UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO

INSTITUTO DE INVESTIGACIONES BIOMEDICAS

APORTACIONES DE LA CISTICERCOSIS MURINA EXPERIMENTAL POR *Taenia crassiceps* AL CONOCIMIENTO DE LOS FACTORES BIOLOGICOS QUE PARTICIPAN EN LA SUSCEPTIBILIDAD A LA INFECCION POR METACESTODOS Y AL DIAGNOSTICO Y PREVENCION DE LA CISTICERCOSIS POR *Taenia solium*.

Tesis presentada por Edda Sciutto para optar por el grado de Doctor en Ciencias Biomédicas en el Proyecto de Licenciatura, Maestría y Doctorado en Investigación Biomédica Básica del Colegio de Ciencias y Humanidades de la UNAM.

México, 1990

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I. RESUMEN

El trabajo reportado en esta tesis identifica como factores criticos en la instalación y desarrollo del parásito (cisticerco) en su hospedero (ratón) a factores sexuales, genéticos, inmunológicos y a los asociados al compartimento en que el parásito se desarrolla. Estos estudios se han realizado utilizando el modelo experimental de cisticercosis murina por Taenia crassiceps. Este modelo ha permitido explorar sistemáticamente la complicada red de eventos que se suceden en el fenómeno de la infección parasitaria que termina en una relación de cierta convivialidad entre la especie parásita y la especie hospedera.

Las observaciones y resultados reportados en esta tesis consolidan la propuesta de considerar a T. crassiceps como un modelo de cisticercosis por Taenia solium. A pesar de las limitaciones propias de un modelo experimental de infección y la diferencia entre ambos céstodos, el modelo de cisticercosis por T. crassiceps ofrece la esperanza de generar caminos propicios en el estudio de la relación entre el cisticerco de la Taenia solium y sus hospederos naturales.

Además, en esta tesis se reportan los estudios que demuestra una extensa antigenicidad e inmunogenicidad cruzadas entre T. crassiceps y T. solium, lo que ofrece la posibilidad de utilizar los cisticercos de Taenia crassiceps como una fuente alternativa de antigenos en el diagnóstico y la prevención de la cisticercosis humana y porcina.

Este trabajo intenta contribuir al conocimiento de la relación hospedero-parásito en la cisticercosis, tanto en el inmunodiagnóstico epidemiológico e individual como en la fenomenología molecular y celular que subyace en esta forma de convivencia biológica entre dos organismos.

II. INTRODUCCION

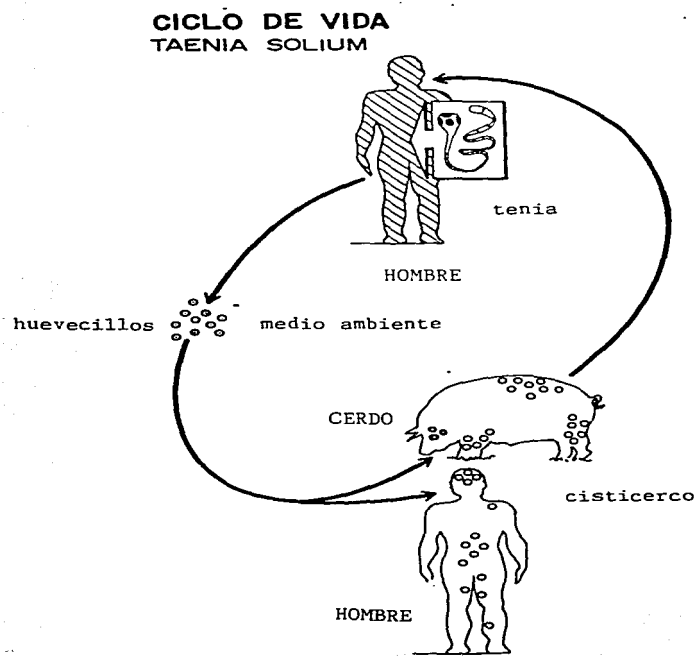


Figura 1. Ciclo de vida de la Taenia solium.

La cisticercosis causada por Taenia solium es una enfermedad parasitaria que afecta principalmente al hombre y al cerdo. Cuando individuos de alguna de estas dos especies se infectan con huevecillos de Taenia solium, los huevecillos se diferencian a metacestodos (cisticercos) en algún compartimento del hospedero. El ciclo del parásito se completa cuando el hombre come carne de cerdo parasitada con cisticercos, estos llegan al intestino delgado, y allí se desarrollan y dan lugar a la forma adulta del parásito. Por reproducción sexuada el parásito adulto produce millones de huevecillos los que son expulsados al medio ambiente en las heces fecales del hospedero (Figura 1).

FRECUENCIAS DE LOCALIZACION DE CISTICERCOS EN 2188 CASOS EN LATINOAMERICA

TABLE VII. Location of the Parasite in 2188 Cases of Cysticercosis in Latin America[a]

Location[b]	*Number*	*%*
Encephalon	*1719*	*78.6*
Spinal cord	*72*	*3.3*
Eye and annexes	*368*	*16.8*
Subcutaneous tissue	*159*	*7.3*
Muscle	*79*	*3.6*
Heart	*31*	*1.4*
Other	*124*	*5.7*
Generalized	*23*	*1.1*

[a] *Data taken from Refs. 2, 8, 16–19, 21–23, 27, 29, 32–34, 38, 42, 46, 52–63.*
[b] *Some patients had larvae in more than one location.*

Tabla I. Schenone et al, 1982.

La cisticercosis humana y porcina constituyen un serio problema socioeconómico en países de Asia, Africa y Latinoamérica.

Las consecuencias patológicas de la cisticercosis dependen principalmente del sitio de alojamiento del cisticerco, de la carga parasitaria y de la reacción del hospedero al parásito.

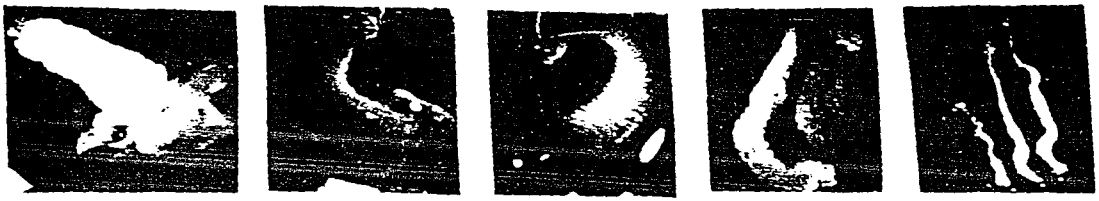
En el humano la forma más grave y aparentemente más frecuente de enfermedad es la neurocisticercosis (Tabla I), la que se establece cuando la larva se aloja en el sistema nervioso central. La neurocisticercosis es un problema de salud frecuente en México con grandes implicaciones sociales y económicas. El impacto económico causado por la teniasis-cisticercosis se extiende a la porcicultura donde genera grandes pérdidas por decomiso de carne parasitada.

