the endothelium of the BBB and allowing the passage of the other cells. Activated T cells with a TH1 cytokine profile have been isolated from CNS [4,6], they also secrete proinflammatory cytokines like TNF-α and IFN-γ [4,7]. These T cells also express chemokines (MIP-1α/CCL3, MIP-1β/CCL4) and increase the expression of other chemokines (RANTES/CCL5, IP-10/CXCL10, MIP-1α/CCL3 and MCP-1/CCL2) in astrocytes and perivascular macrophages [7]. The role that chemokines could have in MS or in EAE is variable, since several factors may influence: type of chemokine produced, cellular type producing it, the site of the CNS where it is produced, the receptor's expression and how it is associated to the severity of the disease or the clinical form.

TNF-α and IFN-γ secreted by T cells, and activated TH1, may have an effect on the endothelium of the BBB. TNF-α induces a greater recruitment of leukocytes to the perivascular space and to the cerebralspinal fluid (CSF) when it is present in the ventricles [8] and together with IFN-γ it has cytotoxic properties on the vascular endothelium mediated by nitric oxide [9]. Also it has been observed that TNF-α induces the pro-

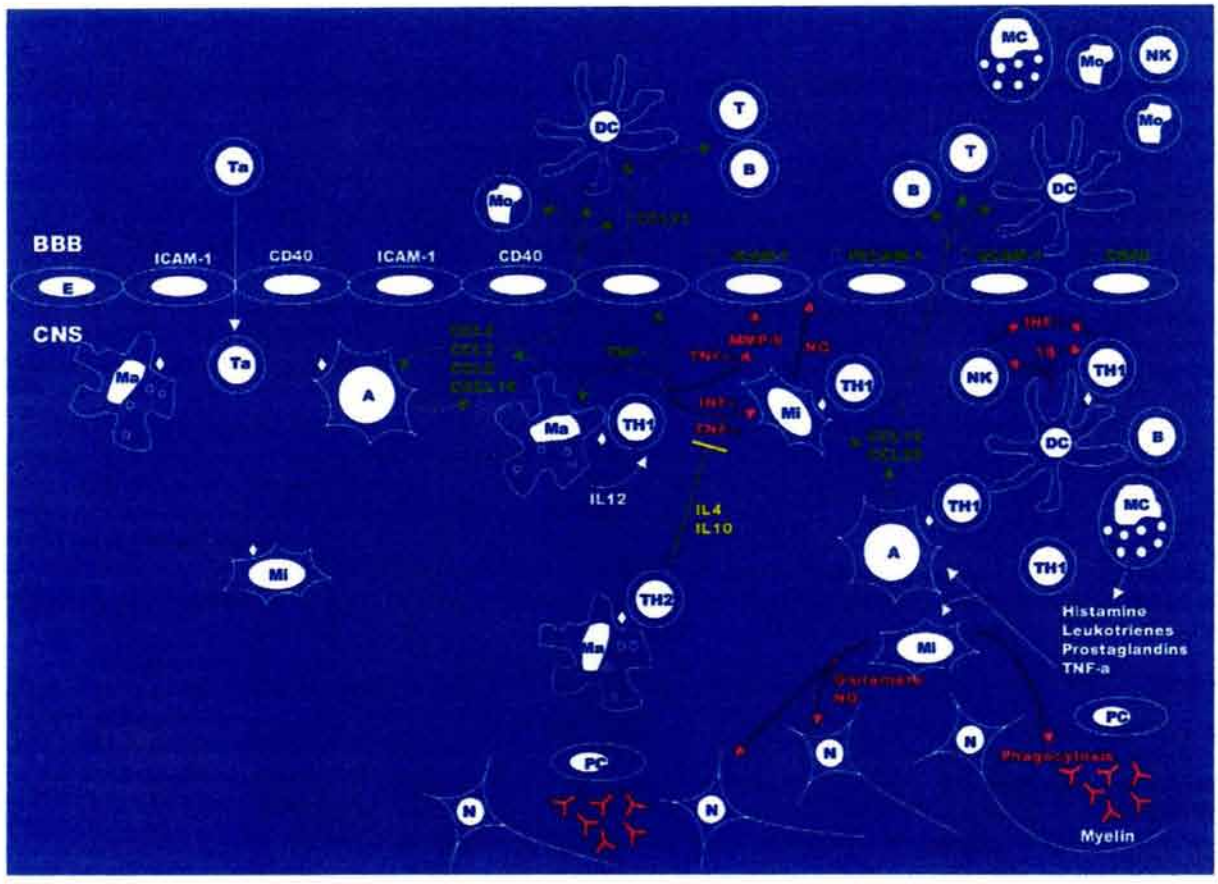

Fig. 2. Development of central nervous system inflammation and damage. After antigen recognition by T cells, several cytokines and chemokines are produced which have several effects on cell recruitment (represented in green), in damaging CNS cells and structures (represented in red) and in the exceptional occasion with anti-inflammatory properties (represented in yellow). BBB, bloodbrain barrier; CNS, central nervous system; E, endothelium; T, T cell; B, B cell; NK, natural killer cell; N, neuron; Ta, activated T cell; Ma, macrophage; A, astrocyte; Mi, microglia; Mo, monocyte; DC, dendritic cell; MC, mast cell; PC, plasma cell; NO, nitric oxide; MMP, metaloproteinase.

duction of MCP-1/CCL2, which induces the expression of the MMP-9 metalloproteinase as well; both, TNF-α and MCP-1/CCL2, increase the expression of adhesion molecules in the endothelium facilitating, therefore, the adhesion and the passage of activated T lymphocytes that express the CCR2 receptor and monocytes; also MMP-9 metalloproteinase facilitates the transmigration through the endothelium [10,11]. Probably these effects of TNF-α, MCP-1/CCL2 and IFN-γ contribute to the rupture of the BBB and the important infiltration of leukocytes that is observed in the EAE.

In the last years it has been demonstrated that other leukocytes also have the ability to enter the CNS and to contribute to the pathology of MS or EAE as are B lymphocytes, dendritic cells, NK cells, mast cells and other members of the macrophage/monocyte family [4].

The participation of B lymphocytes in the pathology of MS or EAE has been proposed after observing the presence of autoantibodies directed against myelin [12], the 90% increase of the intrathecal IgG production in MS patients and the presence of B lymphocytes in the active lesions where the demielinization process is present [13].

Although B lymphocytes are not necessary for the development of EAE, nevertheless they contribute to the severity of the disease and the pathology destroying the myelin more than participating in the inflammation [14]. Their presence in the CNS are due to several factors, an activated state allows them to pass the intact BBB [5], on the other side, the expression of the CCR7 receptor to SLC/CCL21 and ECL/CCL19 chemokines has been described in B lymphocytes, which could explain their migration to the CNS since these chemokines are produced by the BBB endothelium and microglia–astroglia, respectively [15].

The presence of mast cells also has been described in the CNS in MS and EAE; these are an important source of histamine, leukotrienes, prostaglandins and TNF-α, among other molecules, they could have an effect on vascular endothelium of the BBB and by this way they could be contributing to its rupture or permeability with the subsequent massive infiltration of leukocytes [16].

The role of NK cells in inflammation in CNS is controversial. These cells respond to the production of IL18 and IL12 by activated macrophages and dendritic cells increasing their production of IFN-γ and favouring a TH1 profile in the EAE [17,18]. On the other side, it has been observed that their presence is not frequent in the CNS in EAE, and the administration of antibodies anti-NK cells increases the clinical symptomatology of EAE, that is why some groups have proposed that they could function as suppressors or regulators of the inflammation [19].

Dendritic cells also participate in the pathology of the disease in EAE. Dendritic cells as professional antigen-presenting cells (APC) can activate autoreactive T cells with a TH1 profile in lymphatic nodules and in the CNS [20]. Also it has been described that their location within the CNS usually is in strategic places of antigen entrance like choroid plexus and meninges, and not in the parenchyma [20,21]. Their capacity of migrating outside the CNS possibly transporting antigens with them out of the CNS could explain the specific activation of autoreactive T cells in the periphery [20]. The interaction of dendritic cells is not limited to T lymphocytes, they also can favour the clonal expansion of B cells and the subsequent production of antimyelin antibodies and chemokines in MS and EAE [14]. Dendritic cells can arrive at the CNS by chemotaxis when expressing CCR6 and CCR7, both receptors to the chemokines ECL/CCL19, MIP-3α/CCL20 and SLC/CCL21, which can be produced by microglia and the astrocytes [20,22]. Another source of dendritic cells is the microglia, these after GM-CSF stimulation can turn into immature dendritic cells and after CD40 binding to mature dendritic cells able to activate T lymphocytes [23].

The cells of the immune system participate in an important way in the beginning and development of MS and EAE, contributing to the severity of the clinical picture and to the secondary damage of the inflammatory response.

4. The participation of the CNS cells in neuroinflammation

During the last decades it has been observed that not only cells of the immune system participate actively in the inflammation, but also cells belonging to the CNS are a fundamental part of this process. It has been shown that neurons, astrocytes and microglia are also able to produce a great repertoire of immunological and inflammatory molecules, including cytokines, chemokines and their respective receptors, complement molecules, coagulation factors, proteases, among others [2].

It is known that in CNS astrocytes, in a more important way, microglia and perivascular macrophages function as APC, since they express the necessary coreceptor molecules: MCH class II, B7-1 and LFA-3 molecules [24]. In inflammation it has been seen that astroglia and microglia when activated express increased levels of their coreceptor molecules of MHC class II, glial fibrillary acidic protein (GFAP) and microglia response factor-1 (MRF-1), respectively; and with the subsequent production of pro-inflammatory cytokines [24].

Astrocytes, microglia and perivascular macrophages are an important source of IL12, which favours a TH1 profile with IFN-γ production by T and NK cells [18]. The cerebral expression of IL12 is fundamental in the development and the

severity of the EAE, it facilitates the recruitment of antigen specific cells to the CNS [18]. Nevertheless, a recent report mentions that IL23, which shares the p40 subunit of IL12 but has a different p19 subunit, is fundamental in the EAE and not IL12, due to its effects on memory T cells and macrophages [25].

Perivascular macrophages continuously enter into CNS, they reside behind the basal membrane of the endothelium and may return to peripheral lymphoid organs. These cells usually have an important role in EAE, they contribute to the development of the disease when they present antigens to T lymphocytes, activate microglia, produce chemokines (MCP-1/CCL2, MIP-1α/CCL3), and express ICAM-1 and VCAM-1 [26].

The neurons are less important in inflammatory processes, although they have the ability to express class I molecules, to produce several cytokines like IFN-γ and even to induce apoptosis of T cells through the CD95–CD95L interaction [27,28].

The endothelium as an essential structure of the BBB has an important role in CNS inflammation. Under physiological conditions endothelial tight junctions control the flow of cells of the blood to the CNS, nevertheless activated lymphocytes may pass the intact BBB and contribute this way to the immune surveillance. Cerebral endothelial cells in basal state express ICAM-1, and after the TNF-α, IFN-γ and IL1 stimulation ICAM-1, VCAM-1, PECAM-1 and E-selectin are increased [29]. It has been demonstrated that the interactions VLA-4/VCAM-1 and LFA-1/ICAM-1 is critical in differential CD4+ T cells migration, and in cytokine production by the endothelium [4]. Another important interaction for the adhesion and migration is CD40/CD40L, due to the fact that human cerebral endothelium expresses constantly CD40, this expression is increased after TNF-α, LPS or CD40L stimulation [30]. Also microglia, astrocytes and neurons express CD40; B cells, perivascular macrophages and activated T cells express the CD40 ligand [30,31]. In MS patients it was observed that T cells in CNS express CD40L, whereas CD40L is not detected in controls [31]. It seems that the CD40/CD40L interaction is important for the cell passage through the BBB and in the microglia and astroglia activation.

The neuroinflammatory response involves peripheral cells of the immune system and resident cells of the CNS, all participating in a complex orchestra of cytokines, chemokines and receptors, being this response in most of the occasions pathological.

5. How does inflammation contribute to damage in CNS?

Different observations have been mentioned through the text that could be participating in damage associated to MS and EAE. Most of the data have been obtained from the experimental model due to the difficulty for obtaining samples of MS patients with, being the more easily obtained peripheral blood, CSF and cerebral tissue in necropsies.

MS and EAE are inflammatory diseases mediated by CD4+ TH1 cells, which favour and amplify an inflammatory profile. TNF-α and IFN-γ production in CNS damage the BBB integrity due to their cytotoxic effect on the endothelium and, therefore, favour an important leukocyte infiltration [8,9]. Also IL18 has been detected in CSF and serum of MS patients [32], this cytokine has been associated to a TH1 profile by its capacity because it induces IFN-γ production in NK cells and T lymphocytes, contributing to the persistence of elevated levels of IFN-γ and favouring in this way its functions.

Another important effect of IFN-γ that causes damage is macrophage activation with the subsequent expression of the nitric oxide synthetase, increasing the levels of nitric oxide, which has neurotoxic effects and favours permeability of the BBB [33]. Another important nitric oxide source is the microglia [34].

Microglia also contributes to damage producing glutamate, which is toxic for neurons and oligodendrocytes [35]. As an APC with fagocitic properties microglia participates in the removal of myelin in EAE [34]. Also it has an important regulatory function when it induces apoptosis in autoreactive T lymphocytes, nevertheless it has been observed that this process is defective in autoreactive T lymphocytes of MS patients since they express increased bcl2 levels, this could explain its diminished susceptibility to the apoptosis, which would favour the presence of autoreactive cells

Table 1
Molecules involved in neuroinflammation

Molecule	Cells	Functions in CNS	Reference
TNF-α	(a) TH1 cells	(a) Increases leukocyte traffic to perivascular space and CSF	[8,9] [29,30]
	(b) Macrophages	(b) Damages endothelium	
	(c) Endothelial cells	(c) Induces the expression of CCL2 and MMP-9	
	(d) Mast cells	(d) Increases the expression of ICAM-1, VCAM-1, PECAM-1, E-selectin and CD40 in BBB endothelium	
		(e) Induces the production of nitric oxide	
IFN-γ	(a) TH1 cells	(a) Damages endothelium	[9,29,30]
	(b) NK cells	(b) Increases the expression of ICAM-1, VCAM-1, PECAM-1, E-selectin and CD40 in BBB endothelium	
	(c) Neurons	(c) Induces the production of nitric oxide	
IL10	(a) TH2 cells	(a) Anti-inflammatory	[40]
	(b) B cells	(b) Regulator	
		(c) Participates in recovery phases in MS	
IL12	(a) Macrophages	(a) Favours a TH1 profile	[17,18]
	(b) Astrocytes	(b) Induces INF-γ production in T and NK cells	
	(c) Microglia	(c) Facilitates antigen specific cell recruitment	
		(d) Promotes the development of naïve T cells	
IL18	(a) Macrophages	(a) Induces a TH1 profile	[17,18]
		(b) Induces INF-γ production in T and NK cells	
IL23	(a) Dendritic cells	(a) Induces IL1β and TNF-α production	[18,25]
		(b) Mediates the late phases of inflammation	
		(c) Favours chronic inflammation	
MCP-1/CCL2	(a) Perivascular macrophages	(a) Chemotaxis of macrophages and monocytes	[7]
	(b) Astrocytes		
MIP-1α/CCL3	(a) Perivascular macrophages	(a) Increases the expression of RANTES/CCL5, IP10/CXCL10, MIP-1α/CCL3 in astrocytes and perivascular macrophages	[7]
ECL/CCL19	(a) Microglia	(a) Chemotaxis of T, B and dendritic cells	[15]
	(b) Astrocytes		
MIP-3α/CCL20	(a) Microglia	(a) Chemotaxis of dendritic cells	[15,20,22]
	(b) Astrocytes		
SLC/CCL21	(a) BBB endothelium	(a) Chemotaxis of T, B and dendritic cells	[15]
CD40	(a) BBB endothelium	(a) Favours leukocyte recruitment to CNS	[30]
CD40L	(a) T cells	(a) Favours activated T cells, dendritic cells and B cells transendothelial migration	[30]
	(b) B cells		
	(c) Microglia		
	(d) Astrocytes		
	(e) Neurons		

maintaining or prolonging a chronic inflammatory state [36].

As well, dendritic cells contribute to the damage when inducing an inflammatory response towards a TH1 profile, activating autoreactive T cells and maintaining a continuous local stimulation contributing this way to chronic inflammation [22].

Several chemokines have been detected to be increased in serum and/or CSF in different clinical profiles of MS [37,38], nevertheless, MIP-1α/CCL3 and MCP-1/CCL2 apparently are the most important ones. It has been observed that MIP-1α/CCL3 is present in the acute phases of the disease with a predominant TH1 response, whereas MCP-1/CCL2 is associated to remission phases and a TH2 response [7]. Mice lacking MCP-1/CCL2 or mice treated with antibodies directed against MIP-1α/CCL3 do not develop EAE, this would support the hypothesis that these chemokines contribute to the development and damage of the disease [1]. On the other hand, vaccination experiments for EAE prevention have shown the importance of a TH2 response, since its induction suppresses the development of the disease [39]. We could suggest that a TH1 response in MS or EAE produces damage, since a TH2 response, with the important effect of IL4 even on TH1 cells, changes the evolution of the disease completely [39].

Another important observation is that IL10, a powerful TH2 anti-inflammatory regulating cytokine, can be produced after the microglia–T cell interaction, nevertheless IL10 is detectable rather in the recovery phases of the disease [40]. IL10 deficient mice develop a chronic inflammatory disease that affects predominantly colon and are more susceptible to the most severe forms of arthritis induced by collagen. This supports the important anti-inflammatory effect of some cytokines avoiding the subsequent damage produced by inflammation.

6. Summary

Neuroinflammation is a complex process that involves cells of the immune system and of the CNS, nevertheless, unlike other compartments of the body the inflammatory response favours more the damage than the repairing mechanism.

In Fig. 2 and Table 1 some important points commented throughout the text are summarized, standing out the interactions between endothelial cells–T lymphocytes and APC–T cells, the effects of some cytokines and chemokines favouring the recruitment of more cells and thus contributing to the pathology of MS and EAE.