Los estudios de la transmisión de esta enfermedad se han concentrado al análisis de factores sociales, económicos y culturales - que tienen evidentemente un rol fundamental en la persistencia de su ciclo - pero se han descuidado los factores biológicos que afectan su transmisión.

Debido a que la cisticercosis por T. solium afecta al hombre y al cerdo, resulta muy dificil y costoso el análisis sistemático de los factores biológicos que participan en la susceptibilidad del hospedero al parásito.

En este sentido nos ha interesado conocer las estrategias de convivencia que se establecen entre dos organismos complejos, y los fenómenos biológicos que subyacen en la determinación de las condiciones más propicias y las más adversas para convivir. El conocimiento de la intimidad de esta relación podria ofrecer alternativas para modificarla exacervando aquellos fenómenos asociados a la resistencia.

LARVAS EVAGINADAS DE Taenia solium A DISTINTOS TIEMPOS DE CULTIVO (ASPECTO Y TAMANO)



24 hrs | 3 días | 12 días | 15 días | > 20 días

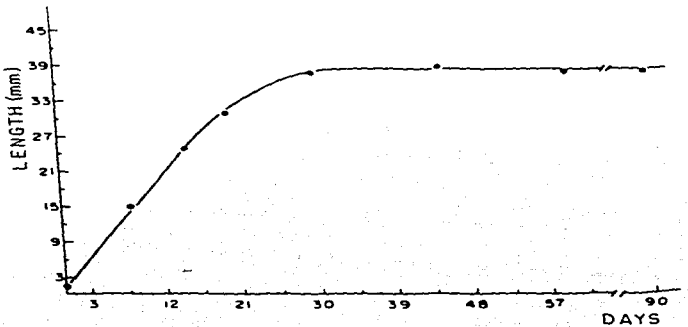


Figura 2. Morales, E. and Canedo, L., 1982

Existen muchas estrategias para abordar el estudio experimental de la cisticercosis, las que varian en las fases de la infección que reproducen y el tipo de información que generan. Entre ellas las técnicas de cultivo de parásitos "in vitro" permiten estudiar efectos de intervenciones experimentales directamente sobre el parásito, pero aislan a los cisticercos de su nicho ecológico. Por otra parte, las estrategias "in vivo" adolecen de falta de detalle molecular, dada la complejidad de las redes interactuantes de un organismo, si bien el significado biológico de estos experimentos pareceria más relevante.

Como ejemplo de la pérdida de contexto biológico en los experimentos "in vitro", consideremos el fenómeno de evaginación que ocurre en la mayoria de los cisticercos después de 3 o 4 dias de separados de su hospedero. Solo un pequeno porcentaje de los cisticercos permanece como tal en el cultivo, mientras la mayoria de ellos evagina (Laclette, comunicación personal) y la larva evaginada crece hasta alcanzar un máximo de 3 o 4 cm (Figura 2). Mientras tanto si los cisticercos hubieran permanecido en su hospedero intermediario, la gran mayoria de ellos hubiera permanecido como tal indefinidamente hasta ser ingeridos por el humano y diferenciarse al estado de tenia. Estas observaciones permiten suponer que el cambio de ambiente de los cisticercos dispara senales de diferenciación que modifican el destino natural de estos parásitos. Asi, el mantenimiento de los cisticercos en cultivo, resulta más atractivo como modelo de diferenciación que como modelo para el estudio del parásito en la fase de cisticerco.

MODELO EXPERIMENTAL DE TENIASIS

POR Taenia solium EN HAMSTERS

(Diferencias en eficiencias de infección con distintos tratamientos inmunosupresores del hospedero)

TABLE 4 Comparative susceptibility of male and female hamsters to infestation with *T. solium*

Treatment	Dosage (mg)	Males		Females	
		No. infested	No. positive (%)	No. infested	No. positive (%)
Imuran	5,0	5	40,0	5	20,0
Imuran	2,5	5	60,0	5	40,0
ALS + Depo-Medrol	5,0	19	89,5	29	89,7
ALS + Betnesol	0,025	8	62,5	10	40,0
ALS + Imuran	5,0	4	50,0	5	40,0
Total	—	41	70,7	54	64,8

Tabla II. Vester, A., 1974

Respecto a la instalación de Taenia solium (cisticerco y/o tenia) en animales de laboratorio; los resultados más alentadores distan aún de establecer a estos métodos como las formas más apropiadas para reproducir el desarrollo completo del parásito, según ocurre en las condiciones naturales de transmisión.

Uno de los modelos experimentales de Taenia solium consiste en la instalación del parásito adulto en hamsters inmunodeprimidos. Los primeros intentos exitosos fueron realizados por Verster (1971, 1974) en hamsters dorados previamente inmunosuprimidos químicamente (Tabla II) o por irradiación. Después de 10 días de infectados por vía oral se recuperan tenias con proglótidos estériles del intestino delgado del hamster. En la Tabla II se ilustra la eficiencia de infección utilizando diferentes drogas inmunosupresoras en distintas dosis y combinaciones para suprimir al hospedero y facilitar la instalación de la tenia. Este modelo permite estudiar una fase crítica en la diferenciación del parásito, como es la transformación del cisticerco en tenia aunque las reglas que rigen esta relación son probablemente diferentes a las que rigen la relación entre el cisticerco y el hospedero (distinta fase del parásito, distinto compartimento del hospedero).

PROTECCION POR VACUNACION FRENTE AL DESAFIO DE RATONES CON ONCOSFERAS DE Taenia solium

Table 1 Number of Cysticerci (larva of *Taenia solium*) in lung and liver from mice immunized with oncospheral antigens and challenged with 736 *Taenia solium* oncospheres by intravenous route.

Dosis (ug)	No. of cysticerci					Parasitized mice	
	Lung	Liver	Total	X	Range	No./Total	%
Control	24	1	25	4.16	2-9	6/6	100
15 (O)	0	0	0	0.0	0-0	0/6	0
25 (O)	0	0	0	0.0	0-0	0/6	0
15 (OB)	0	2	2	0.33	0-2	1/6	17
15 (S)	2	1	3	0.5	0-2	2/6	33
25 (S)	2	0	2	0.33	0-2	1/6	17

All results had a high statistical significance $P < 0.005$.
O = Antigens from isolated oncosphperes.
OB = Antigens from the oncospheres and blocks fraction.
S = Antigens from the sediment fraction (containing: complete eggs, scarce oncospheres and blocks)

Tabla III. Molinari et al (1988)

Entre los intentos de instalar al parásito T. solium en el estado larvario de cisticerco, cabe destacar a un modelo murino publicado recientemente por Molinari y col. de gran interés. Los autores desafian ratones con oncosferas obtenidas a partir de huevecillos de Taenia solium, procedentes de tenias recuperadas de pacientes tratados con niclosamida. Los ratones son desafiados por vía endovenosa con 736 oncosferas por ratón, y después de un mes los ratones son sacrificados. La carga parasitaria es cuantificada macroscópicamente por recuento del número de larvas implantadas en los distintos tejidos (Tabla III). La implantación de los parásitos ocurre casi exclusivamente en pulmón en un promedio de 6 larvas por ratón. Si bien las larvas no alcanzan a desarrollarse completamente, como se observa en su hospedero intermediario natural, el 100% de los ratones infectados presentan implantación de larvas en un promedio de 4 por ratón y esta implementación es susceptible a modificarse a través de inmunización previa del hospedero con antigenos del parásito como se observa en la Tabla III. El empleo de este modelo se encuentra limitado a la disponibilidad de las oncosferas de Taenia solium, lo que implica un alto grado de dificultad y riesgo en su utilización, pero sin duda es de sumo interés buscar las condiciones que lo optimicen.

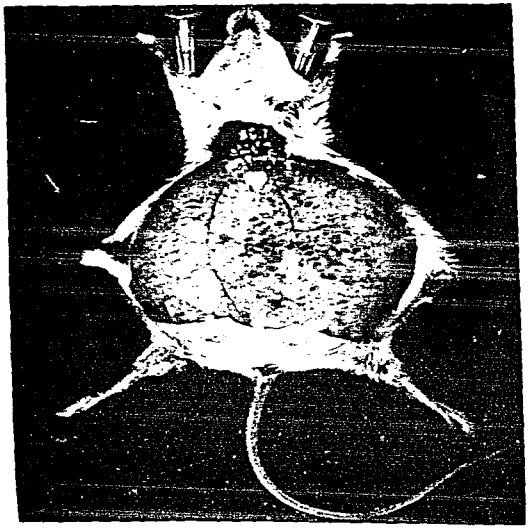


Figura 3. La Cisticercosis Experimental Murina causada por Taenia crassiceps.

Intentando avanzar en el conocimiento de los factores biológicos más prominentes en la relación cisticerco-hospedero, y ante las limitaciones para instalar la cisticercosis por Taenia solium experimentalmente decidimos estudiar otro cestodo (Taenia crassiceps) parásito natural del ratón (Fig. 3). Según lo reportado en la bibliografía, esta cisticercosis murina presenta características atractivas para ser considerado como un buen modelo experimental, comenzando por su capacidad de crecer en ratones, especie muy estudiada genéticamente, y por crecer óptimamente en peritoneo, compartimento muy accesible a estudiar y a ser modificado. En la figura 3 se ilustra el aspecto de un ratón infectado intraperitonealmente con cisticercos de Taenia crassiceps y puede observarse la enorme capacidad para alojar parásitos de este compartimento. En principio este trabajo se concentró en estudiar el crecimiento de los cisticercos en ratones y en recopilar la información necesaria para establecer las similitudes y diferencias entre ambos parásitos así como entre la cisticercosis por Taenia crassiceps y la cisticercosis por Taenia solium.

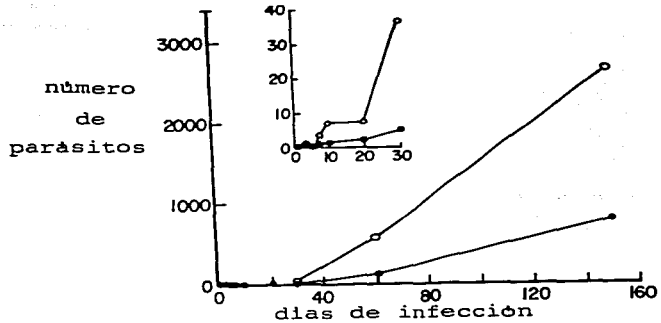


Figura 4a. La gráfica ilustra el crecimiento de la carga parasitaria (número de parásitos recuperados en peritoneo) después de distintos tiempos de infección de ratones BALB/c hembras (o) y machos (.) infectados con 10 cisticercos por ratón. Cada punto resulta del promedio del número de cisticercos obtenido en 10 ratones.

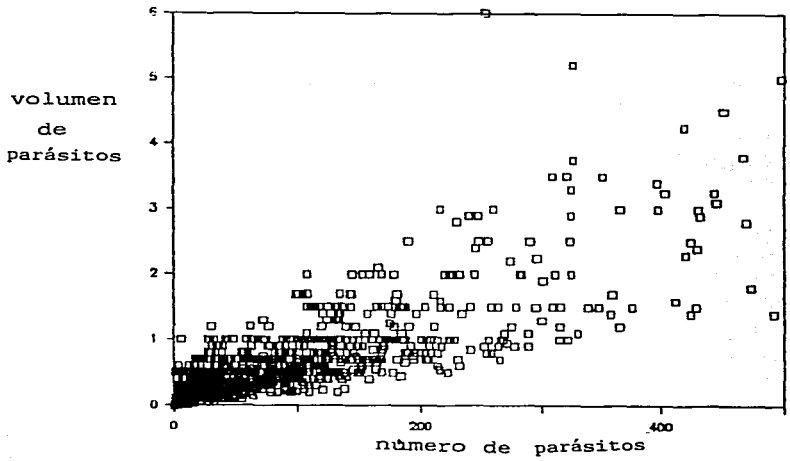


Figura 4b. Correlación entre dos parámetros utilizados en la cuantificación de la carga parasitaria (datos no publicados obtenidos en el laboratorio del Dr. Larralde).

Respecto a su crecimiento, el cisticerco de Taenia crassiceps se divide de manera sexuada y asexuada por gemación múltiple. Infectando intraperitonealmente el ratón con algunos parásitos podemos recuperar cientos de cisticercos en unas cuantas semanas.