Inflammation in CNS usually does not exert its repairing function, rather, its tendency is to decline to damage, which would explain most of the CNS pathologies. Interesting would be to explore in which ways this pathological response in CNS can be declined to a beneficial, repairing or anti-inflammatory response, which could help in medical therapy benefit the patient in its clinical course having less sequels that diminish the quality of its life.

Take-home messages

- Neuroinflammation is a complex process involving cells of the immune system and of the CNS.
- The interactions between endothelial cells–T lymphocytes and APC–T cells, the subsequent production of some cytokines and chemokines are crucial in favouring the recruitment of more cells and thus contributing to the pathology of MS and EAE.
- MS and EAE are inflammatory diseases mediated by CD4+ TH1 cells, which favour and amplify an inflammatory profile. TNF-α and IFN-γ production in CNS damage the BBB integrity due to their cytotoxic effect on the endothelium and, therefore, favour an important leukocyte infiltration.
- The inflammatory response in CNS favours more the damage than the repairing function which would explain most of the CNS pathologies.

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The World of Autoimmunity; Literature Synopsis

Anti-Helicobacter pylori antibodies in Henoch-Schonlein purpura

Henoch-Schonlein purpura (HSP) is a systemic vasculitis characterized by deposition of mainly IgA-containing immune complexes in the skin, gastrointestinal mucosa, joints, glomeruli and small vessels. Novak et al. (Autoimmunity 2003;36:307) tested the levels of antibodies towards *Helicobacter pylori* (HP) in 11 patients having HSP. Ten of 11 patients with HSP had anti-HP antibodies compared with 11 of 20 healthy controls. Four of these 11 patients had concurrent HP infection, whereas nine of 20 controls also had actual HP infection. HSP patients in the acute phase had significantly higher levels of IgG anti-HP antibodies, serum CRP, circulating IgA and tumor necrosis factor-alpha compared to healthy controls. Of note is that the ratio of IgA/IgG anti-HP antibodies during remission was significantly higher in patients versus controls. These results indicate that HP might be somehow associated with HSP, even though the nature of this association is not clear enough. It is possible that HP infection could contribute to the development and progression of HSP.

Autoimmunity and mutation in rasgrp1

Layer et al. (Immunity 2003;19:243) report on a mouse strain with a recessive genetic lesion, which spontaneously developed a lymphoproliferative autoimmune syndrome exhibiting features of systemic lupus erythematosus. This mouse strain had a lesion in Rasgrp1 that prevented the translation of the RasGRP1 protein. The following alterations in T cells accompanied this spontaneous mutation: T cells failed to activate Ras or proliferate vigorously following antigen encounter and showed defects in positive selection, peripheral RasGRP1(lag) T cells spontaneously adopted a memory phenotype and were able to transfer disease to lymphopenic recipient mice, and CD4(+) T cells accumulated in the lymphoid tissues of older RasGRP1(lag) mice and were resistant to activation-induced cell death. Moreover, whereas RasGRP1(lag) B cells were functionally normal, activated B cells were detected in older mice, as were autoantibodies directed against self-antigens.

Valve replacement surgery in antiphospholipid syndrome

Heart valve disease is one of the most common manifestations of the antiphospholipid syndrome (APS). Berkun et al. (J Thorac Cardiovasc Surg 2004;127:414) retrospectively analyzed the clinical data regarding 10 patients having APS who underwent valve replacement (mitral valve replacement in 7, aortic valve replacement in 2, and replacement of both valves in the remaining patient). The immediate mortality was 20%, and during a follow-up period of up to 8 years 2 patients required repeat operation for valve-related complications with subsequent another death of a patient. An additional patient died of cardiac causes 13 months post-surgery. The authors concluded that valve replacement in APS patients carries significant early and late mortality and morbidity, especially in advanced valvular heart disease. Heart valve disease is very frequent in APS, but includes various aspects such as heart valve vegetations and thickening which might be milder in their course.

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HOST GENDER IN PARASITIC INFECTIONS OF MAMMALS: AN EVALUATION OF THE FEMALE HOST SUPREMACY PARADIGM

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ABSTRACT: A review of current literature on mammalian hosts' sexual dimorphism (SD) in parasitic infections revealed that (1) it is a scarcely and superficially studied biological phenomenon of considerable significance for individual health, behavior, and lifestyles and for the evolution of species; (2) there are many notable exceptions to the rule of a favorable female bias in susceptibility to infection; (3) a complex network of molecular and cellular reactions connecting the host's immuno-neuro-endocrine systems with those of the parasite is responsible for the host–parasite relationship rather than just an adaptive immune response and sex hormones; (4) a lack of gender-specific immune profiles in response to different infections; (5) the direct effects of the host hormones on parasite physiology may significantly contribute to SD in parasitism; and (6) the need to enrich the reductionist approach to complex biological issues, like SD, with more penetrating approaches to the study of cause–effect relationships, i.e., network theory. The review concludes by advising against generalization regarding SD and parasitism and by pointing to some of the most promising lines of research.

THE FEMALE HOST SUPREMACY PARADIGM IN PARASITIC INFECTIONS

It is widely held that female mammals are more resistant to parasitic infections than males because of gender-associated differences in exposure and of testosterone's immunosuppressive properties. The paradigm implies that sexual dimorphism (SD) to parasitism is mediated only, or principally, by the host's immune system and usually disregards the parasites' direct response to the distinct sex-steroid profiles of their female and male hosts.

The female supremacy paradigm in parasitic infections has existed for a long time (Addis, 1946), and despite thoughtful recommendations against simplification (Zuk and McKean, 1996), it is rarely questioned. In several prestigious journals, however, it has recently been a matter of debate, particularly in association with mortality trends in humans (Moore and Wilson, 2002; Owens, 2002) and several infectious diseases (Zuk and McKean, 1996; Doprado et al., 1998; Watanabe et al., 1999; Klein, 2000; Ganley and Rajan, 2001; Hughes and Randolph, 2001; Roberts et al., 2001; Verthelyi, 2001). It has also been associated with a number of broader subjects, i.e., evolution of sexual reproduction (Zuk, 1994), decision making of the host, social hierarchy (Barnard et al., 1998; Gourbal et al., 2002), mating behavior (Kavaliers and Colwell, 1993; Morales et al., 1996; Willis and Poulin, 2000), and energy costs of infection and the immune response (Hansen et al., 2003). We decided to reexamine the paradigm in the light of the current understanding of the immune and endocrine systems of potential hosts because we found that it conflicted with our observations regarding experimental murine cysticercosis caused by *Taenia crassiceps*.

EXPERIMENTAL MURINE *TAENIA CRASSICEPS* CYSTICERCOSIS CONFLICTS WITH THE PARADIGM

Taenia crassiceps is an intestinal cestode of canines (definitive host) and of various extraintestinal tissues of rodents (intermediate host) in its larval (cysticercus) stage (Freeman, 1962). Experimental cysticercosis caused by *T. crassiceps* in mice simply requires the intraperitoneal injection of live cysticerci (Culbreth et al., 1972). Intraperitoneal cysticerci reproduce asexually by exogenous budding, developing massive parasite loads in a few months (Smith, Esch et al., 1972; Smith, Parrish et al., 1972) that may even approximate the host's body weight, without causing it apparent discomfort (Larralde et al., 1995). The cysticerci also survive and reproduce in vitro under usual culture conditions in media free of fetal calf serum. These features of experimental murine cysticercosis have made it a convenient model in studying the immunological, genetic, and sexual factors involved in susceptibility to infection and parasite proliferation (Sciutto et al., 2002). Sexual differences to infection in mice are still a matter of research. Thus, experimental findings have shown that in different congenic and syngenic strains of mice, females become infected more often than males and carry more cysticerci than males, with significant between-strain variations (Sciutto et al., 1991; Huerta et al., 1992; Larralde et al., 1995; Terrazas et al., 1998; Morales-Montor, Baig et al., 2001; Morales-Montor, Baig, Hallal-Calleros et al., 2002; Morales-Montor, Baig, Kabbani et al., 2002; Morales-Montor, Hallal-Calleros et al., 2002). Estrogens favor parasite reproduction, whereas androgens appear to inhibit it (Bojalil et al., 1993; Terrazas et al., 1994; Morales-Montor, Baig, Hallal-Calleros et al., 2002). Gonadectomy and thymectomy equalize parasite loads between sexes by greatly increasing those in males and slightly decreasing those in females (Huerta et al., 1992; Terrazas et al., 1994; Morales-Montor, Baig, Hallel-Calleros et al., 2002). Male mice are better protected by vaccination than females (Cruz-Revilla et al., 2000). Externally administered 17β-estradiol and dihydrotestosterone (DHT) are able to restore parasite loads to their normal levels in castrated animals. T cells, but not antibodies, also restore the effects of thymectomy (Bojalil et al., 1993). The TH1 response hinders parasite growth early in infection (Terrazas et al., 1999; Toenjes et al., 1999; Spolski et al., 2000; Rodriguez-Sosa et al., 2002), whereas the TH2 response prevails at later times of infection but is incapable of slowing parasite growth (Terrazas et al., 1998; Toenjes et al., 1999). In chronic infections, the male mouse is feminized (estrogenized and deandrogenized) to a degree that

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inhibits male sexual behavior (Morales et al., 1996). Feminization is apparently caused by overexpression of P-450 aromatase (Morales-Montor, Hallal-Calleros et al., 2002), triggered by the high levels of interleukin-6 (IL-6) in late infections (Morales-Montor, Baig et al., 2001; Morales-Montor, Mohamed et al., 2001). Cytokine profiles of infected male and female mice do not show major differences, except for the levels of IL-4, which are higher in males during early infection only (Terrazas et al., 1998).

These findings led to the initial proposal of a sex steroid, immunoendocrine interaction that controls the reproduction of cysticerci, one in which androgens were postulated to favor a TH1 response that limits parasite growth and in which estrogen favors a TH2 response that permits parasite reproduction (Huerta et al., 1992; Bojalil et al., 1993; Terrazas et al., 1994; Morales-Montor, Baig et al., 2001; Morales-Montor, Baig, Hallal-Calleros et al., 2002; Morales-Montor, Baig, Kabbani et al., 2002). Other studies also support the purported correlation between androgens and TH1 response and between estrogen and TH2 response (Morales-Montor, Baig et al., 2001; Morales-Montor, Baig, Hallal-Calleros et al., 2002; Morales-Montor, Baig, Kabbani et al., 2002; Morales-Montor, Hallal-Calleros et al., 2002).

Signs of SD in cysticercosis were recently reported for other host and taeniid species. For example, sex steroids have been implicated in porcine cysticercosis caused by *Taenia solium* because both castration and pregnancy nearly double the prevalence of naturally acquired cysticercosis in rural pigs (Morales et al., 2002). In humans, women are more frequently afflicted than men by severe neurocysticercosis (Del Brutto et al., 1988) and show higher inflammatory profiles (Fleury et al., 2003). Because sex steroids affect experimental *T. crassiceps* infections in laboratory mice and extend to natural infections of *T. solium* in humans and pigs, as well as *Trichinella spiralis* in rats (Klein et al., 1999), our initial suspicions regarding the general validity of the female supremacy paradigm were strengthened.

Other ongoing research has shown that in *T. crassiceps* murine cysticercosis several physiological systems of the host respond to the host's sex hormones, and so does the parasite either by limiting or by prompting its reproduction. For example, the central nervous system of infected and feminized male mice responds to intraperitoneal infections by overexpresssion of the *c-fos* gene in the hypothalamus, hippocampus, and preoptic area (Morales-Montor, Arrieta et al., 2003). It would appear that hormonal changes induced in the host act to promote the overexpression of the *c-fos* gene involved in cellular differentiation and proliferation of both parasite and host cells (Escobedo et al., 2004), as they do in other stress and immune challenges (Pacheco-Lopez et al., 2002). Thus, in murine cysticercosis, parasite proliferation is responsive not only to the host immune system and testosterone but also to a complex network that integrates the nervous, immune, and endocrine systems of the host and the parasite's physiological systems. The conflict between male biases in *T. crassiceps* cysticercosis with the host female supremacy paradigm expectations is undeniable and requires explanation.

TESTING THE FEMALE HOST SUPREMACY PARADIGM IN A WIDER REPERTOIRE OF PARASITIC INFECTIONS

The paradigm of female host supremacy in parasitic infections of mammals, as well as the robustness of the corresponding endocrinological and immunological factors postulated as its mechanisms, was evaluated using current literature (Medline, n = 110: 1995–2002), as well as several frequently cited classic articles and a few recent ones published in 2003. After examining this literature, however, the general validity of the female supremacy paradigm was seriously weakened by too many exceptions. What emerged was a complex host immuno-neuro-endocrine network that was related to the parasite physiologically and that seems more likely to control the complexities involved in certain host–parasite interactions than testosterone alone.

When infections that documented sex bias in infection parameters (Infection-SD) or immune profiles (Immune-SD) were found, the possibility of a cause–effect relationship was examined further. Each infection was classified as either sexually dimorphic, noting the sex favored by the bias (females > males or females < males), or undefined (females = males). Infection-SD was evaluated in terms of prevalence, intensity, severity, morbidity, mortality, hormonal profiles, or behavioral changes in infected animals. The biological meaning of these parameters differs substantially. Thus, some relate to the probability of infection, i.e., prevalence, and others to the outcome of infection, i.e., mortality, but they were assumed to be equivalent indications of sex bias. Immune-SD parameters include antibody production, lymphoid cell responses to mitogens or antigens, cytokine production, hypersensitivity reactions, and protective effects of vaccination. Immune-SD parameters also have important functional differences in their nature and context of expression, i.e., populations, individuals, cells, and molecules, in their role as effectors or mediators of immune responses, in their operation under in vitro or in vivo conditions, and in their ability to protect from infection. These immune parameters were scored as "greater than" or "smaller than" with respect to the opposite gender. The Immune-SD and Infection-SD data collected were used to examine how the 2 are connected.

FIRST GENERAL SIGNS OF PARADIGM WEAKNESS

The total number of references examined is relatively small, i.e., only 110. The search identified just 46 different parasite species occurring in 10 species of mammalian hosts, a minute sample considering the many thousands of parasite (Hoberg, 1997) and mammalian species (Anderson et al., 1984). Forty-three references reported SD (Table I), and the rest (67) were concerned with molecular interactions between the endocrine and immune systems or with hormonal, behavioral, and immune effects on the host (Fig. 1).

The human medicine bias in SD research is obvious because 56% of all the articles were oriented to the study of infections affecting humans or experimental animal infections (usually in rodents) having a human counterpart, i.e., malaria, schistosomiasis, trypanosomiasis, toxoplasmosis, and cysticercosis. Most references in the list do not directly explore SD but rather describe the in vitro effects of sexual hormones or cytokines on the immediate response of some immunological or endocrinological component derived from hosts of either sex in rather

TABLE 1. Infection-SD or Immune-SD in different host–parasite relationships documented to date (some parasites infect more than 1 host species).

Parasite	Host	Dimorphism	Prevalence	Intensity	Severity	Mortality	Mechanisms	Other observations	Reference
Brachylaima cribbi	Mice	Yes	♀ < ♂	♀ < ♂				Expulsion of worms in C57 BL/6J mice is mediated by an immune response	Butcher et al. (2002)
Brugia malayi	Human	Yes	♀ < ♂	♀ < ♂					Ganley and Rajan (2001)
Brugia pahangi	Rat	Yes	♀ < ♂	♀ < ♂					Bell et al. (1999)
Dipetalonema vitae	Hamster	Yes	♀ < ♂	♀ < ♂			17β-estradiol and progesterone are associated with protection in females		Reynouard et al. (1984)
Eimeria vermiformis	Mice							Females distinguish between infected and noninfected males	Kavaliers and Colwell (1993), Kavaliers (1995)
Heligmosomoides polygrus	Mice						Peripheral immune response is reduced in infected males and it is associated to higher levels of corticosterone	High-ranking infected males are less aggressive	Barnard (1998)
Heterakis spumosa	Mice	Yes	♀ < ♂	♀ < ♂			Testosterone favors the development of the parasite and its survival		Harder et al. (1992)
Hymenolepis diminuta	Rat	Yes in response to treatment					Infected males have decreased levels of testosterone in plasma	A deficient diet in vitamin G complex inhibits growth of the parasite in females only. Females distinguish between infected and noninfected males	Addis (1946), Wills and Poulin (2000)
Ixodes ricinus	Voles						Testosterone reduces innate and acquired resistance to tick feeding	Tick feeding favors transmission of other parasites	Hughes and Randolph (2001)
Leishmania donovani	Mice	Yes	♀ < ♂	♀ < ♂				Macrophages treated in vitro with testosterone have an increased number of promastigotes	Zhang et al. (2001)
Leishmania major	Mice	Yes	♀ < ♂	♀ < ♂				Testosterone treatment in females increases parasite number and orchidectomy in males decreases it	Mock and Nacy (1988)

TABLE I. Continued.

Parasite	Host	Dimorphism	Prevalence	Intensity	Severity	Mortality	Mechanisms	Other observations	Reference
Leishmania mexicana	Mice	Yes	♀ < ♂	♀ < ♂	♀ < ♂		Infected females produce more IFN-γ and infected males more TNF-α	Lesion growth as a result of treatment with IFN-γ neutralizing antibody in females equaled that in males	Satoskar and Alexander (1995)
Leishmania spp. (*Leishmania viannia guyanensis* and *Leishmania viannia panamensis*)	Hamster	Yes	♀ < ♂	♀ < ♂	♀ < ♂		The increased severity in males was associated to a greater intralesional expression of IL-4, IL-10, and TGF-β, which are disease promoters	Testosterone treated females have larger lesions than untreated females	Travi, Osorio et al. (2002)
Nippostrongylus brasiliensis	Rat	Yes	♀ < ♂	♀ < ♂			Testosterone affects goblet cell function and proliferation, delaying parasite expulsion		Tiuria et al. (1995)
Plasmodium chabaudi	Mice	Yes	♀ < ♂	♀ < ♂	♀ < ♂	♀ < ♂	Testosterone decreases the levels of total IgG, IgG1, and IgG2b, increases CD8+, and decreases Ig+ cells in infected females	Oral treatment with testosterone increases mortality in females	Benten et al. (1997), Mossmann et al. (1984)
Plasmodium chabaudi	IFN-γ R KO mice	Yes	♀ < ♂	♀ < ♂		♀ < ♂	Male sex hormones modulate the TH1–TH2 cell function		Zhang et al. (2000)
Plasmodium chabaudi	IL-4 KO mice	Yes	♀ < ♂	♀ < ♂		♀ < ♂	Male sex hormones modulate the TH1–TH2 cell function		Zhang et al. (2000)
Plasmodium chabaudi	IL-10 KO mice	Yes	♀ > ♂			♀ > ♂	The pathology may be due to direct stimulation of TNF-α by the parasite		Li (1999)
Schistosoma haematobia	Human	Yes		♀ = ♂			Females have higher levels of specific IgA, TGF-β, and IL-10 with a low specific proliferation compared with males		Remoue et al. (2001)
Schistosoma mansoni	Human	Yes	♀ = ♂	♀ < ♂	♀ < ♂				Mohamed-Ali et al. (1999)
Schistosoma mansoni	Mice	Yes	♀ = ♂	♀ > ♂	♀ > ♂	♀ > ♂		Testosterone treatment in females or castrated males reduces mortality. DHEA treatment reduces parasite number in females	Nakazawa et al. (1997), Fallon et al. (1998)

TABLE 1. Continued.