En la Figura 4a vemos cómo, después de la inoculación de 10 cisticercos de T. crassiceps en la cavidad peritoneal del ratón obtenemos cientos de cisticercos en unas cuantas semanas. Además, los cisticercos son fácilmente cuantificables macroscópicamente por conteo individual de los mismos o por medición del volumen que ocupan. Estas variables presentan un alto índice de correlación, y pueden ser utilizadas indistintamente cuando se trata de medir altas cargas parasitarias. En la Figura 4b se observa la relación entre estos parámetros utilizando cientos de valores experimentales que hemos obtenido en el laboratorio.

El principal atractivo de este modelo radica en que es el ratón una de las especies hospederas naturales de T. crassiceps y su fisiología, así como su genética es la más conocida de los mamíferos. Existe además disponibilidad de cepas singénicas, congénicas y recombinantes en porciones del genoma de gran interés inmunológico, como es el Complejo Mayor de Histocompatibilidad (H-2) y la posibilidad de modificar su genoma creando ratones transgénicos.

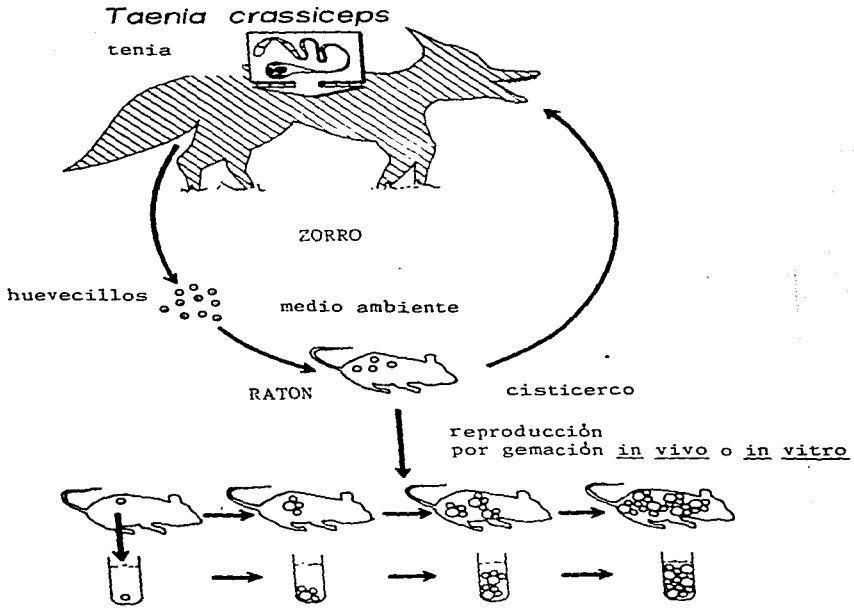
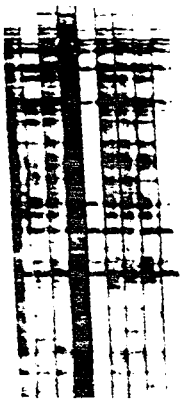


Figura 5. Ciclo de vida de Taenia crassiceps

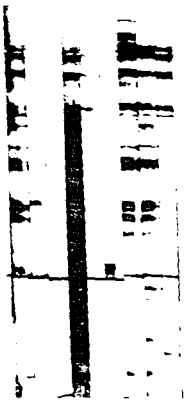
Figura 6. Imagen de la forma gemante de un cisticerco de Taenia crassiceps (ORF) en microscopio esteroscópico (cortesía de la Dra. Teresa Rabiela).

Comparando los ciclos de vida de T. crassiceps con T. solium (Fig. 1 y 5) podemos observar que en la fase sexuada de reproducción ambos requieren de un hospedero intermediario (porcinos o roedores) y un hospedero definitivo (humanos o cánidos y felinos). Los cisticercos y tenias de ambas especies se parecen en su estructura macroscópica, si bien tanto los cisticercos como la tenia de Taenia crassiceps son más pequeños. Una diferencia notable pero muy ventajosa experimentalmente es que, además de la forma sexuada de reproducción, los cisticercos de Taenia crassiceps tienen la capacidad de dividirse por gemación polar múltiple como se ilustra en la Figura 6. Así, un cisticerco emite gemas que crecen (Fig. 6) y se separan del cuerpo gemante dando lugar a una población muy numerosa de cisticercos en poco tiempo. Esta forma de división permite mantener tanto in vivo (Freeman, 1962) como in vitro (Taylor, 1963; Siebert and Good, 1979) a los cisticercos. In vivo, los cisticercos crecen y se multiplican óptimamente en la cavidad peritoneal de ratones, lo que permite un fácil acceso experimental a la relación hospedero-parásito. In vitro, si bien su crecimiento es mucho mas lento, pueden también mantenerse durante un par de meses en los medios de cultivos convencionales.

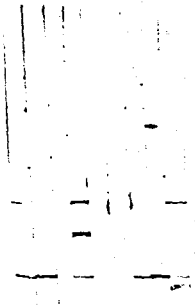
ANTIGENOS DE FLUIDO VESICULAR DE CISTICERCO
DE Taenia crassiceps



Suero de ratones cisticercosos (T. crassiceps)



Suero de humanos neurocisticercosos (T. solium)



Suero de humanos normales

Figura 7. Datos obtenidos en el laboratorio del Dr. Larralde por Rosa Ma. Montoya.

En el intento de identificar similitudes y diferencias entre ambos cestodos estudiamos el repertorio de antigenos reconocidos por sueros humanos de neurocisticercosos (T. solium) confirmados y sueros de ratones cisticercosos (T. crassiceps) utilizando como fuente de antigenos el liquido vesicular del cisticerco de T. crassiceps e identificandolos por inmunoelectroransferencias. En la Figura 7 puede constatarse la gran reactividad cruzada entre los antigenos de ambos parasitos.

Peso Total de ratones parasitados (grs)	Peso de ratones (grs)	Peso de la masa parasitaria (grs)
51	23.5	27.5
44.6	23.5	21.2
48.5	21.9	26.7
47.2	23.4	23.8
46.4	23.7	22.7
55.4	25.1	30.3
49.3	23.8	25.5
51.7	22.6	29.1

Tabla IV. Registro de pesos de ratones Balb/c hembras después de 300 dias de ser inoculadas por via intraperitoneal con 10 cisticercos por ratón.

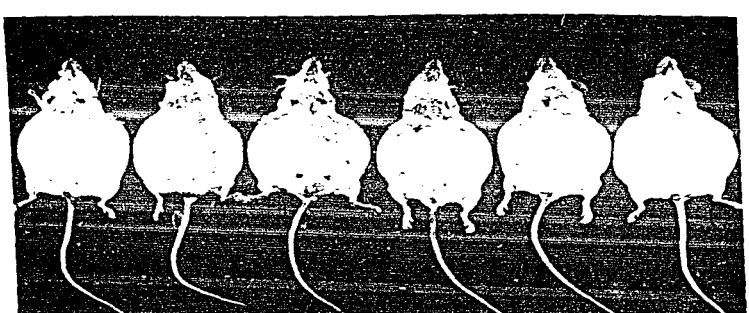


Figura 8. Aspecto de ratones BALB/c hembras después de 300 dias de infectados con 10 cisticercos de Taenia crassiceps.

Esta inmunoreactividad cruzada permitió considerar a los cisticercos de Taenia crassiceps como fuente alternativa para provisión de antigenos, lo que seria de una enorme ventaja teniendo en cuenta la alta carga parasitaria que es capaz de alojarse en la cavidad peritoneal de los ratones infectados, la que puede superar la masa corporal del ratón (Tabla IV, Fig. 8). La utilidad de estos antigenos en el inmunodiagnóstico de la cisticercosis humana forma parte del contenido de esta tesis.

El antecedente de la gran inmunoreactividad cruzada entre ambos céstodos sumado a la rapidez en la instalación de la cisticercosis murina ofrece condiciones óptimas para la evaluación de antigenos interesantes para vacunación. Para ello utilizamos como antigenos extractos provenientes de T. crassiceps y de T. solium para inducir protección inmunológica a los ratones frente al desafio con cisticercos de T. crassiceps. Estos experimentos de evaluación experimental de una vacuna contra la cisticercosis se detallan en el contenido de esta tesis y fundamentan nuestra esperanza de inducir inmunoprotección en cerdos vacunándolos con antigenos provenientes de T. crassiceps.

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III. DESCRIPCION DE LAS TECNICAS Y LA METODOLOGIA UTILIZADA

Esta sección constituye una descripción detallada de las técnicas utilizadas. Si bien ya se encuentran, aunque más sintéticamente descriptas en los articulos que constituyen esta tesis, este apéndice de metodologias intenta favorecer su reproducción.

MANTENCION DE LOS PARASITOS

Los cisticercos de Taenia crassiceps empleados son de la cepa ORF y los de la cepa HYG. La cepa ORF fue la primera aislada y es mantenida en el laboratorio desde 1952. En 1972 se reportaron las primeras anomalias en la constitución de su escolex y en la actualidad esta estructura se ha pérdido completamente. La cepa HYG, mientras tanto, mantiene sus caracteristicas estructurales normales y su capacidad de completar el ciclo sexuado de división.

Estas dos cepas de parásitos están siendo mantenidas en ratones Balb/c hembras. Los ratones se infectan inoculando por via intraperitoneal 10 cisticercos de un tamano de aproximado de 2 mm suspendidos en 0.5 ml de PBS con jeringas de insulina con aguja de 25 x 16 mm. Previo a la inoculación, los cisticercos se lavan con PBS, en relacion 1 volumen de parásitos en 20 vol de PBS. A partir del mes de infección y en los siguientes 4 meses se utilizan los parásitos generados en la cavidad peritoneal de estos ratones para toda la experimentación. En nuestra experiencia los parásitos provenientes de infecciones más prolongadas tienen alteraciones en su capacidad de reproducción por lo que no recomendamos su uso.

OBTENCION DE ANTIGENOS DE CISTICERCOS

Para la obtención de antigenos se parte de los cisticercos lavados con solución fisiológica y mantenidos en hielo para reducir posibles actividades proteoliticas. Asi tratados, los utilizamos en la obtención de tres preparaciones antigénicas

1. Extracto Total

Se resuspenden los cisticercos en PBS frio adicionado con inhibidores enzimáticos (EDTA 2.5 mM(0.93 g por litro de PBS), PCMB 0.04% (0.4g disueltos en 10 ml de H20 destilada adicionada con 4 gotas de NaOH 5 M), PMSF 0.006% (0.006 g disueltos en 4 ml de etanol o 2-propanol), en una relación de 2 volumenes de solución por volumen de cisticercos. En estas condiciones se homogeinizan los parásitos en un Polytron (Brikmann Instruments) manteniendo durante 10 segundos el émbolo en la mezcla y repitiendo 10 veces esta operación. Se determina la concentración proteica del extracto por el método de Lowry y se congela a -70°C hasta su uso en alicuotas.

2. Fluido Vesicular

Los cisticercos obtenidos y lavados con PBS mantenidos en bano de hielo. Se colocan sobre un papel de filtro para quitar el exceso de PBS, se pasan uno por uno a un tubo de centrifuga. Se centrifugan a 10.000 rpm durante 60 min. El liquido vesicular mas componentes del parásito solubilizados en el sobrenadante se separan del paquete celular, se determina su concentración proteica y se congela en alicuotas a - 70°C hasta ser utilizado.

3. Extracto antigénico enriquecido con antigenos de superficie

Los cisticercos obtenidos mantenidos en hielo se lavan 3 veces en PBS adicionado con inhibidores enzimaticos en una relación 1 de cisticercos con 20 ml de solución, después del último lavado se resuspenden en la misma relación de volumen y en la misma solución de lavado y se dejan en agitación suave durante 18 horas a 4°. Se centrifugan a 15.000 rpm durante 60 min, se separa la capa de lipidos que queda como una pelicula en la parte superior del sobrenadante. El sobrenadante se ultracentrifuga una hora a 50.000 rpm, se separa el sobrenadante obtenido se determina su concentración proteica, se preparan alicuotas y se mantienen en congelación a - 70° hasta su uso.

CUANTIFICACION DE ANTICUERPOS POR ELISA

Se utilizan placas de fondo en U de 96 pozos tipo immulon I (Dynatech) como soporte de reacción. Se sensibilizan con 100 ul por pozo de una solución de 10 ug/ml de proteinas totales del fluido vesicular en Tris-ClH 0.01 M, pH=7.5 incubando durante 2 horas a 37 grados y toda la noche a 4 grados. La placa se sella herméticamente o se mantiene en cámara húmeda durante los tiempos de incubación. Las placas se lavan 5 veces con 200-250 ul de PBS-Tween al 0.05% y se bloquea con 100 ul por pozo de una solución de albúmina sérica bovina al 1% en PBS durante 60 min a temperatura ambiente. Se repiten los lavados de la manera descrita. Se adicionan 100 ul por pozo de los sueros diluidos 1 : 200 en PBS -Tween 0.01 % incubando a temperatura ambiente durante 90 min. La placa vuelve a lavarse y los anticuerpos fijados se revelan utilizando inmunoglobulinas de suero anti-ratón

biotinilado (100 ul por pozo de la dilución recomendada durante 60 min a temperatura ambiente) y avidina peroxidasa (100 ul por pozo de la dilución recomendada durante 60 min a temperatura ambiente). La placa se lava y la actividad de peroxidasa se revela agregando como sustrato o-phenilendiamine (0.04% en buffer citrato-fosfato(acido citrico 0.1M / PO4HNa2 0.2M, pH=5). Se detiene la reacción a los 30 min. con 100 ul de SO4H2 4 N, leyéndose en el lector de ELISA a OD495nm inmediatamente. Toda reacción debe incluir además de los sueros en estudios, sueros provenientes de animales no infectados(controles negativos) y sueros provenientes de animales infectados (controles positivos) indicadores de la reproducibilidad de los valores obtenidos.