Parasite	Host	Dimorphism	Prevalence	Intensity	Severity	Mortality	Mechanisms	Other observations	Reference
Schistosoma mansoni	Hamster	Yes	♀ < ♂	♀ < ♂					Barrabes et al. (1980)
Strongyloides ratti	Rat	Yes	♀ < ♂	♀ < ♂				Testosterone treatment or macrophage blocking increases worm recovery in females	Watanabe et al. (1999)
Strongyloides venezuelensis	Rat	Yes	♀ < ♂	♀ < ♂			Testosterone restitution increases susceptibility and estradiol restitution decreases it	Gonadectomy in male rats decreases parasite loads, whereas it increases the loads in females	Rivero et al. (2002a, 2002b)
Taenia crassiceps	Mice	Yes	♀ > ♂	♀ > ♂			During infection there is a TH1–TH2 shift; in the acute infection IL-12 promotes CD4+-specific proliferation; in the chronic infection, IL-6 predominates and stimulates the aromatase activity, which increases serum estradiol in males	Chronically infected males lose their sexual behavior. Infection changes the response to the predator, facilitating the parasite cycle. Vaccination is more effective in males than in females	Sciutto et al. (1990, 1991), Larralde et al. (1995), Morales et al. (1996), Terrazas et al. (1998, 2002), Gourbal et al. (2001), Morales-Montor, Baig et al. (2001)
Taenia solium	Pigs		♀Nonpregnant > ♀pregnant; ♂noncastrated > ♂castrated				Low levels of androgens or high levels of estrogens probably influence susceptibility to infection		Morales et al. (2002)
Taenia solium	Human	Yes			♀ > ♂			Women develop a greater degree of inflammation when cysticerci are found in brain parenchyma and have more CSF* inflammation and increased cellularity in the CSF than men	Del Brutto et al. (1988), Fleury et al. (2003)
Toxoplasma gondii	Mice	Yes	♀ > ♂	♀ > ♂	♀ > ♂	♀ > ♂	Male SCID* more rapidly produce IL-12 and higher levels of IFN-γ. Males produce higher levels of TNF-α and IFN-γ at the onset of the infection, controlling parasite multiplication	Testosterone treatment reduces parasite numbers and mortality in females. Infection produces infertility in females	Stahl (1994), Roberts et al. (1995), Walker et al. (1997), Liesenfeld et al. (2001)

TABLE I. Continued.

Parasite	Host	Dimorphism	Prevalence	Intensity	Severity	Mortality	Mechanisms	Other observations	Reference
Trichinella spiralis	Voles	Yes	♀ < ♂	♀ < ♂				Polygamous males have higher testosterone levels than monogamous males concomitant to infection	Klein et al. (1999)
Trypanosoma cruzi	Mice	Yes	♀ < ♂	♀ < ♂			Intact males have higher levels of lytic antibodies	Dominant males have higher levels of testosterone and are less parasitized. Ovariectomy increases infection; estrogen replacement reduces the parasitemia. Orchiectomized males have fewer parasites than controls; testosterone replacement increases parasitemia	Deprado et al. (1998, 1999), Schuster and Schaub (2001)

* CSF, cerebrospinal fluid; SCID, severe combined immunodeficiency disease.

unrealistic in vitro conditions, i.e., cell culture media containing (contaminated with) fetal calf serum rich in growth factors and antibiotics. The biochemical results were then mistakenly assumed to operate in a similar manner in the more complicated context of an in vivo infection.

The analysis developed by the literature search casts doubt on the validity of the general female supremacy paradigm. The most notable shortcomings relate to (1) the poor representation of host–parasite systems among cited references, (2) the heavy human medical bias of the more thoroughly explored infections, (3) the unequal meanings of infection and immune parameters measuring SD in host susceptibility to infection, (4) the questionable protective function for many of the immune parameters, and (5) the excessive use of the reductionist approach in explaining events occurring at higher levels of complexity by way of the direct extrapolation of events occurring in vitro.

THE PREVALENCE AND MECHANISMS OF SD IN SPECIFIC PARASITE INFECTIONS

Table I summarizes the 43 references describing 32 infections in 8 host–parasite systems (some parasite species infect more than 1 host species, and 1 host species is infected by more than 1 parasite species) that provided information on the subject of Infection-SD or Immune-SD. In this data set, in 22 of 32 instances (68%) of the 8 systems, females fared better than males in prevalence, intensity, or consequence of infection (severity), varying from insignificant to pronounced. In 5 of 32 instances (16%), males scored better than females, and in 5 of 32 cases (16%), results could not be defined one way or another. In effect, 32% were exceptions to the paradigm. Furthermore, it is of interest to note (Table I) that severity of infection and mortality indicators were not studied as extensively as prevalence and intensity of infection. Severity of infection was reported in only 28% of the cases cited, and in 60% of these situations, female hosts fared better than males. Only in human schistosomiasis, by *Schistosoma haematobium*, was severity the same for both sexes. Mortality, in contrast, was only reported in 19% of the infections, half of which favored female hosts and the other half males. It is clear, therefore, that SD in severity and mortality have been insufficiently explored to make general and categoric statements.

Sex-associated immunological differences (Immune-SD) were reported in only 10 of 32 infections, and multifeatured immune profiles are described in only 5. Table II focuses on the 5 host–parasite systems in which several infection and immune parameters were evaluated at the time of infection. No uniform pattern or sex bias is discernable. In malaria, toxoplasmosis, and cysticercosis, infections are more prevalent and intense in female than in male mice. However, in leishmaniasis and schistosomiasis, it is the male host that is more frequently and intensely parasitized. The immune parameters studied also vary in each infection, without clear association to infection parameters. Comparison of immune profiles in all 5 infections is possible only for interferon-γ (IFN-γ), which was found to be more elevated in males than in females in all but 1 infection (murine leishmaniasis). Levels of tumor necrosis factor-α (TNF-α) were greater in females than in males with malaria; however, in leishmaniasis, schistosomiasis, and toxoplasmosis, males exhibited higher levels of TNF-α than females. In leishmaniasis

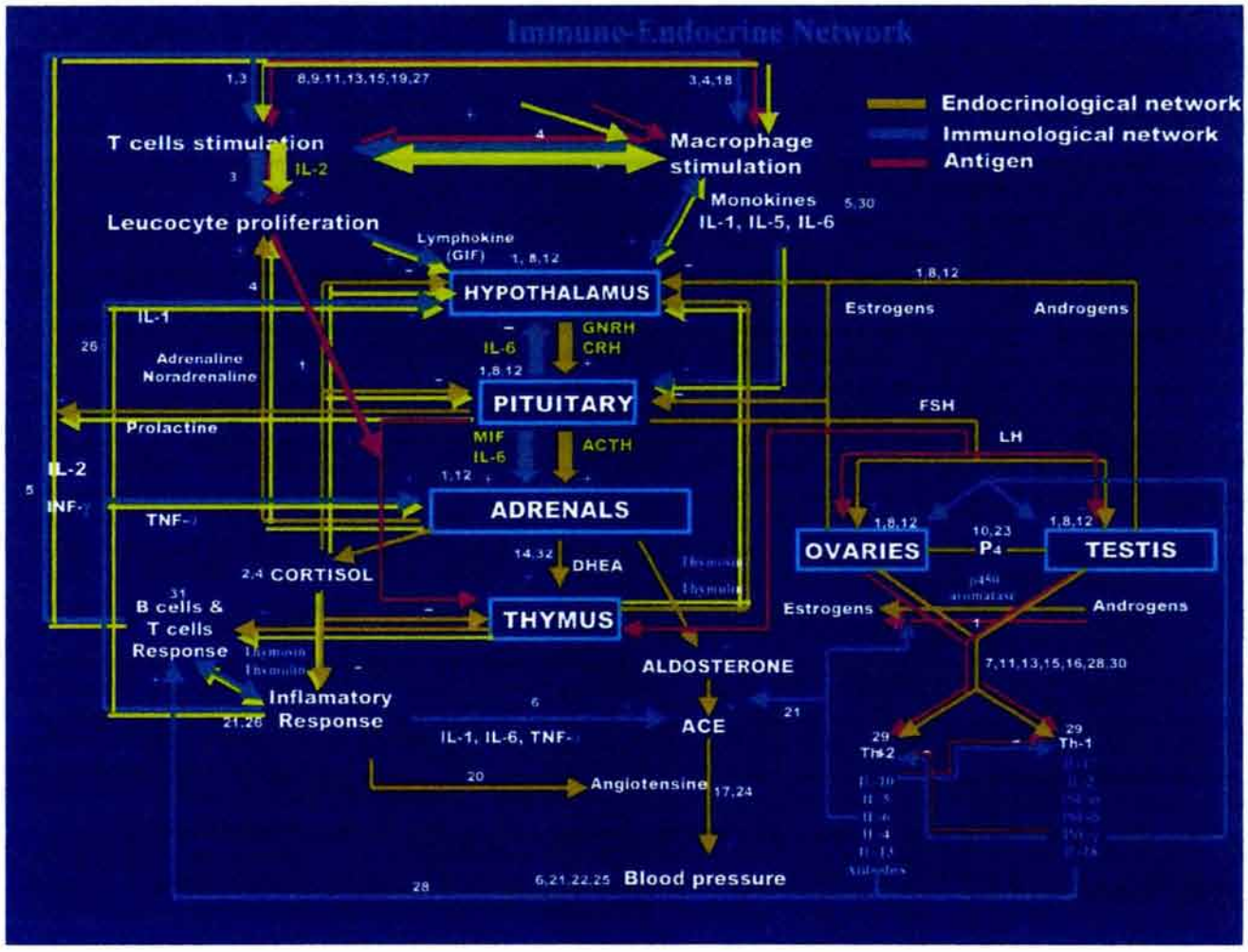

FIGURE 1. The HINEP network circuitry. The magnitude and complexity of the network includes practically all the host's sexual hormones and many of the immunological components described connecting among themselves and with the parasites systems. Arrows (←, ↑, →, ↓) denote connections between nodes; each points to the direction of the signal. ± Signs refer to stimulatory or inhibiting effects. The numbers near each connection code denote the references cited in the figure: (1) Spinedi et al. (2002), (2) Esch (2002), (3) Medzhitov and Janeway (2002), (4) Murtaugh and Foss (2002), (5) Henri et al. (2002), (6) Pramparo (2002), (7) Hughes and Randolph (2001), (8) Verthelyi (2001), (9) Roberts et al. (2001), (10) Thaker et al. (2001), (11) Liesenfeld et al. (2001), (12) Morales-Montor, Baig et al. (2001), (13) Taylor-Robinson (2001), (14) Kurtis et al. (2001), (15) Remoue et al. (2001), (16) Ganley et al. (2001), (17) Salzet and Verger-Bocquet (2001), (18) Zhang et al. (2000), (19) Soliman et al. (2001), (20) Feterowski et al. (2001), (21) Peeters et al. (2001), (22) Chae et al. (2001), (23) Barnea (2001), (24) Franco et al. (2001), (25) Gavras (2001), (26) Weinstock and Elliott (2000), (27) Grossman (1989), (28) Zhang et al. (2000), (29) Balemba et al. (1998), (30) Benedetto et al. (2000), (31) Hunter and Reiner (2000), and (32) Freilich et al. (2000).

and schistosomiasis of mice and humans, respectively, Immune-SD is observed in IFN-γ and TNF-α. In contrast, infection by *Leishmania mexicana* shows no dimorphism in IL-4, IL-10, and IL-12, whereas in infections with other species of *Leishmania*, there is a clear increase in IL-4 and IL-10 in males measured at the site of the lesions. In schistosomiasis, IL-10 production is clearly dimorphic (higher levels favoring females). Murine leishmaniasis exhibited the least dimorphic TH2 cytokine profile of the 5 infections, which contrasts with its very significant favorable female bias toward infection. In male-biased murine cysticercosis, INF-γ was higher in males; IL-2, IL-6, and proliferative responses were equal in both sexes, and IL-10 was higher in females. In addition, in murine cysticercosis, there was a reversal in the sexual bias toward IL-4 with time of infection. Males have higher amounts of IL-4 than females in early infection, but this is reversed in chronic infection. No change in profiles with time of infection was reported for the other 4 parasitic infections. These observations support the suspicion that the relationship between SD to infection, and the immune system's mediating effects are not simple and clearly involve many of the immune effectors. The host's immune response does not seem to be gender specific because no clear sex-related strategy can be detected. One would expect that hosts would have evolved immunological responses that are complementary to parasite strategies at different times of infection, number of parasites, location in the host's tissues, and offensive and defensive mechanisms. For example, extracellular stages of the parasite would be vulnerable to antibodies and

TABLE II. Host–parasite relationships in which several infection and some immune parameters were measured at some point during infection. The immune parameters collected varied in each infection.

Parasite	Host	Dimorphism	Prevalence	Intensity	INF-γ	TNF	IL-2	IL-4	IL-6	IL-10	IL-12	IgA	Specific proliferation	Reference
Leishmania mexicana	Mice	Yes	♀ < ♂	♀ < ♂	♀ > ♂	♀ < ♂		♀ = ♂		♀ = ♂	♀ = ♂			Satoskar and Alexander (1995)
Leishmania spp.	Hamster	Yes	♀ < ♂	♀ < ♂				♀ < ♂		♀ < ♂				Travi, Arteaga et al. (2002)
Schistosoma haematobia	Human	Yes	♀ < ♂	♀ < ♂	♀ < ♂	♀ < ♂				♀ > ♂		♀ > ♂	♀ < ♂	Remoue et al. (2001)
Taenia crassiceps	Mice	Yes	♀ > ♂	♀ > ♂	♀ < ♂*		♀ = ♂	♀ < ♂* ♀ > ♂+	♀ = ♂	♀ > ♂*			♀ = ♂	Terrazas et al. (1998)
Plasmodium chabaudi	Mice IL-10	Yes	♀ > ♂	♀ > ♂	♀ < ♂	♀ > ♂		♀ = ♂			♀ = ♂			Li (1999)
Toxoplasma gondii	Mice	Yes	♀ > ♂	♀ > ♂	♀ < ♂	♀ < ♂					♀ < ♂		♀ < ♂	Roberts et al. (1995), Walker et al. (1997)

* In acute infection.
+ In chronic infection.

complement because of their direct effect on the parasite's external surface (Philipp et al., 1980). Such antibody-mediated damage has been documented to affect some parasite life cycle stages but certainly not all, i.e., tachyzoites in toxoplasmosis (Johnson and Sayles, 2002), early larvae in cysticercosis (Restrepo et al., 2001), merozoites in malaria (Daly and Long, 1995), trophozoites in amoebiasis (Ghosh et al., 1998), and promastigotes in leishmaniasis (Rafati et al., 2001). However, some of the extracellular stages of parasites manage to escape from the circulation and become sequestered inside their target cells, apparently unscathed by antibodies or other harmful immune effectors. An immune response against intracellular parasites would largely depend on the expression of parasite antigens on the infected cell's membrane (Kyes et al., 2001) and the triggering of innate immune effectors. Attraction of effector leukocytes (polymorphonuclear leukocytes, eosinophils, and basophils), cytotoxic T lymphocytes, and natural killer (NK) cells to the site of parasite infection should aid in parasite destruction or at least in containment of their growth or dispersal. Thus, an inclination toward TH2 profiles, with high levels of antibody production, would be most appropriate against extracellular stages of parasites. A TH1 response, which kills infected cells, would be most effective against intracellular parasites (Sher et al., 1992). If female hosts truly favor TH2 responses, they should be more resistant to extracellular parasites and to intracellular infections in their initial stages when the parasites are migrating toward their protected intracellular locations. In contrast, if androgens favor TH1 responses, then males should handle intracellular parasites more effectively, especially during late stages of infection. Despite the attractive congruence of these speculations on immunity and SD to infections, Table II shows no clear sign of such patterns in the strategies of hosts or parasites. Lack of congruity between expected and observed results most likely come from unsound expectations regarding the role of the immune system in sexually dimorphic parasitic infections.

A detailed understanding of the mechanisms leading to the destruction of host or parasite or to a mutually tolerable stalemate requires further research and the application of a more potent and discerning technology. It might also be useful to use other conceptual approaches in exploring host–parasite systems, i.e., SD and host–parasite specificity, among others. Most research on these topics uses a reductionist approach, attempting to link microscopic events and effectors. However, the network or systems approach, where some functions are distributive to the entire network and not localized in some of its parts, is rarely considered (Oltvai and Barabasi, 2002; Strohman, 2003).