INMUNOELECTROTRANSFERENCIAS

Para el desarrollo de esta técnica empleamos geles de poliacrilamida medianos (1.5 mm) al 7% con SDS (28 ml) y un gel concentrador al 3% (3 ml). Se utiliza 3 mg/ml de proteina por gel si se usa el gel sin peine y 300 ug de proteinas por pozo si se usa el peine de 10 pozos, pudiendo tratarse de cualquiera de las tres preparaciones antigénicas mencionadas. La muestra se mezcla con un volumen igual de coctail para electroforesis, se tapa perfectamente el tubo donde se preparó y se hierve a bano maria durante 5 minutos. La muestra asi tratada se coloca sobre el gel superior cuidando de que no se remueva con el buffer de corrida, y se realiza la electroforesis a corriente constante (20 mA por gel de 1.5 mm para el gel concentrador una vez que la muestra entra en el gel de corrida). El tiempo total de corrida varia entre 4 y 6 horas y se utiliza como indicador para finalizar la corrida que el frente del colorante esté a 1 cm del final

del gel.

Concluida la electroforesis, se desmontan los geles y se les corta una tira para tenir con azul de Coomasie y el resto se coloca en 200 ml de amortiguador de transferencia durante 15 min.

Para realizar la transferencia se utiliza papel de nitrocelulosa (13 x 13 cms) al que se le marcan los segmentos que se utilizarán de manera individual con la punta de una aguja (3 mm) en los dos extremos de la hoja. Todo el procedimiento se realiza con los papeles de protección de las hojas y con guantes. Se coloca el papel de nitrocelulosa, el de filtro y las fibras scoth con amortiguador de transferencia. Se prepara el sistema de transferencia y se corre a 25 volts durante 60 min, cuidando de que la corriente no pase de 1 A. Después de la transferencia se corta una tirita se lava con PBS-Tween al 0.3% y se tine con tinta india al 0.1% en PBS-Tween durante toda la noche. El resto del papel se deja bloqueando con BSA al 3% en PBS-Tween toda la noche a 4 grados. Se lava 5 veces con cantidad suficiente de PBS-Tween al 0.3% , 5 min. cada lavado y se pone a secar sobre un papel absorbente con la cara donde están las proteinas hacia abajo. Una vez seco se cortan las tiritas de 3 mm. y se enumeran una por una en el extremo inferior. Cada tirita se moja en PBS-Tween y se coloca sobre un vidrio en una cámara húmeda, sobre cada una de las tiritas se colocan 100 ul de una dilución de 1/10 a 1/20 de los sueros problemas y se mantienen en incubación durante 5 horas a temperatura ambiente. Terminada la incubación se lavan las tiritas y se incuban con un anti-ratón biotinalado en la dilución comercial recomendada durante 90 min., vuelven a lavarse las tiritas y se incuban con avidina-peroxidasa en la dilución recomendada durante 1 hora en

agitación constante, después de lavadas se incuban con sustrato para peroxidasa durante 30 min. protegiéndolas de la luz, la incubación se termina lavando exhaustivamente con agua corriente y se procede luego al análisis de resultados.

SOLUCIONES Y REACTIVOS UTILIZADOS

Amortiguador de Tris-HCl 0.01M, pH=7.5

Trisma - HCl anhidro	1.27 g
Trisma base anhidro	0.236 g
H2O destilada para	1.00 l.

Ajustar el pH=7.5

PBS - pH =7.2 (+0.2)

Na2HPO4 anhidro	2.044 g
NaH2PO4.H2O	0.772 g
NaCl	14.80 g
H2O destilada para	2.00 l

PBS-Tween 0.03% - pH=7.2 (+0.2)

PBS	1.00 l
Tween 20	0.3 ml

Composición de Geles

	Gel Separador 7 %	Gel Concentrador 3 %
Acrilamida-Bis (1)	14.0 ml	2.0 ml
Amortiguador (2)	15.0 ml	-
Amortiguador (3)	-	5.0 ml
SDS 10% (4)	0.6 ml	0.2 ml
H2O destilada	30.1 ml	12.86 ml
Persulfato de amonio (5)	300 ul	100 ul

Desgasificar con vacio y agitación 1 minuto.

Temed	40 ul	20 ul

Desgasificar con vacio y agitación 1 minuto.

(1) Acrilamida monómerica(30% T, 2.7% C bis)

Acrilamida	29.2 g
Bisacrilamida	0.8 g
H2O destilada para	100.0 ml

Almacenar a 4 grados cubierta de la luz
La acrilamida es neurotóxica y debe manejarse con cuidado, se recomienda usar cubreboca y guantes.

(2) Amortiguador para gel separador 4x
(Tris-Cl 1.5 M, pH=8.8)

Tris base	36	g
H2O destilada para	200	ml

Se ajusta a pH=8.8 con ClH concentrado antes de aforar.

(3) Amortiguador para el gel concentrador 4 x
(Tris -Cl 0.5 M, pH=6.8)

Tris base	3	g
H2O destilada para	50.0	ml

Se ajusta el pH=6.8 con ClH concentrado antes de aforar.

(4) SDS al 10% (Dodecil sulfato de sodio)

SDS	5	g
H2O destilada para	50	ml

(5) Persulfato de amonio al 10%

Persulfato de amonio	100	mg
H2O destilada	1	ml

Se prepara inmediatamente antes de usarse

Amortiguador para guardar el gel separador

(Tris-Cl 0.375 M pH=8.8 0.1% de SDS)

Tris-Cl 1.5 M pH=8.8	2.5 ml
SDS al 10%	0.1 ml
H2O destilada para	10.0 ml

Coctel para el tratamiento de la muestra, 2x

(Tris-Cl 0.125 M pH=6.8, SDS 2%, glicerol 20%, 2-mercaptoetanol 10%, EDTA 0.074%)

Tris-Cl 0.5M, pH=6.8	2.5	ml
SDS al 10%	2	ml
Glicerol	2	ml
EDTA	7.4	mg
Pironina (1%)	20	ul
2- mercaptoetanol	0.1	ml
H2O destilada para	10	ml

El 2-mercaptoetanol se agrega al descongelar cada alicuota (10 ug/ml)

Amortiguador de corrida

(Tris 0.025 M pH=8.3, glicina 0.192 M, SDS 0.1%)

Tris base	15	g
Glicina	72	g
SDS	5	g
H2O destilada para	5	lt.

Solución para tenir los geles

(Azul de Coomasie R-250 0.06%, metanol 30%, ácido acético 10%)

Azul de Coomasie R-250	300	mg
Metanol	150	ml
Acido acético glacial	50	ml
H2O destilada para	500	ml

Se filtra antes de usar

Solución para destenir geles

(Acido acético al 10%)

Acido acético galcial	100	ml
H2O para	1	l.

Sustrato para peroxidasa (en inmunoelectrotransferencia)

(O-cloronaftol 0.05 mg/ml, H2O2 0.03%)

O-cloronaftol	30	mg
metanol	10	ml
PBS	50	ml
H2O2 al 30%	50	ul

El O-cloronaftol primero se disuelve en el metanol, se mezcla con el Pbs e inmediatamente antes de usarlo se le agrega el H2O2.

Sustrato para peroxidasa (en ELISA)

(O-phenilendiamina 0.04, citrato 0.1 M fosfato 0.2M, pH=5)
O-phenilendiamina 40 mg amortiguador
Amortiguador necesario para 100 ml

Se prepara en el momento de usar

IV. ARTICULOS PUBLICADOS

From: CELL FUNCTION AND DISEASE
Edited by L. E. Canedo, L. E. Todd, L. Parker and J. Jaz
(Plenum Publishing Corporation, 1989)

BIOLOGICAL DETERMINANTS OF HOST-PARASITE RELATIONSHIP IN MOUSE CYSTICERCOSIS CAUSED BY Taenia crassiceps: INFLUENCE OF SEX, MAJOR HISTOCOMPATIBILITY COMPLEX AND VACCINATION*

Larralde, C.+, Sciutto, E.+, Grun, J.°., Díaz, M.L.+, Govezensky, T.+, and Montoya, R.M.+

+Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, AP 70-228, México, D. F. 04510,
°Dept. Biochemistry, Th. Jefferson U., Philadelphia, PA. USA

The metacestode of Taenia solium is frequent cause of serious neurological illness of humans in Mexico and other countries of Latin America, Asia and Africa. Man is the only carrier of the adult worm and sole responsible of transmission to pigs and other humans via inadequate disposal of faeces. Recent years have see advancement in therapy (1,2) diagnosis (3-5) and pathology of human and porcine disease (6,7). Copious accounts of recent developments in cysticercosis are collected in (8), (9) is an exhaustive review of all literature on taeniasis/cysticercosis, while (10) is the most authorative and comprehensive account of human cysticercosis.

Factors determining the risk of humans contracting cysticercosis are thought to be mainly related to magnitude and frequency of exposure to eggs of T. solium. Evidence implicating biological factors in susceptibility is tenuous. No impressive association of human cysticercosis with histocompatibility antigens was found in a doubtfully representative study performed recently (11), nor do the few terminal cases studied make a strong case for immunosupression determining human disease (12). However, inklings of biological factors being involved are present in a recent report of women showing more frequently than males severe inflammation in neurocysticercosis (13). Also suspicious of biological mediation is the lack of correlation between positive serology and social factors conventionally associated to high risk of infectious disease, such as low income and scholarity and defective personal hygiene (14). Further, the very heterogeneous clinical pictures and forms of evolution of the disease, some curing spontaneously while others progress relentlessy to fatal outcomes or live on essentially asymptomatic (10), together with the parasites'sensitivity to drugs, some resistant to praziquantel and others to albendazol (15), all argue for a complicated network of factors and events belonging to parasite (16), host and environment, concurring in the pathogenesis of cysticercosis.

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Systematic exploration of the role of biological factors in susceptibility to cysticercosis is hardly possible studying man, and most laborious and costly in pigs. However, there are other tapeworms - T. crassiceps and T. taeniformis - whose metacestodes affect mice, that are most suitable for experimentation and have already provided with some evidence for the genetic background of the host influencing the outcome of infection with T. taeniaformis (17) and of immunity affecting installation of T. crassiceps (18). Mice harbor the cysticerci of T.crassiceps in their peritoneal cavities as a chronic infection causing some inflammation in serose intestinal surfaces at late

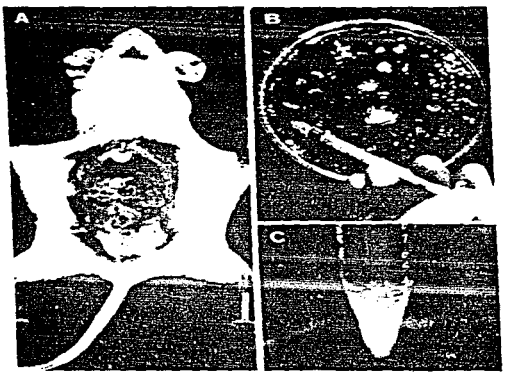

Figure 1. T. crassiceps cysticercosis in the peritoneal cavity of mouse infected six months before with five live cysticerci. The disease's accesibility to experimentation and measurement of parasite load is immediately apparent.

stages of disease quite like that caused by T. solium in basal meninges of man. Furthermore, anatomically, the murine parasite resembles that of T. solium, although somewhat smaller, and shares the seemingly convivial behavior of the human parasite, not causing major damage to neighboring structures of the host, other than space occupation and scant inflammation, as evidenced by conventional light microscopy and NMR imaging (19). In nature mice contract the disease ingesting eggs present in the environment contaminated with faeces from small carnivores -like foxes, cats, and perhaps others (20)- bearing the adult worm in their intestine.

Experimental infection is simply attained by injecting metacestodes in the peritoneal cavity of mice, where they reproduce by budding, presumably asexually. In normal conditions one may harvest them by the hundreds a couple of months after infection (21). If truly asexual, T. crassiceps capability to multiply by budding, allows for control and uniformity of the parasite's genetic characteristics. Most conveniently, cysticerci of T. crassiceps fare well for weeks in conventional tissue culture conditions and for days in minimal media.