THE HOST–IMMUNO-NEURO-ENDOCRINE–PARASITE NETWORK IN CHARGE OF INFECTION AND SD

The usual experimental strategy for examining the mechanisms of immunoendocrine interactions is to add a sexual hormone or a cytokine to an isolated component of the immune, neurological, or endocrine system and then to measure its response. With results obtained by this approach and also with some of the in vivo data in Tables I and II, we constructed a flowchart of the immune and neuroendocrine systems that included all interactions across species, sexes, cells, and types of responses. The result was an all-encompassing host–immuno-

neuro-endocrine-parasite (HINEP) network connecting components within and between the systems of the host and the parasite (Fig. 1). The magnitude and complexity of the HINEP network includes practically all sexual hormones and many of the known immunological components. Simple inspection suggests the networks possible relevance to many other biological scenarios other than infection. Some of the events in the immunoendocrine network involve cellular differentiation, reproduction or death and de novo synthesis of receptors. The prominent and varied connections of the HINEP network with other hormones, well known to be related to stress and the inflammatory process (Besedovsky and del Rey, 2002; Dantzer et al., 2002), suggest that the network can also influence innate immune mechanisms (Yokoyama and Scalzo, 2002). The HINEP network of the host is related directly with the parasite (Morales-Montor, Baig et al., 2001; Morales-Montor, Mohamed et al., 2001), affecting its reproductive capacity through sexual and adrenal steroids that favor the expression of genes related to cellular differentiation and proliferation (Escobedo et al., 2004). The HINEP network contains circuits with forward and backward regulation, producing a great range of effects on the parasite or the host using several venues. Because some of the events in the HINEP network involve cellular reproduction and de novo synthesis of receptors, the network would seem capable of adapting and evolving.

There are other immunoendocrine networks described in the literature, which focus on the connections of the immune system with adrenals and nervous system, but secondarily or not at all with the gonads (Besedovsky and del Rey, 2002). The HINEP network presented in this study adds to the existing networks by incorporating the gonads and sex steroids as intrahost connections and the parasites' physiological systems as interhost connections.

Sex steroids act on a variety of immunocompetent cells affecting clonal expansion, phagocytosis, apoptosis, antigen presentation, and physiological responses to cytokines and chemokines. Thus, there is no question regarding the capacity of sex hormones to modulate the immune response. The significant question is, rather, what is their end effect on the host–parasite relationship and at which point does it act in each gender of host? A node in the network likely to be present under a strong sex-steroid modulation of acquired immunity includes the TH1–TH2 immune responses (Rook et al., 1994; Martin, 2000). Conflicting effects of androgens and estrogens on TH1–TH2 may possibly adjust the relationship of each host sex with the parasite and achieve either "pacifist" coexistence or "belligerent" confrontation. Antigen presentation, clonal expansion, cell activation, or apoptosis and effector macrophage functions, inflammation, and chemotactic responses are also likely candidates for significant hormonal control. The exploration of direct sex-steroid effects is as yet incomplete, and their end effects on the whole immune system, especially when acting in unison, are seldom studied. As can be gathered from the summary of a single hormone's actions on some of the immune parameters cited in Table III, estradiol seems to stimulate TH2, but there is no proof that it shuts down TH1 other than in experiments using mitogens instead of antigens. Similarly, testosterone decreases some B-cell associated effector functions by reducing the levels of some TH2 cytokines (IL-1, IL-6, TNF-α) but has not been shown to interfere with TH1 functions. Dehydroepiandrosterone (DHEA) stimulates TH1 immune parameters without apparent effect on TH2. DHT has effects similar to testosterone. Progesterone downregulates effector mechanisms (NK cytotoxic activity and macrophage cytokine and nitric oxide production), and prolactin also acts on the TH1–TH2 modulation node. Based on these observations and considering they probably are not independent effectors, however, it would be adventurous to predict a single hormone's end effect on a host's immunological protection or vulnerability to infection. This is even more likely when many of the sex hormone levels are not independent effectors and some hormones are probably operating simultaneously on the host's immune system when confronted with a parasite. Furthermore, more complexity and less predictability are to be expected from the likelihood of immune cytokines acting directly on the parasite, as do the sex steroids.

Notwithstanding the problem in understanding how real physiological networks actually work, their nodes might differ in terms of the number of connections. Herein lies the only hope for understanding relatively simple cause effect relationships in parasitism. Firing of the most connected nodes may extend widely and rapidly throughout the network, inducing a significant change of phase in its equilibrium state and prompting the emergence of new properties (Oltvai and Barabasi, 2002; Strohman, 2003). Identification of the most connected nodes would be a way to begin their study as principal participants in SD to infection. To clarify the relationship existing between a host's SD and immunity with susceptibility to infection, we must look for these hierarchic nodes in the HINEP network. Some of them may be apparent at sexual maturity, but others might be more difficult to identify, having operated in the early ontology of the female or male immune and endocrine systems and then disappearing by the time of sexual maturation. One could hypothesize that important neuroendocrine system connections with the immune system are established during embryonic development, when gonadal differentiation occurs (Klein et al., 2002; Sinisi et al., 2003) and principal criteria for immunological self- and danger signal recognition also appear to be set (Matzinger, 2002; Medzhitov and Janeway, 2002).

To illustrate the HINEP network's explanatory and predictive properties of parasite infections, circuits that are turned on in experimental cysticercosis and schistosomiasis are illustrated with different colors in Figure 1. The circuits are not identical, but in both, IL-6 is a prominent feature, and the consequences of its overexpression, i.e., feminization, a TH2-leaning immune response, would be expected in other infections with all the rest being equal. In the network, there are also circuits capable of masculinizing the infected female if P-450 aromatase is directly inhibited, or by inhibition of GNRH in the hypothalamus, mediated or not by IL-6, or by enhancing the expression of 5α-reductase type II by means of an IL yet to be identified. In the opposite direction, the DHEA upregulation of TH1, for instance, endows the endocrine system with an ability to participate in immunological defense, a prediction that has yet to be verified in TH1-sensitive infections (Baszler et al., 1999; Suzuki, 1999; Rogers et al., 2002). In the network's schistosomiasis example, the parasite actively induces an immune response, which progressively leans toward TH2. Then, the increase in IL-β expression in the hypothalamus stimulates CRH production, which, in turn, stimulates pituitary adrenocorticotropin hormone (ACTH) (Morales-Montor, Newhouse et al.,

TABLE III. Effects and mechanisms of action by hormones on immunocompetent cells.*

Hormone	Effect on immune system cells	References
Estradiol	Polyclonal B cell activator; promotes B cells into plasma cells; ↓ bone marrow and thymus mass; ↑ IL-10 and IL-6 secreting cells; ↓ IFN-γ and IL-2 production; downregulates NK activity; upregulates phagocytocis by macrophages; ↑ serotonin and histamine release	Mandrup-Poulsen et al. (1995), Gaillard and Spinedi (1998), Chen et al. (2001), Roberts et al. (2001), Verthelyi (2001), Spinedi et al. (2002), Kitaya et
DHT	↓ T-cell response to mitogen; ↓ mast cell secretion; ↓ IL-1, IL-6, and TNF-α production; ↑ IL-2, TNF-α, and IFN-γ mRNA	Bijlsma et al. (2002), Morales-Montor, Baig, Hallal-Calleros (2002), Tanriverdi et al. (2003) al. (2003), Marct et al. (2003), Ou et al. (2003)
Testosterone	↓ B-cell response to mitogen; ↓ mast cell secretion of histamine and serotonin; ↓ IL-1, IL-6, and TNF-α production	Zhang et al. (2000), Bijlsma et al. (2002), Morales-Montor, Baig, Hallal-Calleros (2002), Tanriverdi et al. (2003)
Progesterone	↓ NK cytotoxic activity; ↑ TNF-α secretion; ↓ macrophage cytokine secretion; ↓ NO production	Mandrup-Poulsen et al. (1995), Gaillard and Spinedi (1998), Verthelyi (2001), Spinedi et al. (2002)
Cortisol	↓ Prostaglandins and leukotrienes production; modulates T- and B-cell maturation; affects trafficking and activation of proinflammatory cells; ↓ the production of IL-1, IL-2, IL-6, IL-8, IL-10, IL-12, and TNF-α	Derijk and Berkenbosch (1991), Mandrup-Poulsen et al. (1995), Loria et al. (1996), Nussdorfer and Mazzocchi (1998), Feterowski et al. (2001), Besedovsky and del Rey (2002), Esch (2002), Morales-Montor, Mohamed et al. (2003)
DHEA	↑ IL-2 production; ↑ IFN-γ production; ↓ IL-6 secretion; ↓ TNF-α production; protects against neurooxidative damage; ↑ T-cell immunity; ↑ DTH reaction	Derijk and Berkenbosch (1991), Mandrup-Poulsen et al. (1995), Loria et al. (1996), Nussdorfer and Mazzocchi (1998), Feterowski et al. (2001), Besedovsky and del Rey (2002)
CRH	↑ IL-1 and IL-6 production; ↑ chemotaxis and superoxide production; ↑ B-cell proliferation; ↑ expression of T cells IL-2 receptors	Derijk and Berkenbosch (1991), Mandrup-Poulsen et al. (1995), Nussdorfer and Mazzocchi (1998), Besedovsky and del Rey (2002), Esch (2002)
ACTH	↑ Antibody production; cytokine secretion and proliferation	Panerai and Ottaviani (1995), Nussdorfer and Mazzocchi (1998), Ottaviani et al. (1999)
Prolactin	↑ Lymphocyte proliferation in response to antigen and mitogens; ↑ IFN-γ and IL-2 secretion; ↓ cell death mechanisms in immune cells; induces NK cells to their differentiation to prolactin-activated killer cells	Derijk and Berkenbosch (1991), Matera et al. (2001), McMurray (2001), Yu-Lee (2002)
VIP	↓ Production of proinflammatory agents; ↑ production of anti-inflammatory cytokines; both functions in activated macrophages; ↑ Th2 cell differentiation	Delgado et al. (2001), Voice et al. (2002), Ganea and Delgado (2003)
GH	↑ Adhesion of thymocytes to thymic epithelial cells; ↑ release of thymocytes from thymic nurse cells; ↑ intrathymic T-cell traffic	Sternberg (1997), Weinstock and Elliott (2000)
Thyroid hormones	Affects primary B-cell development because of reduced proliferation of immature B-cell precursors	Dorshkind and Horseman (2001)
Vasopresin and oxitocin	↑ Cell proliferation	Dorshkind and Horseman (2001)
Encephalins	Low doses: ↑ activates B and T cells; high doses: immunosuppression	Dorshkind and Horseman (2001)
Endorphins	↓ Antibody production and proliferation	Machelska and Stein (2002)
hCG	↓ Proliferation of T and NK and induction of T suppressors	Pope (1990)
Melatonin	Affects thymocyte maturation and differentiation	Hotchkiss and Nelson (2002)

* Abbreviations and symbols: DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; CRH, corticotrophin; ACTH, adrenocorticotrophic hormone; VIP, vasointestinal peptide; GH, growth hormone; hCG, human chorionic gonadotrophin; DTH, delayed-type hypersensitivity; ↓, decrease activity; ↑, increase activity.

2001). Concomitantly, IL-6 and migration inhibitory factor could be regulating ACTH production directly in the pituitary gland. The ACTH production, in turn, stimulates adrenal glands to produce cortisol and DHEA, together with TNF-α, which can directly inhibit parasite growth. Infection triggers the immune response of the host by mediating the neuroendocrine system at HPA axis level (Morales-Montor, Mohamed et al., 2003). The consequent microenvironment could be more permissive for the establishment and growth of the schistosome. The network also exhibits the possibility of HPA axis hormones to act directly on the parasite, affecting its reproductive capacity through adrenal steroids that favor the expression of genes related to cellular differentiation and proliferation (Morales-Montor, Mohamed et al., 2001). On the other hand, in cysticercosis, the nodes of interaction are by way of the hypothalamus pituitary gonadal axis. Infection with the metacestode results in a feminization process and in a TH1 TH2 shift of the host's immune response. The cysticerci actively induce a TH2 immune re-

sponse. Then, the increase in IL-6 production in turn stimulates estradiol production by directly activating the enzyme P-450 aromatase and at the same time increasing pituitary follicle-stimulating hormone levels. Estradiol would then cycle back to favor the TH2 response further. In turn, the decreased testosterone production inhibits the TH1-dependent cellular response. The consequence is the change of the host's hormonal microenviroment from restrictive (male) to permissive (female) for cysticerci growth. The network also illustrates the possibility that the host's sex hormones may act directly on the parasite, affecting its reproductive capacity through sex steroids that favor the expression of genes related to cellular differentiation and proliferation in many animal species (Fig. 1).

The HINEP network's prowess, with its manifold forward and backward regulations in operation, is congruent with the variegated profiles of Infection-SD and Immune-SD in the various host parasite systems. It does not, however, necessarily imply or deny that there will be differences between sexes in the final results of a given confrontation with a parasite, reached perhaps by different mechanisms in different host sexes. A functional feature of complicated, random, or scale-free networks is its stability before random perturbations, whereas strategic perturbations "break" them to pieces (Oltvai and Barabasi, 2002; Strohman, 2002). This could explain why there are some sex-unbiased parasitic diseases. Perhaps only a few parasites are capable of triggering a profound general change in the state of the network that results in Infection-SD, a property limited to those parasites connecting with the complex hierarchical immunological nodes of the network and the sex steroids.

IS THE HINEP NETWORK INVOLVED IN OTHER MEDICAL AND BIOLOGICAL PUZZLES?

The literature search revealed an extremely complex HINEP network, involving hormones and cytokines that predict potent interactions in events generally attributed to the exclusive operation of single systems in response to simple precepts (reproduction and defense). Therefore, much plasticity and multifunctionality in a network are not without risk. Absence of control could lead to the loss of tolerance and autoimmune problems (Derijk and Berkenbosch, 1991; Lechner et al., 1996) or be involved in the immune compromise of aging (Panerai and Ottaviani, 1995), in the pathophysiology of some infections in which inflammation is a prominent effector of pathology (Mandrup-Poulsen et al., 1995; Henri et al., 2002), or even in some combination of all the above. Moreover, the HINEP network could connect parasite infections with other diseases that seem alien to the immunological and endocrinological domains, such as arterial hypertension (Peeters et al., 2001), atherosclerosis (Chae et al., 2001), and cancer (Herrera and Ostrosky-Wegman, 2001; Polat et al., 2002).

Many other biological questions emerged from the review of the literature on SD, each pointing to avenues for future research. We shall focus on 2 of the more prominent ones, which, in turn, involve many subsidiary possibilities. First, why is there SD in the immune response? Second, is there evidence to suggest that parasites have influenced the evolution of their hosts' Immune-SD?

The very complexity of the HINEP network hints at reasons for Immune-SD other than the self or foreign concept. Perhaps it has evolved as the best mechanism for individuals of either sex to confront infection successfully, even if by different mechanisms, and also to solve with precision gender-specific challenges, like pregnancy (Grossman, 1989), or perhaps the consequences of their territorial, mating, and social behaviors (Zuk, 1994; Kavaliers et al., 2001).

The selective pressure driving evolution toward Immune-SD, matching in importance the defense of the host against infection, is to permit reproduction in a dioecious species without much immunological compromise (Grossman, 1989; Gaillard and Spinedi, 1998; Agrawal and Lively, 2001; Charles et al., 2002; Moore and Wilson, 2002; Owens, 2002; Potti et al., 2002; Tella et al., 2002). This compromise could be achieved by a transient, immunologically specific allowance of female pregnancy with an offspring that is half-foreign, designed in terms of immunoendocrine signaling that does no damage to an effective response to a pathogen (Martal et al., 1995; Matzinger, 2002; Medzhitov and Janeway, 2002). Pregnancy demands for immunological allowance would originate from the advantage of species diversification gained through gender dichotomy. Its satisfaction would call for occasionally fastidious but transient immunoendocrine regulation by hormones and cytokines so that the fetus is not damaged (Barnea, 2001). Immune-SD may provide males with the specialized ability to better cope with their more stressful and dangerous lives when displaying their sex-specific behaviors (Kavaliers et al., 2001; Spinedi et al., 2002). Thus, the hosts that get the best trade-off between the need to diversify and the need to survive would appear to have the better chances to evolve. Even the parasite could benefit from the host's Immune-SD. For example, the parasite-restrictive males in the case of murine cysticercosis may be regarded as behaviorally enhanced vehicles toward the parasite's final destiny in the gut of carnivores (Willis and Poulin, 2000; Gourbal et al., 2001). In turn, the cysticercus-permissive females, when infected with the eggs of *T. crassiceps*, would act as the optimal hosts for their massive reproduction (Poulin and Thurn, 1996; Zuk and McKean, 1996; Panhuis et al., 2001).

The level of complexity introduced in the decision-making process of immune events by the powerful HINEP network regulatory capacity promises to enlighten persistent immunological puzzles such as tolerance and autoimmunity, the connection with infection of seemingly unrelated physiopathological events such as hypertension and cancer, and the role of Immune-SD in species diversification and individual behavior. The roles of sex steroids in the ontological development of the immune system and in acquired and innate immune responses promise invaluable insights and beg for more research.

CONCLUSIONS

There are many exceptions to the female host supremacy paradigm in parasitic infections of mammals, too many to leave unquestioned. Indeed, testosterone is involved in the immunoendocrine interactions triggered by infection, but so are many other hormones and cytokines that act as a network in which the contributions of its single effectors are unclear. Instead, important properties, like infection and immune sex-associated differences, may emerge from the network as a whole. The role of the host's immune system as the only effector of SD in parasitism is not clear; it is insufficiently explored, and it is not

uniformly implemented, even in the most studied host parasite systems. Finally, the parasite's direct response to the hormonal environment of each host sex has been overlooked as a significant contributor to host SD in parasitic infections. In fact, it would appear that the conflicting findings in murine *T. crassiceps* cysticercosis with the female supremacy paradigm in parasite infections provoke even greater sensitivity to the host's sex steroids, i.e., parasite driven estrogenization and deandrogenization of infected male mice and permissiveness of the female mice TH2 inclined immune profile toward cysticercus proliferation.

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MOLECULAR MECHANISMS INVOLVED IN THE DIFFERENTIAL EFFECTS OF SEX STEROIDS ON THE REPRODUCTION AND INFECTIVITY OF *TAENIA CRASSICEPS*

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ABSTRACT: The in vitro exposure of *Taenia crassiceps* cysticerci to 17-β estradiol (E_2) and progesterone (P_4) stimulated their reproduction and infectivity. Testosterone (T_4) and dihydrotestosterone (DHT) inhibited their reproduction and reduced their motility and infectivity. E_2 and P_4 increased, whereas T_4 and DHT reduced, the expression of parasite *c-os* and *c-jun* and DNA synthesis. In vitro exposure of cysticerci to sex steroids before their inoculation into recipient noninfected mice resulted in large parasite loads when pretreated with E_2 and P_4 and in smaller loads when pretreated with T_4 and DHT. To determine the possible molecular mechanisms by which sex steroids affect *T. crassiceps*, sex steroid receptors were amplified. *Taenia crassiceps* expressed estrogen receptors (both α and β isoforms) and androgen receptors but no P_4 receptors. These results demonstrate that sex steroids act directly on parasite reproduction by binding to a classic and specific sex steroid receptor on the parasite. The differential response of cysticerci to sex steroids may also be involved in their ability to grow faster in the murine female or feminized male host. This is the first report of direct sex steroid effects on the parasite possibly through sex steroid receptors in the cysticerci.

Host and parasite sex-associated biases may be combined to favor their evolution toward a mutually acceptable relationship.

In experimental *Taenia crassiceps* cysticercosis, female hosts of various inbred mouse strains bear larger parasite loads than males in the first 4 wk of infection (Sciutto et al., 1991). Later on during infection, however, the sex-associated differences tend to disappear as male parasite loads approach those of females. Chronically infected males become feminized and develop high serum estradiol (E_2) and low testosterone (T_4) levels (Larralde et al., 1995; Morales et al., 1996; Morales-Montor et al., 2001) with a negative impact on their sexual and aggressive behavior (Morales et al., 1996; Gourbal et al., 2001; Morales-Montor, Baig et al., 2001; Morales-Montor, Mohamed et al., 2001). These results strongly suggest that estrogens promote and androgens inhibit parasite reproduction and are well in line with gonadectomy and hormonal reconstitution experiments (Huerta et al., 1992; Larralde et al., 1995; Morales-Montor et al., 2002). Because preliminary experiments failed to detect a direct response of the parasite when exposed in vitro to sex steroids (C. Larralde, pers. comm.), it was assumed that sex steroids exert their actions on parasite reproduction by way of the host's immune system, perhaps by inducing a T-helper type 1/T-helper type 2 (TH1/TH2) imbalance in favor of TH2, in the case of estrogens, and of TH1 in the case of androgens (Terrazas et al., 1994; Bojalil et al., 2002; Morales-Montor et al., 2002). Notwithstanding the intervention of the host's immune response in dealing with the parasite, the possibility of additional direct effects of sexual steroid hormones on the parasite's physiology should not be hastily discarded. Direct effects of sex steroids have been invoked in explaining the relation between androgen treatment and increased number of larval and adult stages of intestinal helminths or of various other parasites in other organs of vertebrate hosts, i.e., *Ancylostoma caninum* grows better and increases egg production when the host is injected with T_4 (Bhai and Pandey, 1982); T_4 increases viability of *Nematospiroides dubius* larvae in the gut of the rat (Dobson, 1961), and it also does so with *Nippostrongilus brasiliensis* located in the hamster gut (Soloma, 1969). *Leishmania major* systemic infections in mice are strongly affected by T_4 (Mo and Nacy, 1998) as well as by the accelerated larval development of intestinal cestodes such as *Echinococcus granulosus* (Frayha et al., 1971) and *Mesocestoides corti* (Novak, 1975). However, none of the above observations indicate which are the targets of the sex steroids or if they emanate from hosts or parasites or both. Certainly, the complex interactions existing between the endocrine and the immune systems of mammals (Besedovsky and Del Rey, 2002; Morales-Montor et al., 2001) provide examples of opportunities for sex steroids to exert their parasite-promoting or -restrictive actions through the physiological systems of the host. Indeed, early experiments with *T. crassiceps* showed that sex-associated differences in parasite loads tend to disappear after thymectomy (Terrazas et al., 1999; Bojalil et al., 2002). However, the direct effects of sex steroids and other hormones in a variety of other parasites (Maswoswe et al., 1985; Lingnau et al., 1993; Zeitlich et al., 2000; Morales-Montor et al., 2001) indicate caution in excluding helminths from the general rule of sex steroid capacity to act directly on them. Only a consistent failure of sensitive technology to show the effects of sex steroids on cysticerci outside the host, in vitro, could weaken the possibility of direct action of the hormone on the parasite.

Thus, the present in vitro experiments were designed to thoroughly explore the possibility that sexual steroid hormones have a direct effect on *T. crassiceps* reproduction, viability, and infectivity and to look for preliminary evidence of the possible molecular mechanisms involved.

MATERIALS AND METHODS

Harvesting and preparing cysticerci for experimentation

A new stock of *T. crassiceps* cysticerci (ORF-Kuhn2 strain) (Culbreth et al., 1972; Freeman, 1985) was maintained in our laboratory by serial intraperitoneal passage in BALB/c Ani female mice approximately every 4 mo. Cysticerci for each experimental session were obtained from intraperitoneally infected female mice and placed in tubes containing sterile phosphate-buffered saline (PBS 1×) supplemented with 100 IU/ml of antibiotics-fungizone (Gibco, Grand Island, New York) (Eschan & Smyth, 1976). The tubes were centrifuged for 10 min at 1,500 rpm at 4 C and the supernatant was discarded. The packed cysticerci were incubated in AIM-V serum-free medium (Gibco BRL, Rockville, Mary-

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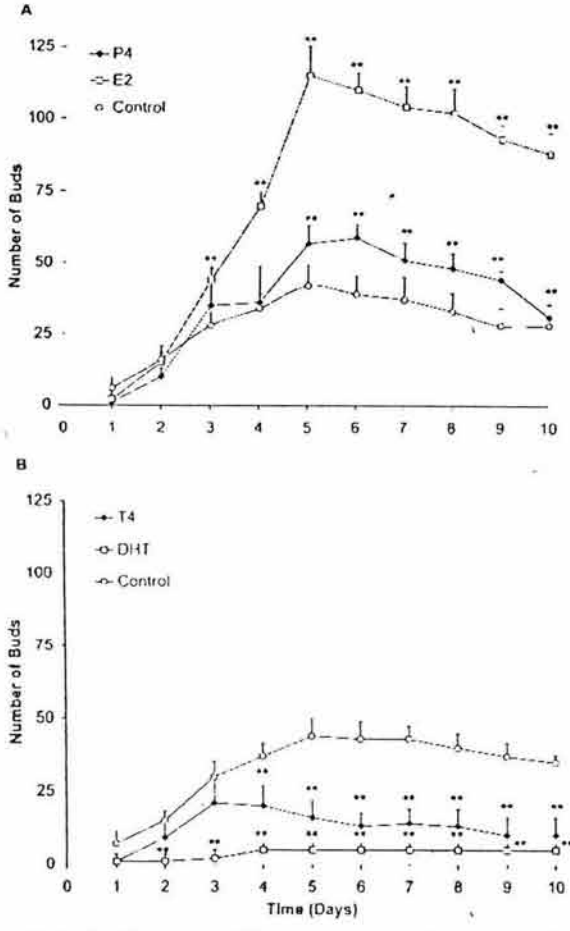

FIGURE 2. Time curves of *Taenia crassiceps* cysticerci reproduction after exposure to estradiol and progesterone (A) or testosterone or DHT (B). Each point represents the mean (SD) of 5 assays counting the number of buds in each parasite contained in each cultured well. Data were pooled, and the mean was obtained. $^{*}P < 0.05$, $^{**}P < 0.01$ with respect to control and vehicle-treated cysticerci. The concentration of hormones are as follows: E_2, 40 μg/ml, P_4, 20 μg/ml, T_4, 4μg/ml, and DHT, 6 μg/ml.

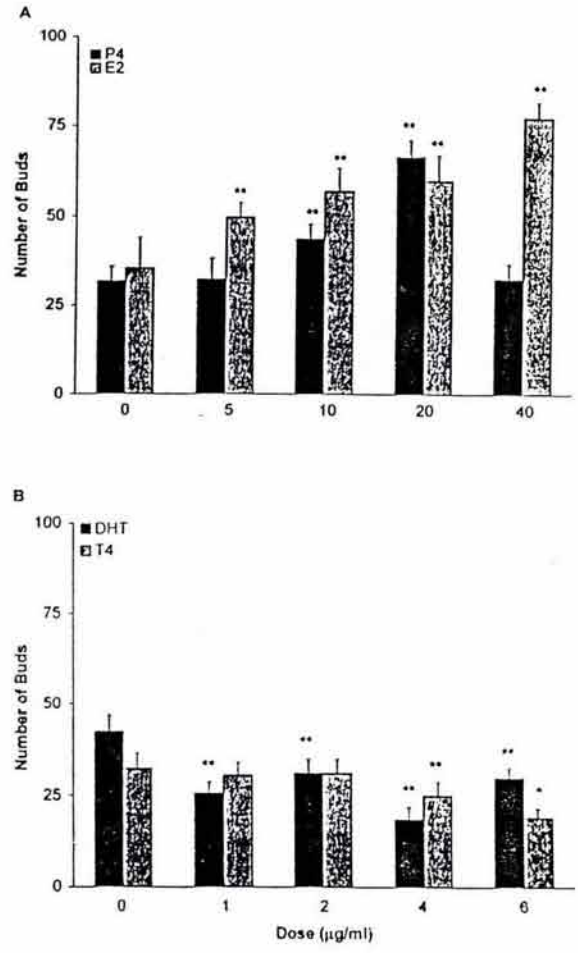

FIGURE 3. Dose-dependent effect of sex steroid hormones on in vitro reproduction of *Taenia crassiceps*. Ten cysticerci were incubated for 5 days under different concentrations of E_2 and P_4 (A) or T_4 and DHT (B). The bars represent the average of determinations (SD) of the number of buds. $^{*}P < 0.05$, $^{**}P < 0.01$ with respect to control and vehicle groups. Each point represents the mean (SD) of quintuplicate determinations of the number of buds counted and viability of parasites.

Expression of c-fos and c-jun genes in response to hormonal treatment: In view of the known effects of sex steroids on the expression of several gene families, including the AP-1 complex genes (*c-fos* and *c-jun*), which are involved in the control of cell differentiation, reproduction, and apoptosis, these 2 genes were prime candidates for exploration (Hyder et al., 1992, 1995; Jochum et al., 2001). The expression of β-actin as an internal control was also measured to control for differences in amplification procedures and gel staining in each experiment. RNA (mRNA) expression, which was clearly augmented in E_2- and P_4-treated cysticerci, but DHT treatment had no effect and T_4 treatment slightly inhibited its expression (Fig. 4C). All these values were significant at $P < 0.01$ in comparison with the expression of both genes in control cysticerci.

DNA quantity and ^{3}H-thymidine incorporation: Figure 6A shows the E_2, P_4, T_4, and DHT stimulation effects on ^{3}H-thymidine uptake in its DNA fraction with respect to controls. E_2 and P_4 treatment augmented ^{3}H-thymidine uptake 5-fold and 3-

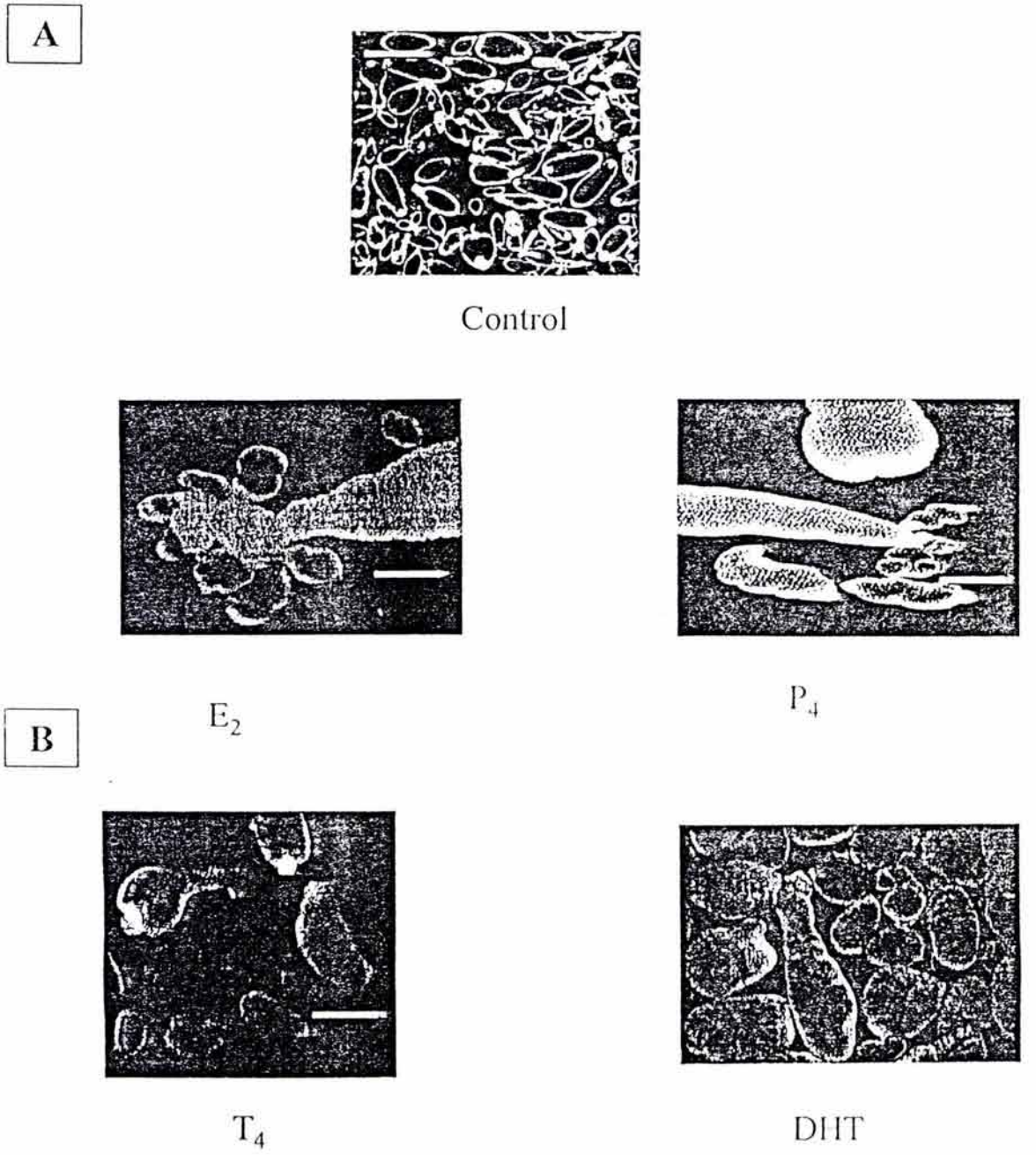

FIGURE 4. Micrographs of *Taenia crassiceps* cysticerci treated in vitro with sexual steroid hormones. The parasites were selected to faithfully represent the mean effect on the number of buds for each treatment. White bars in each photograph, 1 mm. (A) *T. crassiceps* cysticerci control. (B) *T. crassiceps* cysticerci treated with estradiol (40 μg/ml), (C) *T. crassiceps* cysticerci treated with progesterone (20 μg/ml), (D) *T. crassiceps* cysticerci treated with testosterone (4 μg/ml), and (E) *T. crassiceps* cysticerci treated with DHT (6 μg/ml).

thesis when compared with untreated control cysticerci. Thus, E_2 treatment doubled the amount of DNA synthesized, whereas P_4 treatment increased the same 1.5-fold. No effect on DNA synthesis was seen when cysticerci were treated with either T_4 or DHT.

Infectivity assays: Infectivity studies of cysticerci exposed to sex steroids before their inoculation to recipient mice indicated that estrogens promoted and androgens inhibited their infectivity. Figure 7 shows the parasite burdens obtained at 4 wk of infection with cysticerci previously exposed to optimal dose of E_2, P_4, T_4, and DHT 5 days before being inoculated into male and female mice. In male recipients, E_2-treated parasites triple

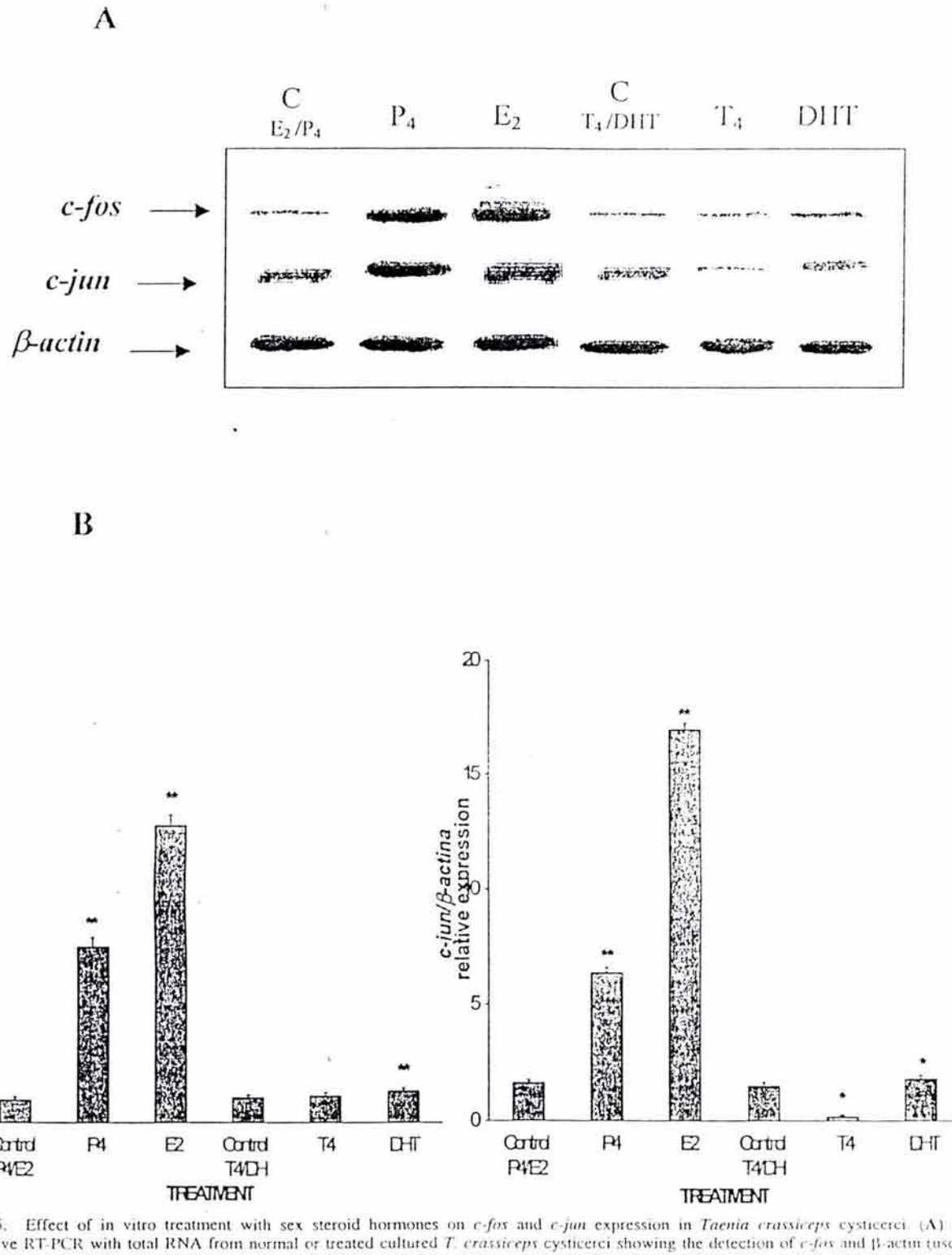

FIGURE 5. Effect of in vitro treatment with sex steroid hormones on *c-fos* and *c-jun* expression in *Taenia crassiceps* cysticerci. (A) A representative RT-PCR with total RNA from normal or treated cultured *T. crassiceps* cysticerci showing the detection of *c-fos* and β-actin (used as a control expression gene). (B) Results of *c-fos* and *c-jun* expression are reported as densitometric data of the autoradiographic signals. Data represent individual wells by treatment, and each experiment was done in quintuplicate. The relative expression was obtained by correcting the expression of *c-fos* or *c-jun* to that of β-actin. $^{*}P < 0.05$, $^{**}P < 0.01$ with respect to their respective control group.

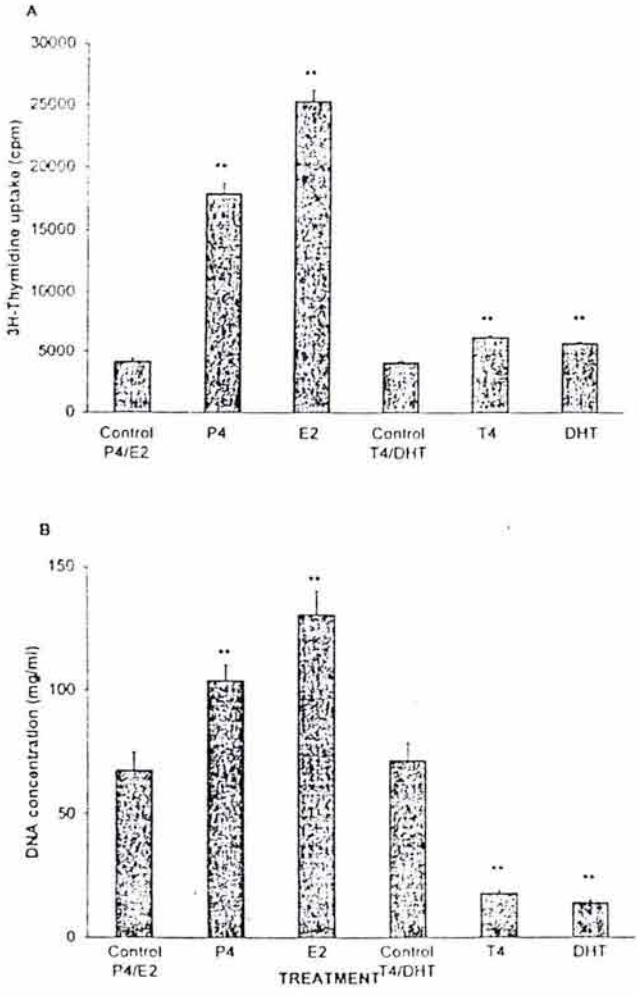

FIGURE 6. Specific proliferation of *Taenia crassiceps* cysticerci measured as ^{3}H-thymidine incorporation (A) and total DNA quantities (B). Data are represented as the mean SD of 3 different experiments (n = 5). Each cyst culture was done in triplicate. Cysticerci were cultured separately in the presence of each sex steroid and proliferation was measured. DNA was extracted, purified, and quantified from the cysticerci obtained after different treatments. $^*P < 0.05$, $^{**}P < 0.01$ as compared with their respective control group.

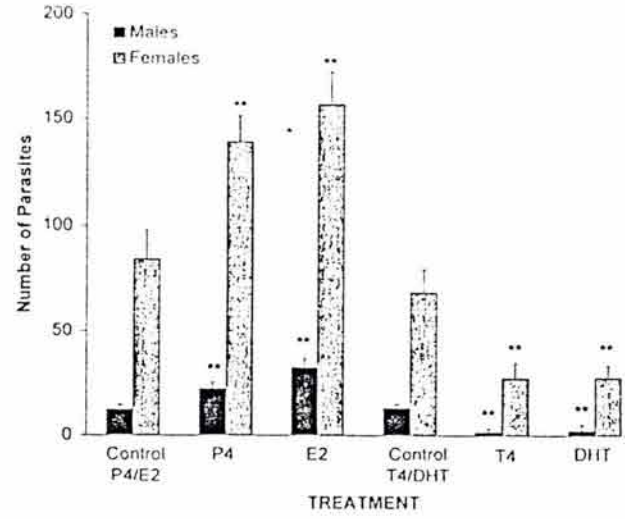

FIGURE 7. Number of *Taenia crassiceps* cysticerci obtained fro the peritoneal cavity of BALB/c AnN mice of both sexes with differe experimental treatments. Cysticerci were cultured first with a sing dose of estradiol, progesterone, testosterone, or DHT, and then reino ulated (10 parasites per mouse) into female and male mice. Bars re resent the mean SD of individual parasite loads of a total of 2 exper ments (n = 5). $^*P < 0.05$, $^{**}P < 0.01$ as compared with control mic

in number as compared with controls, and in female recipients they were doubled. P_4 pretreatment also doubled the expected parasite load in both genders. In contrast, prior exposure of parasites to T_4 and DHT significantly decreased the expected parasite load in male and female hosts.

Sex steroid receptors detection in Taenia crassiceps: Because different mRNAs can have varying half-lives and may undergo selective degradation, we determined the quantity and integrity of total RNA extracted from the different sources used in this study. It was clear that the same amount of RNA (1 μg) that was not degraded was used for reverse transcription (RT)-PCR amplification in each studied tissue (data not shown). The amplification by RT-PCR of the ER-α and ER-β, AR, PR-A, and PR-B and β-actin genes for *T. crassiceps* is shown in Figure 8. Specific fragments that correspond in molecular weight to those of ER-α, ER-β, and AR were obtained from both *T. crassicep* and control tissues, whereas PR-A and PR-B fragments wer only detected in the mouse uterus (control tissue) and not in *T. crassiceps*. Sequencing of the *T. crassiceps*–amplified frag ments, as well as of control tissues, demonstrated their identit to ER-α and ER-β, AR, and β-actin (data not shown).

DISCUSSION

This work shows that sex steroids act directly on *T. crassi ceps* cysticerci proliferation and viability without need of th host's participation, and E_2 and P_4 promote parasite reproduc tion without affecting their viability. In contrast, T_4 and DH significantly inhibit parasite proliferation and may lead to thei destruction. These effects depend both on the concentration o hormones and the duration of exposure. The effects on repro duction and viability began at 24 hr of culture but were maxi mally different between experimental and control groups at 7 days of culture and later. DHT was more drastic in its delete rious effects on cysticerci than T_4 ($P < 0.05$) and E_2 mor stimulatory than P_4 ($P < 0.05$). The involvement of the AP-1 complex genes (*c-fos* and *c-jun*) of the parasite was shown be cause the treatment with the 4 sex steroids had an impact o their expression in a way congruent with the proliferation an viability changes. The effects of E_2 and P_4 stimulated the ex pression of both *c-fos* and *c-jun* and those of T_4 or DHT inhib ited their expression.

Previously, we had found that gender and circulating E_2 and T_4 levels in host mice crucially affected the dynamics of para site loads in mice infected with cysticerci of *T. crassiceps* (Mo rales-Montor et al., 2002). The very fact that infection of male mice with *T. crassiceps* leads to striking increases in estrogen levels of the host is consistent with the idea that cysticerci fare better in high estrogen conditions and somehow induce the host

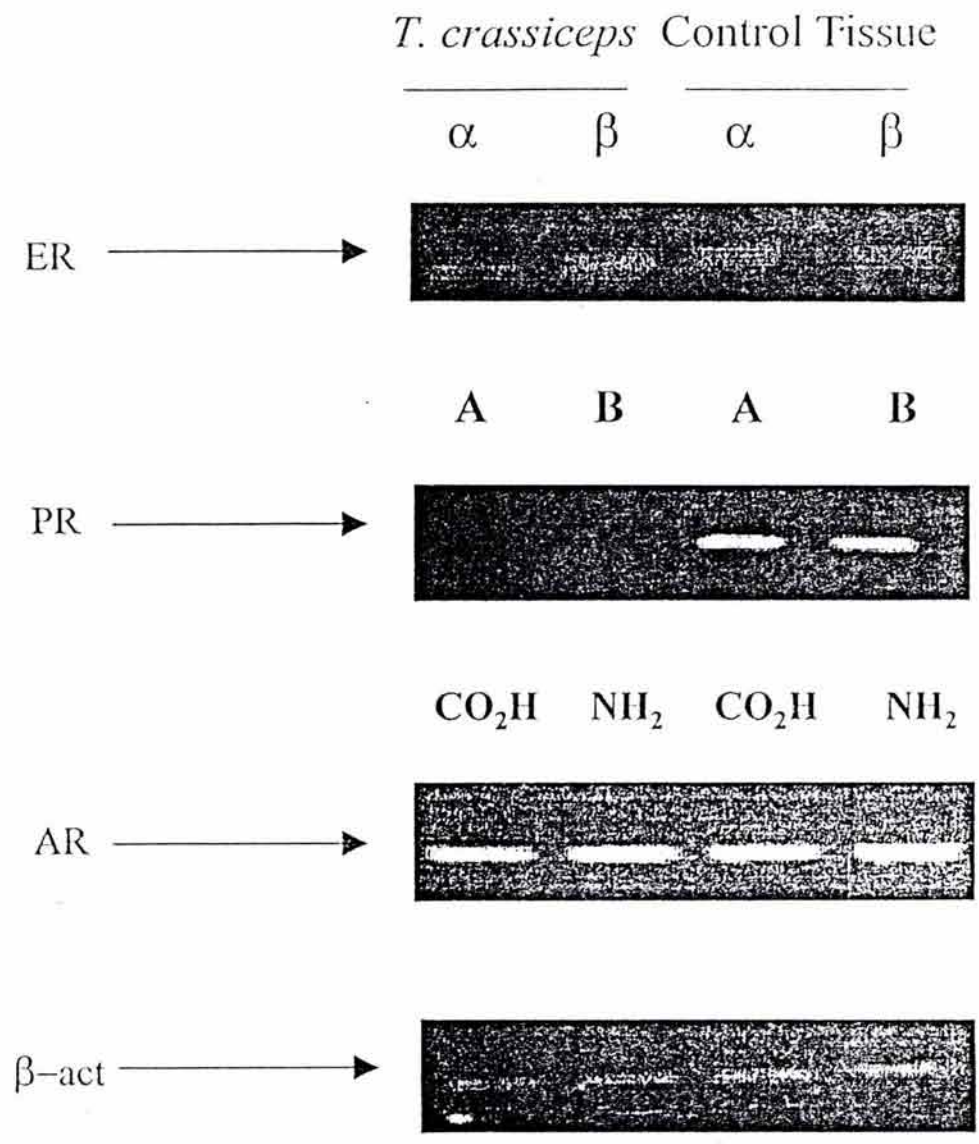

FIGURE 8. Expression of sex steroid receptors in *Taenia crassiceps* cysticerci. In the vertical axis, we show the expression of the estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), and β-actin in *T. crassiceps* and control mouse tissue. In the horizontal axis, we show the distinct isoforms of the receptors. The positive expression control tissue for the ER was mouse ovary, for PR it was mouse uterus, and for AR we used mouse testes. For amplification of AR, we used 2 different sets of primers defined in the figure like the –COOH (carboxilic) and $-NH_2$ (amino) terminal domain of the gene. β-actin was the expressed constitutive gene.

to produce them. However, a more intricate strategy of parasite activity must now be considered. Perhaps low androgen levels are also necessary for the parasite because they are so stunted by them. The basis for the parasite's preference or avoidance of sex steroids is more understandable if overexpression of AP-1 genes favors asexual reproduction, whereas underexpression does not. An inadequate hormonal environment may lead to apoptosis of crucial parasite cells, as has been proposed for other parasite infections, i.e., retinoic acid has been shown to affect female *Litomosoides carinii* and microfilariae of *L. carinii*, *Brugia malayi*, *Brugia pahangi*, and *Acanthocheilonema viteae* (Zahner et al., 1988). Cercariae, schistosomula, and adult worms of *Schistosoma mansoni*, when treated with 2 hypothalamic–pituitary–adrenal axis hormones, show reduced viability, whereas dehydroepiandrosterona and cortisol significantly inhibit in vitro schistosoma oviposition (Morales-Montor et al., 2001) and T_4 does likewise with the mitochondrial function of *S. mansoni* (Fantappie et al., 1999).

Because there is a great deal of conserved sequence homology among most hormone receptors, especially in the ligand and DNA-binding domains (Damián, 1997), we were able to show that cysticerci expressed an androgen receptor, with homology to the androgen receptor that binds both T_4 and DHT. Such commonalities between host and cysticercus metabolism

should come as no surprise, when extensive homologies between species are being documented in other systems as well. The same argument applies for the direct action of estrogens, although their effects are opposite to those of androgens. Interestingly, we showed that both isoforms of the classic estrogen receptor (ER-α and ER-β) are expressed in *T. crassiceps*, but there is no expression of P_4 receptors (neither isoform A nor B). It appears that the effects of estrogens and androgens are caused by the binding of E_2 and T_4 to their respective receptors. The small effects of P_4 could result from nonexpression of its specific receptor or be caused by nongenomic effects or merely reflect its transformation to E_2 as previously shown for androgens (Gomez et al., 2000). Binding of the ER to the classic estrogen-dependent elements could be responsible for the activation of AP-1 complex genes in the normal metabolism of *T. crassiceps*. Previous studies have demonstrated that the genome of *Onchocerca volvulus* encodes at least 3 members of the nuclear receptor family (Unnasch et al., 1999), and this could also be the case for *T. crassiceps*.

With respect to the significance of the differential effects of sex hormones on host and parasite, we found it interesting to speculate on the evolutionary impact of the host gender differential specialization in dealing with the parasite's different stages. Thus, it would appear that gender specialization of the kind described here for *T. crassiceps* (females favoring and males hindering larval asexual reproduction) could be evolving to a more benign host–parasite relationship.

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Taenia solium cysticercosis of humans and pigs: A review of our contributions and perspectives in the research of its complexities

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Abstract

This is a brief account of some of the problems posed by Taenia solium cysticercosis that we have approached since the early 70's and a summary of our principal results and/or present position about each of them.

In Human neurocysticercosis (NC): NC is a clinically pleomorphic disease in symptoms and severity that range from clinical silence to life threatening and sometimes deadly conditions. The rural population of Mexico shows higher NC prevalence (aprox 10%) than is generally recognized, but most of the cases are clinically silent. Serologic diagnosis is still unsatisfactory but immunodiagnosis

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in CSF of symptomatic NC performs somewhat better. The use of synthetic peptides derived from recombinant antigens open a new possibility to improve diagnosis. 90% of people with positive serology in Mexico do not cluster around the household, which speaks of far reaching mechanisms of transmission that operate in highly endemic situations in addition to the importance of living in close proximity with tapeworm carriers that operates in both high and low endemic situations. There are hints that severity associates with inflammation, gender and genetic background of the human host but not with level of exposure to infection. There are also indications that the TH1/TH2 balance of the immune response may be decisive for the outcome of an infection.

In Pig-cysticercosis (PC): PC prevalence is alarmingly high (5 to 30%) in rural villages of Mexico. A synthetic peptide vaccine against PC was shown to reduce 50% the prevalence and 98% the intensity of PC in a realistic field trial of pigs exposed for one year to natural infection, enticing its application as an adjunct in transmission control. Antipeptide antibodies of vaccinated pigs cripple the ability of cysticerci to develop unto adult tapeworm parasites, a subtle yet important and applicable effect of antibodies otherwise considered inoperative. Castration and pregnancy of pigs double their expected prevalence from about 25 to 50%, an indication of hormonal involvement in susceptibility to infection. Experimental infections hint to genetic factors in controlling parasite establishment.

In Taenia solium: Cysticerci collected from different donor pigs naturally infected in different geographic areas of Mexico show substantial antigenic diversity peaking out of a comparatively highly similar DNA background, an indication that the expression of antigens may be subject to circumstantial factors of the parasite, the host and/or the environment.

Overview: The roots, strategies and threats of *Taenia solium* disease

Taenia solium disease exists in European civilizations since antiquity (1), and some hold that it affects humans since prehistoric times favored by their filthiness and cannibalism It was later extended to wild boars (2, 3). Nowdays the disease emerges in rural areas of developing countries from the coalition of persisting insalubrious social conditions, poor personal hygiene, open air defecation and the practice of feeding pigs with human feces, all factors associated with social injustice and extreme poverty (4, 5, 6). The disease is reemerging in developed areas because of human migration (7, 8). Cysticercosis affects the health of millions of people and causes great economic loss in endemic areas worldwide (9). Besides its huge medical and veterinary impacts, *Taenia solium* disease poses many interesting social and biological problems for scientific study of general relevance for parasitic infections. It also offers varied opportunities to develop strategic technology to apply in controlling its transmission and palliate its effects on the health and economy of those affected, while in waiting for social development to finally arrive in the poor countries of the world.

The life cycle of *Taenia solium*

The larval phase (cysticercus) of the platyhelminth parasite *Taenia solium* may localize in the central nervous system (CNS) of humans causing neurocysticercosis (NC), a severe neurological disease (9, 10, 11, 8, 12) (Figure 1). Cysticerci (Figure 2)

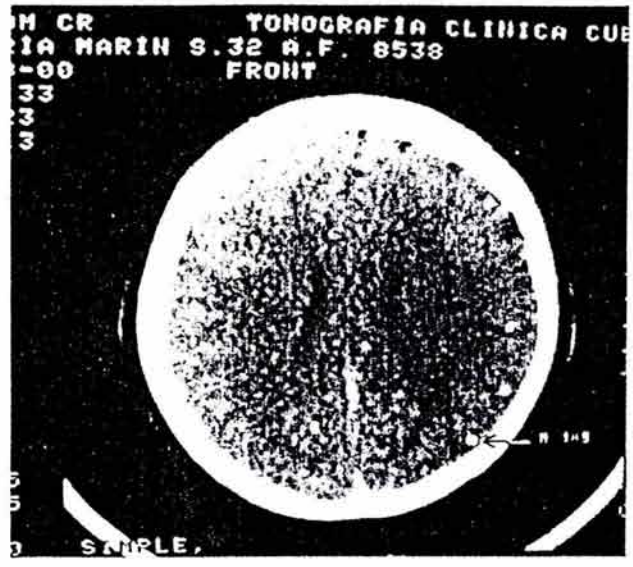

Figure 1. Computerized axial tomography showing massive cysticercosis with calcified cysts. In spite of the inactivity of the parasites, the patient presents a high frequency of convulsive seizures.

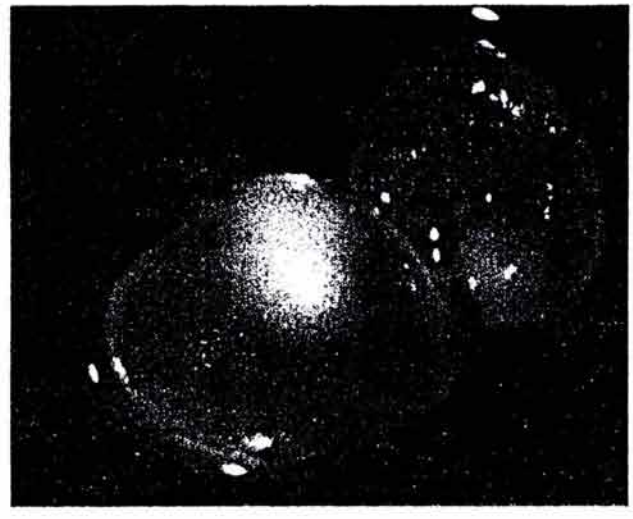

Figure 2. Macroscopic aspect of *T. solium* cysticerci obtained from an infected pig. Picture taken by Ivan Flores

may also infect pigs' skeletal muscle, causing major economic loss in already impoverished rural people of endemic areas. The adult parasite is a tapeworm that lives only in the intestine of humans, in whom the tapeworm may dwell for years causing minor disorders but producing thousands of eggs per day that are shed to the environment in the feces. Each egg has the potential of transforming into a cysticercus

when ingested by human or pig. Upon their ingestion, the eggs hatch and liberate free-swimming hexacanth embryos (one/egg) that penetrate the intestinal mucosa, circulate systemically, and eventually lodge and develop into cysticerci in various tissues. Many of the recently established cysticeri die and are absorbed or become calcified in the short term but some remain alive for indefinite periods (years, perhaps) inducing or not a variable degree of inflammation in its interface with the host (13, 14, 15, 16). For a cysticercus the human host is its terminal stage, as human cannibalism is seldom practiced today (3). In contrast, the porcine host offers the cysticercus with its only possibility of completing its life cycle, this when humans ingest improperly cooked cysticercotic pork meat (17). The pig is then an indispensable intermediate host for *T. solium* and humans are its only definitive hosts (Figure 3).

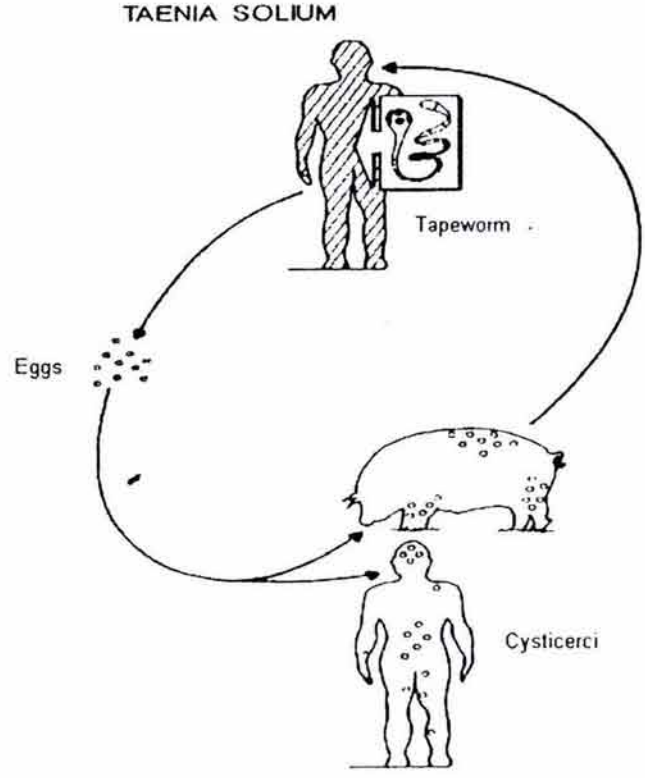

Figure 3. Life cycle of *Taenia solium*

Epidemiology

T. solium disease is highly distributed all over the world, and the manifestations are unequal in different continents. In Africa (Benin, Togo, South Africa, Malawi, Rwanda) (18, 19, 20, 21, 22) and Asia (Japan, Nepal, India, Indonesia) (23, 24, 25, 26, 27,28, 29, 30), as well as in European countries (Spain, France, Germany, Chec Republic, Greece, Italy) (32, 33, 34, 35, 36, 37, 38) there have been reported a lot of combined and separate

cases of NC and SC (subcutaneous cysticercosis). For example, in China the prevalence of SC in patients with NC varies from 54 to 78.5% (39, 40, 41), in a human population in New Guinea 17% of a sample had subcutaneous nodules caused by cysticerci (30, 41), in Togo the prevalence of subcutaneous nodules in patients with NC was 79% (42), and in India SC is more frequent than NC (43). Unlike what was reported in African and Asian countries, in Latin America SC is a less common and/or less documented manifestation. It has been reported that the frequency of subcutaneous nodules in patients with NC in Latin America is very low, and it has been estimated, for example, that in Colombia less than 6% of the patients with NC had subcutaneous nodules (44, 41). In a study based upon a *T. solium* hyperendemic population in Ecuador, just one patient (2.9%) out of 34 examined individuals presented SC (41). In Table I a bibliographic research (www.ncbi.nlm.nih.gov) is presented, which documents the differences in the number of case or study reports about NC and cysticercosis in other locations of the human body, between countries belonging to different continents (key words used: cysticercosis, neurocysticercosis, subcutaneous cysticercosis, cysticercosis Asia, cysticercosis Europe, cysticercosis Africa, cysticercosis Latin America, cysticercosis United States, cysticercosis developed countries). It may be noted that SC reports in America are practically nonexistent (in Brazil there have been cases of cysticercosis in the oral cavity

Table 1. Bibliographic research of cisticercosis reports in several countries

Country	Report	CC in CNS	SCC/muscular	Observations	Reference
Brazil	Study	38		Most frequent diagnostic depression 52.6%	114
Brazil	Study	151		Epilepsy 54.3%	115
Brazil	Case	1			116
Brazil	Case		7	Oral CC, strange kind of lesion	117
Brazil	Study	38/2522 (1.5%)		Autopsy	4
Mexico	Study	100			118
Mexico	Study	122		Mexican children	119
Mexico	Case	1		6 years old child	120
Mexico	Study	50/100 (50%)			121
Honduras	Study	85		In 6 years, in children	122
Honduras	Study	14/61 (23%)		With active NCC or antibodies in vesicular fluid	5
Peru	Study	8			123
Ecuador	Study	72		In rural and urban population, in relatives and children	124
Ecuador	Study	34	1 (2.9%)		41
U.S.A.	Study	15		Immigrant patients from Central and South America	125
Italy	Case	1		Possible immigrant	37
Italy	Case	1		Tourist	38
Spain	Case	4		India and Latin America immigrants	31
Greece	Case	1			36
Germany	Case	1		Ocular, Tourist in India and South East Asia	34
France	Study	29		Tourists, 1988-1999	32
France	Case	1			33
Czech Rep.	Case	8	2	6 Czech citizens and 4 immigrants	35
Togo	Study	5/2064 (0.24%)	33/2064 (1.59%)		19
Benin	Study	57/1443 (3.95%)		Epilepsy 22/1443 (1.52%)	18
Malawi	Case		1		21
S. Africa	Study	239		1975-1989	20
Rwanda	Case		5		22
Nepal	Study		62	Cutaneous nodules, oral mucosa and breast	24
India	Case	1			25
India	Study	25		Ocular, 1990-1998	26
India	Case	1	9	The one with NCC also presented SCC	27
India	Study	23/38 (60.52%)	18/38 (47.36%)	17 ocular, 6 NCC	28
Japan	Case	1			23
Irian Jaya	Study		142	Pectoral region, 1993	29
Irian Jaya	Study		42/242 (17.35%)		30
Australia	Case	2		India and Zimbabwe immigrants	7
Australia	Case	5		Tourists to undeveloped countries and immigrants	126

and in Ecuador it has been reported one case of SC), while in Asia and Africa are very common and frequently presented in combined cases with NC. Ophthalmologists in Mexico are familiar with ocular cyticercosis (10, 13).

The possible explanations on the encountered differences in manifestations between continents may be based on racial differences of the human host or to the genetic and antigenic diversity of the parasite in different continents (3, 9, 39, 45).

Risk factors

Transmission is propitiated by the rustic forms of pig rearing practiced by the poor in rural villages, where pigs are left to roam around the village in search of edibles in garbage, frequently ingesting human feces contaminated with *T. solium* eggs (Figure 4). Many of the resulting cysticercotic pigs are consumed locally to avoid confiscation at abattoirs or as a better alternative to selling them at low prices to clandestine meat markets. At the rural areas, low personal hygiene, poor sanitary conditions at households and lack of sewage disposal at villages coalesce to contaminate hands, foods and soil

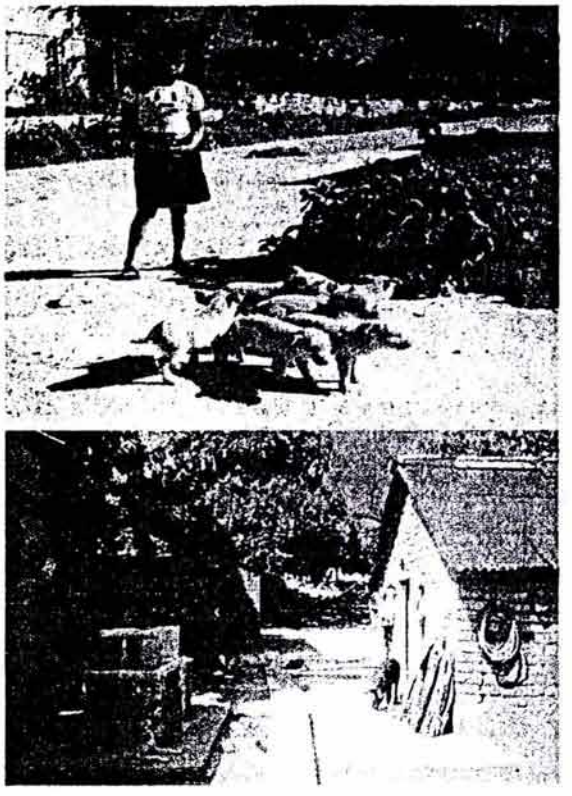

Figure 4. Village of Cuentepec, Morelos, México, showing the way pigs are reared in a rural ambience and their proximity to humans.
Photograph taken by Prof. Michel Dumas and Julio Morales

with *T. solium* eggs for humans and pigs to ingest (17). The parasite life-cycle can not be completed at urban areas because there are almost no roaming pigs and sewage is not as defective but, there being considerable rural-urban migration, domestic employment of rural people and out-of-home eating habits in low hygiene establishments, the eggs may find effective channels to become cysticerci in the human host.

The higher prevalence in the households of tapeworm carriers (17, 46, 47) and the spectacular outbreak of NC cases in a jewish family employing a latin-American cook (11) have have stressed the high risk of living in close proximity to a tapeworm carrier. Notwithstanding its obvious importance, it should not be minimize other mechanisms of egg dispersal and infection that operate in highly endemic situations with high rural/urban migration, touristic and laboral, eating-out habits indiscriminate of the establishment's sanitary conditions and food contamination by irrigation of fields with sewage discharges. The far reaching mechanisms of transmisssion are denounced by the high proportion (around 90%) of the serologically positive not clustering in households but ocurring singly (5) and by the high prevalence of positive serology in Mexico city, in urban conditions that do not favor the completion of the parasite cycle to become a tapeworm. Neglecting the importance of other than living in proximity with a tapeworm carrier may prove imprudent in highly endemic situations (46).

Control of transmission

Strategies to control transmission at the social level in endemic countries are mainly educational and aim to improve personal hygiene, safe eating habits and upgrade sanitary installations in the people's hygiene and homes (47, 48). Meat inspection programs were very effective in Europe in erradicating *T. solium* cysticercosis (49) and are now enthusiastically being designed in Asian countries recently reacting to the disease (50). In Mexico, and we presume that in many other countries afflicted by poverty and social underdevelopment, meat inspection programs have failed in controlling transmission, if they have not fostered it in reality. This because of the high costs of extensive sanitary coverage of huge rural areas, indolent or corrupt inspection practices, home slaughtering and clandestine marketing of infected meat to avoid confiscation at abattoirs. At the human population level, technical control measures aim to reduce egg production by extensive drug treatment of human tapeworm carriers (51, 52, 53, 54) or to prevent pig-cysticercosis in order to reduce the number of tapeworms developing thereof and to improve the quality of pork meat (55, 56, 57).

Therapy

There are a number of effective treatments against intestinal helminthes in the traditional medicine of many cultures (58) and there are also effective pharmaceutical drugs (59). The main problem in treating tapeworm carriers lies in finding them, the parasite causing so little discomfort, coproparasitoscopic studies being so insensitive and massive treatment so costly and intrusive of human rights. Antigen or DNA assays in feces are for the moment out of question because of lack of technical sophistication in most clinical labs of afflicted countries and their costs. Nonetheless, it has been recently developed an specific PCR based on species-specific oligonucleotides which permits the

differential detection of *Taenia saginata* and *Taenia solium* that could be a useful tool in the identification of tapeworm carriers if conditions allowed (60).

Against cysticercosis of both humans and pigs, praziquantel and albendasol are outstandingly effective (61, 62). In the treatment of human neurocysticercosis, when in combination with corticosteroid and anticonvulsive theraphy, they offer a less somber prognosis to those affected with severe forms of the disease (Figure 5, Figure 6), albeit not to all and not without significant neurological sequels in many (63, 64, 65, 66, 67) (Figure 7).

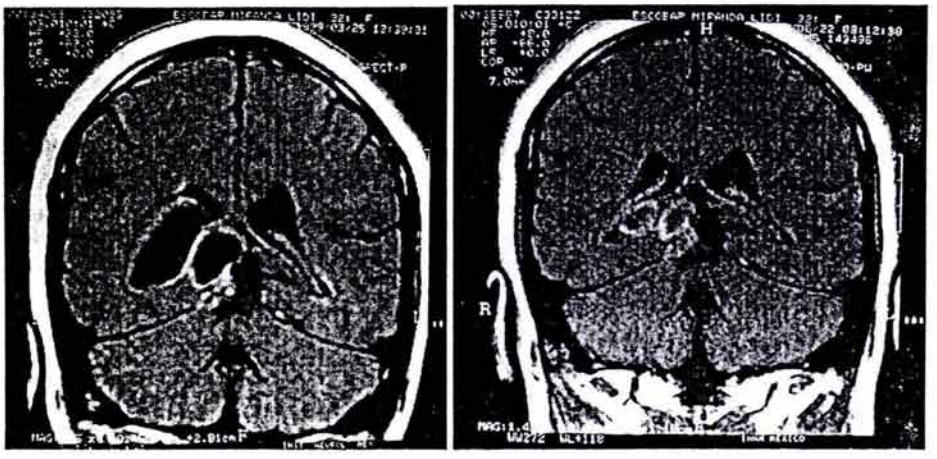

Figure 5. Nuclear magnetic resonance of a patient with sub-ependimal and sub-aracnoidal cyts before and after treatment with Albendazol.

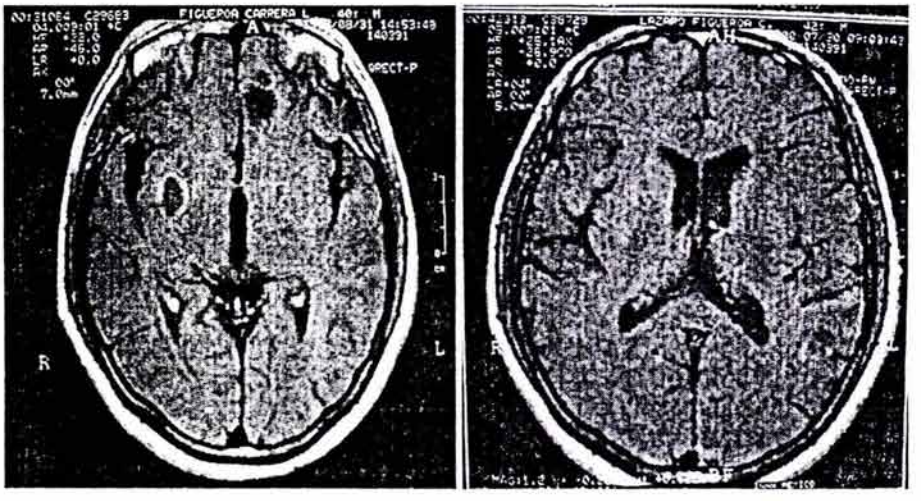

Figure 6. Computerized axial tomography showing different phases of parenchymal cysticerci before and after treatment with Albendazol.

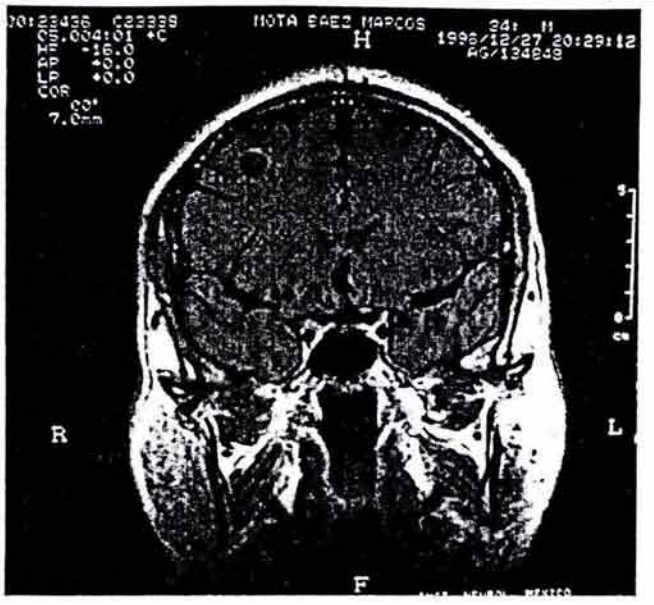

Figure 7. Nuclear Magnetic resonance showing a vesicular cyst with scarce contrast medium uptake.

Cysticercosis research program in studying *Taenia solium* disease

Our research program focuses on the study of this host-parasite relationship at the cysticercus stage in humans and pigs because this is the parasites most vulnerable stage to biotechnological strategies aimed at interrupting its life-cycle, and also because it is the stage that most seriously affects humans in different ways and levels of severity.

1. Immunodiagnosis of human neurocysticercosis

Human neurocysticercosis (NC) manifests through a great variety of symptoms and levels of severity, from sub clinical forms to headache, seizures and to severe intracraneal hypertension that endangers the patient's life (66). Sub-clinical cases are usually unawares of the disease and are found among the seemingly healthy population by epidemiological surveys (5, 45), at necropsy studies (13) and accidentally in radiological cranium studies indicated for other causes.

The vast heterogeneity in the symptoms of NC makes medical diagnosis impossible on clinical grounds alone: image studies of the cranium, such as Computarized Axial Tomography (CAT) and Nuclear Magnetic Resonance (NMR), are the gold standard for NC diagnosis in suspect cases (66, 67). NC should be suspected by medical personnel in endemic areas in patients with neurological complaints like chronic headache, seizures of late onset, and/or signs and symptoms of intracraneal hypertension (68). The high costs and scarcity of image technologies in endemic countries points to the convenience of designing less expensive and more accessible diagnostic procedures, like immunodiagnosis.

Numerous research groups have developed tests for immunodiagnosis of NC through the detection of antibodies or antigens in serum and/or cerebro-spinal fluid (CSF) (69, 70, 71, 72, 73, 74, 75, 76). We have tried to contribute to immunodiagnosis of NC by different means but have not succeeded in developing a stable and reproducible test with high sensitivity and specificity scores (74, 77, 78, 79, 80, 81, 82, 83,). This is the general experience in the field (5, 45). In spite of the early optimism of the authors, all tests eventually are recognized to miss a variable but significant number of NC cases and to yield false positive results (5, 83, 45). Some more recent efforts have reported better scores but have not yet been put to trial in more varied situations and demanding multilab tests (84, 68). We believe that the irregular and suboptimal performance of immunodiagnosis arise from the heterogeneity of the disease and from technical reasons. The main technical causes of variability and reproducibility are the differences among the antigens being used, the majority of which come from antigens obtained from parasites dissected from naturally infected pigs or from other hosts infected with closely related parasites (85, 86, 77, 75). This source of antigens implies heterogeneity associated to the different parasites that infected the different donor pigs (87), and possibly to the different phases of development of the cysticerci at the time of collection. Additionally, the different panels of sera used from patients as true positive controls are very heterogeneous clinically and so are the panels of sera used as putative, but non-certified, negative controls (i.e., lack of neurological symptoms or negative CT scans of the skull do not discard cysticercosis affecting other tissues inducing the production of antibodies as well as liberate antigen to the circulation). The high proportion of false positive results possibly reflects a sum of factors, like cross-reacting antigens among helminths (78), cysticercosis located in tissues other than CNS (88), resolved infections (89) and history of exposure without infection (90). In our experience and that of others the results of immunodiagnosis are more encouraging in CSF, in which the detection of antibodies turns out to be a more reliable tool for clinical diagnosis with sensitivity and specificity at about 90% (70, 72, 91). However, the problems of reproducibility remain because of the complexity and heterogeneity of antigens used (78). Serology might just be useful in detecting contact with the parasite but not prove to be reliable in NC diagnosis.

More recently, our research group as well as others are testing new biotechnological strategies to identify the best performing antigenic epitopes and prepare a more uniform antigen cocktail to use in immunodiagnosis of NC (92, 93, 81, 82). The results with phage display are encouraging since some peptides have been identified that are very frequently recognized by NC individuals (93) but more extensive studies are needed before making a final statement and approach industrial production.

2. Heterogeneous susceptibility to the infection and disease

2.a. Relevance of factors associated to exposure

A considerable number of epidemiological studies on taenosis/cysticercosis by *Taenia solium* have been carried out during the last 20 years. Data of prevalence indicate to the endemic character of this parasite in developing countries in Latin America, Asia and Africa, whilst in Europe, Australia, USA and Canada only a few isolated autochthonous cases are reported (11, 94). The epidemiological studies consider as risk factors all those that favor the establishment and the progression of the parasite's life

cycle. These are mainly associated to extreme poverty (non-existing or inadequate sanitary conditions, malnutrition, lack of education, low income, margination from the national health services, open air defecation, close relation with tapeworm carriers, frequent out of home eating habits) and the practice of rearing pigs rustically with organic rubbish, including human feces (17).

The use of serologic essays in epidemiological studies aims to identify the individuals that have been in contact with the parasite (95, 78, 96, 97). Considering that the higher the exposure to the parasite the higher it is the probability of infection, has led to overemphasize the risk of living with a tapeworm carrier. This conjecture is supported by serological data showing higher prevalence of sero-positive individuals living close to tapeworm carriers than the in the open-population, and to the spectacular finding of autochthonous NC in a Jewish family of New York that hired a Latin-American cook carrying a tapeworm (94). However, the importance of the extensive environment pollution in endemic areas or out-of-home eating habits should not be minimized, especially in high population density areas of endemic countries with high rural inmigration, as they may explain why 90% of all seropositive cases do not group by households but are singularly distributed in each family household (78).

2.b. Relevance of factors associated to the host

In spite of the difficulties that are incurred in experimentation with pigs, studies on porcine cysticercosis provided us with an important approach to evaluate the role of gender and genetic background of the pigs in cysticercosis by *Taenia solium*.

Our studies on murine experimental cysticercosis caused by *T. crassiceps* demonstrated the relevance of the sexual and genetical factors upon parasite intensity: females are manifestly more susceptible than males to parasitosis (98, 99, 100) and the Q9 gene that codifies for a non-classical antigen of the MHC associates with resistance (101). Thus, during a prevalence survey on porcine cysticercosis in the rural community of Cuentepec, Morelos, 1,084 out of the total 1,300 pigs of the community were classified according to their gender and reproductive state (whole/castrated males, non-pregnant/pregnant females), and inspected for tongue cysticercosis. Results were rather staggering: castration doubled the prevalence in males from 23 to 50% and pregnancy increased prevalence from 28 to 59% (102). As for the role of the genetic background of pigs, in genetically heterogeneous pigs our results in controlled experimental infection indicate that there is an overdisperse distribution of parasite intensities: a significant fraction of the infected population (20%) presenting 3-5 times higher amounts of parasites than the remaining population, putatively attributed to different innate susceptibilities among the pigs (103). Moreover, in a recent study of piglets sired by 3 different studs and 20 different females, that were homogeneously exposed to natural infection in a rural community, it was found at necropsy that 80% of the cases grouped in a single stud family, thus strengthening the notion of genetic factors involved in pigs susceptibility to *T. solium* (personal comunication).

The extensive distribution of the parasite in endemic countries, with higher concentrations in rural areas that propitiate the completion of the parasite cycle, is recognized in several studies (5, 17, 91), and yet the number of NC cases is not overwhelming (45, 78). This suggested to us that even though many individuals are exposed to the parasite, only a few develop the infection and much fewer the disease: an indication of biological factors associated to the hosts susceptibility and resistance

participating in both acquiring the infection and in developing the disease. In support of this notion are: the involvement of gender in the severity of NC in at least one of its clinical presentations (women present encephalitic forms more frequently than men (104), and the atypical distribution of some HLA antigens in NC cases, hinting to the participation of genetic factors (105). However, neither hospital case studies nor epidemiological studies have focused systematically on the search for biological factors of the host or the parasite associated with prevalence or severity of NC.

We moved to evaluate the relative importance of biological and exposure factors of the host in the prevalence and severity of NC. An NC epidemiological study was performed in a rural community of the State of Puebla (Tepetzetzintla) with a clear cysticercotic profile and high prevalence of pig cysticercosis. In a random sample that included 155 persons, we found a prevalence of 9.1% NC cases by CT scan, all without neurological symptoms. When evaluating the role of traditional risk factors for cysticercosis, we found that none of them associated to NC (45). These findings show that even though exposure to the parasite is necessary for challenge, in highly endemic situations some other factors independent of exposure participate in the probability of infection and in the severity of the disease.

2.c. Relevance of immunological factors

The majority of immunological studies relative to NC have been performed with the purpose of improving NC diagnosis, more so than with the aim of understanding the immunologic factors comprised in its pathogenesis. About the humoral immune response, it is widely known that the majority of patients present circulating antibodies albeit its relevance in the clinical status remains to be dilucidated. The antibody response is highly heterogeneous in terms of levels of specific immunoglobulins detected both in CSF and sera and in terms of specificity regarding the differential reactivity detected in the recognition of complex mixture of cysticercal antigens in Western Blots (78). There is a significant fraction that does not present antibodies at all at the time of study (80). We tentatively attribute the lack of antibodies in NC cases to cysticerci being calcified or, when alive, to the effects of corticosteroid treatment usually employed. An interesting alternative explanation for lack of anticysticercal antibodies in NC cases would imply innate immunity failing to emit a danger signal necessary to trigger the immune response (107): it is well known from pathological studies that many established cysticerci are surprisingly free of inflammatory infiltrate (16, 14, 15). Even though the inflammatory response associates with symptomatic NC, the cellular immune response in NC had not been explored in a systematic way until recently. The available information is summarized in Table 2, which includes all the reports known to us in the study of the immune response related to human NC. As it shows, studies are still incomplete and spotty (gathered from varied epidemiological, clinical and geographic samples) to support a solid statement about the relevance of the immune response of humans with the state and outcome of their host-parasite relationships with *T. solium*, but the notion is forming that an immune response that associates with proinflammatory cytokines relates with severe NC whilst a balanced TH1/TH2 response results in protection against infection and/or destruction and calcification of the established parasite, with less severe clinical repercussions. However, full understanding of the role of immunity in susceptibility and severity of NC will need of a study design

Table 2. Synopsis of the reports studying the immune response in human neurocysticercosis

NC (# cases)	Biological Material	Immunological Features	Reference
Parenchymatous (17)		Intrathecal specific IgG, IL1β, soluble IL2R and neopterin declined after treatment with praziquantel.	127
Subarachnoid (55)		Increased IgG, IgM, IgE, IL1β and IL6 IgA, TNFαand IFNγ similar values to those of controls.	128
Multiple (14), single (14))		Lower level of chemotaxis in multiple than in single lesions Normal level of CD4+ and CD4+/CD8+.	129
Multiple (12), single (2)	Sera/CSF	Eotaxin, IL5 but not IL8 were elevated in sera, IL5 and IL6 levels were also elevated in CSF.	130
Parenchymatous (2) leptomeninges(12)	Brain specimens	IgM+ plasma cells, predominant NK response, an infiltrate with macrophages, granulocytes and T cells. IL12 was predominant followed by INFγ, IL6 and IL10; IL4 was undetectable.	131
Ventricular (2), parenchymatous(6)	CSF/PB	CD69+% was higher only in CSF from patients. CD3+ % similar in CSF and PB cells from NC and controls. Increased CD8+% in 3 patients with inflammatory NC. CD45+CD19+% was higher in CSF,from patients with anti-cysticercus antibodies in CSF.	132
Multiple (15)	PB	Increased in vitro cell proliferation, decreased CD8+, higher levels of Th1 cytokines (IFNγ and IL2) and no difference in IL4 in NC patients.	133
Vesicular(22),calcified(13)	CSF	Higher IL5 levels in active NC. Higher IL5 and IL10 in inflammatory patients.	134
Vesicular and calcified (6)	CSF	Higher TNFα in children with active NC. Increased IL6 in subarachnoid cases.	135
Vesicular (37).	PB	Similar CD3, CD4, and CD8%, specific lymphoproliferation and mRNA cytokine levels (IL2, IFNγ, IL10, and IL-4).	136
Single vesicular (2), multiple vesicular and calcified (8)	Brain sections	Numerous mast cells. The tryptase mast cells phenotype dominated over the tryptase-chymase phenotype. The tryptase phenotype infiltrated mainly meninges and brain parenchyma around cysts with viable and necrotic parasites. The tryptase-chymase phenotype infiltrated perivascular area of the blood vessels penetrating to the depth of the brain.	137
(8)	Brain specimens	A dying parasite surrounded by a granuloma with associated fibrosis, angiogenesis, and an inflammatory infiltrate. Plasma cells, B and T lymphocytes, macrophages, and mast cells were the most abundant cell types. Th1 cytokines were prevalent (IFNγ, IL18 and TGFβ), the Th2 cytokines (IL4, IL13 and IL10) were also present.	138
Calcified (4), multiple vesicular and calcified (6)	PB	Higher specific proliferation with a glycoprotein or vesicular fluid in NC patients than controls. 80% of the patients had antibodies against the glycoprotein of the cestode.	139
(79)	Sera / PB	NC patients recognized the carboxyl end region of the paramiosin, poor recognition of the central and amino regions. The cellular immune response of patients did not show preferential recognition of any region of paramyosin.	140
Multiple (3), calcified (4), vesicular (4)	PB	Decreased lymphoproliferative response in inactive NC.	132
(5)	Brain specimens	Increased pro-inflammatory (IFNγ and IL18), anti-inflammatory cytokines (TGFβ and IL10) and an up-regulation of MHC II molecules. Active wound-healing process reflected by angiogenesis, collagen deposition and glial scar formation.	141

PB (peripheral blood mononuclear cells), CSF (cerebral spinal fluid).

comprehending the principal clinical categories of the disease and the use of longitudinal studies with a representative panel of immunological and inflammatory indeces.

2.d. Relevance of factors associated to the parasite

The possible differences among individuals of the *Taenia solium* species have received little attention. Parasite heterogeneity could well entail different infectivity and pathogenicity. Considerable antigenic diversity was evident since 1982, even when using immunological tools of low resolution (87). We are now doing antigen taxonomy using WB and finding that indeed there is considerable antigenic variation within *T. solium* species (personal communication). In addition, the heterogeneity of the parasite population is being studied in cysticerci collected in Central and Southeast of Mexico using "RAPDS" (Random Amplified Polimorphic DNA assay technique). A first level analysis suggests that the parasites of Mexico present many differences in its genome at high level of similarity within geographic regions but there is some degree of differentiation between regions (108). More research is necessary to determine the relevance of the parasite heterogeneity observed as an important factor in the clinical heterogeneity and severity of NC.

3. Prevention

The high frequency of cysticercosis by *Taenia solium*, its severe consequences in human health and its economic repercussions wholly justify the attempts to prevent it. Different strategies for this purpose based on social development (48, 50) and/or biologic interventions have been considered (51, 55, 109, 110, 54). Among them, our research has focused on the development of an effective and stable vaccine against porcine cysticercosis. Vaccination of the pig only (intermediate host) could hinder the transmission cycle of the parasite and thus reduce the infection pression upon humans and improve the economy of rural pig rearing.

We have recently developed a vaccine (S3Pvac) constituted by three peptidic epitopes that confer protection against murine cysticercosis, called GK-1, KETc1 and KETc12, of 18, 12 and 8 amino acids respectively. These peptides derive from a group of proteins in a related murine cysticercus (*Taenia crassiceps*) and are shared by *T. solium* (111, 112). S3Pvac was evaluated in its protective capacity against porcine cysticercosis by *T. solium* in pigs rustically reared and exposed to natural challenge in a rural village of Mexico. S3Pvac with saponine as adjuvant was applied to a group of 240 pigs (120 controls, 120 vaccinated). At necropsy, vaccination had reduced 50% the number of infected pigs, and from 66,563 to 1,369 the total number of recovered cysticerci and had damaged 80% of the few cysticerci that had managed to establish in the pigs in spite of vaccination (113). We concluded that S3Pvac appears to be a useful tool to prevent pig cysticercosis and perhaps reduce the infection pressure upon the human population of endemic areas. At the present moment we are evaluating different possibilities to optimize its production and immunogenecity at low-cost, through the expression of the protective peptides in filamentous phages, transgenic plants and vectors of bacterial origin, among other approaches (56).

Perspectives

We have presented a brief description of the advances most recently attained in our research of *Taenia solium* cysticercosis, and our contributions and positions resulting from a multidisciplinary, multinstitutional and multinational effort in the study of *Taenia solium* cysticercosis of humans and pigs in Mexico. We now better know where and how it prevails, how costly it is in human suffering and pig husbandry, its many clinical facets and suspect the involvement of immunoinflammatory and endocrinological factors as well as genetic background of the host in modulating susceptibility and pathogenesis. We have developed a synthetic peptide vaccine proven effective vaccine against realistic natural pig cysticercosis and these are hints that it may work in humans. Some advances have been made in immunodiagnosis. However, the job is far from finished. We expect our ongoing projects will enable us to better understand the immunopathology of the disease as well as the relevance of its genetic components and sexual factors, develop better serological technology and understand the pathogenic strategies of the parasite *T. solium*, that most neglected of the actors involved in this parasite infection.

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