In here we present preliminary observations in mice infected with T. crassiceps indicating that biological factors are indeed involved in host susceptibility to cysticerci. Results point to the significant participation of immunological, endrocrinological and genetical determinants on the rate of parasite growth and replication inside the host, opening very exciting possibilities of studying the interaction of these three prominent organic systems upon the host-parasite relationship, a unique biological phenomenom seemingly heedless of simple rules of thumb concerning immunology, histocompatibility and causation of disease (21).

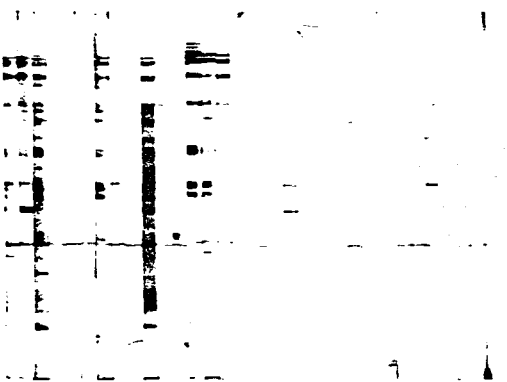

Figure 2. Western blots of protein antigens from the vesicular fluid of T. crassiceps cysticerci reacting with sera of neurocysticercotic patients (left) and of healthy donors (right).

1) Immunological Determinants

Antigenic crossreactivity between the human and the mouse parasite was definitively established by Western Blots (Figure 2), for a number of proteins differing in molecular weight. Furthermore, Tables I and II show that vaccination of susceptible mice with antigen extracts of T. solium cysticerci reduces the parasite load developed from a challange with T. crassiceps.

TABLE I

EFFECTS OF VACCINATION WITH *Taenia solium* ANTIGENS UPON PARASITE LOAD OF MICE CHALLENGED WITH *Taenia crassiceps*

Parasite load ul.	Relative dose of vaccine			
	0.00	0.01	0.10	1.00
Individual Mouse	2000	1000	1500	0
	1500	400	0	600
	2000	400	200	0
	1000	2000	2000	50
	1000	2000	2500	1000
Mean	1500	1160	850	300

Decrement of *T. crassiceps* parasite load with increasing vaccine dose in female (SJL X Balb/c) mice, challanged five months after vaccination with five live cysticerci implanted intraperitoneally. Vaccine was a sterile 50% ammonium sulphate protein extract (3X precipitated) of a single *T. solium* cysticercus dissected from the skeletal muscle of a parasitized pig; ten fold dilutions of the extract were prepared to generate widely different vaccination protocols. Parasite load was measured as volume of harvested parasites one month after challenge.

TABLE II

EFFECTS OF VACCINATION WITH *Taenia solium* ANTIGENS FROM DIFFERENT SOURCES UPON PARASITE LOAD OF MICE CHALLENGED WITH *Taenia crassiceps*

	Saline	Antigens From Vesicular Fluid	Antigens From Complete Parasite
Parasite loads per animal, in ul	100	0	200
	1000	600	600
	1000	1000	400
	1000	900	500
	2000	600	0
		800	10
		300	0

Decrement of *T. crassiceps* parasite load due to vaccination in female Balb/c mice. Vaccine (1 mg total protein of *T. solium* cysticerci/mouse) was administered two months before challange with five cysticerci of *T. crassiceps* implanted intraperitoneally. Parasite load was measured one month after challenge.

These results establish the sharing of antigens between the murine and the human parasites, as well as the effective influence of vaccination in reducing parasite load. These findings are of interest in that T. crassiceps in mice closely resembles T. solium in antigenic constitution, validating it's use as a disease model and as an alternative source for antigens to be used in immunodiagnosis of neurocysticercosis Likewise, crossimmunity from T. solium to T.crassiceps allows some optimism about the inverse also holding, at least in pigs. Reducing the parasite load in pigs, via an effective vaccine, could lower the endemia of adult T. solium tapeworm infection and the number of eggs in the environment, as consequence.

TABLE III

SEXUAL AND HISTOCOMPATIBILITY DIFFERENCES IN SUSCEPTIBILITY OF MICE TO Taenia crassiceps AS MEASURED BY TOTAL PARASITE COUNT

Strain	H 2 Haplotype	Males	Females	Fem/Male
Balb/c	d	200	640	3.2
C57Bl	b	116	750	6.5
C3A	k	130	860	6.6
SWR	q	290		3.1
DBA	q		600	2.1
SJL	s	200	540	2.7

Greater female susceptibility to T. crassiceps is here shown in five strains of mice differing in H2haplotypes. Parasite loads are given as the mean number of parasites collected from each of ten animals. three weeks after challange with five cysticerci in the peritoneal cavity. Statistical analysis (Two Way ANOVA) indicated to significant variation between sexes and among strains.

2) Sexual histocompatibility Determinants

Mouse females' greater susceptibility to T. crassiceps is demostrated in Table III: in all strains of mice tried, the ratios of parasite loads in ten challanged females, as measured thirty days after injecting five cysticerci in the peritoneal cavity of each animal, was always greater than twice that of the respective males. Statistical analysis of results in Table III indicated to significant variation due to strains, thus pointing to the importance of genetic background, presumably the Major Histocompatibility Complex, in susceptibility of mice to T. crassiceps. Experimental design does not allow definitive conclusions on the role of histocompatibility

because the strains used, although differing in their H2 haplotypes, also differ, and to unknown extent, in their genetic complement. Careful selection of mouse strains is required to elucidate this point. However, there is no statistical doubt that the susceptibility associated to sex varied in the different strains used. This we consider an important finding. Although the literature is not lacking in reports of sex associated differences in susceptibility to experimental infections, rarely do they point to females being more susceptible or to histocompatibility association (21,28-33) and the consequent implications for immune reactivity (33). Thus, we have great hopes in T. crassiceps cysticercosis as a potent model for studing the significance of "immunoneuroendocrine interactions" in a macroscopic event such as infection, instead of microscopic phenomena in simplified systems such as isolated cells, subcellular particles and the like (reviewed in 22-27).

In closing, it would be adventurous to claim these results are already relevant to our understanding and dealings with human disease in terms other than the most general: parasitic disease installs and progresses as the net result of a complicated and delicate network of interactions among the organic constitutents of both host and parasite. That sex - presumably hormones - make such a great difference in host-parasite relationship involving helminths was indeed surprising. Dilucidation of the mechanisms involved in the sexual differences in susceptibility is yet to be done; direct hormonal influence on the parasite or through immunological mediation are the most prominent - non-disjunctive - possibilities.

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ACTA LEIDENSIS

INSTITUTO DE INVESTIGACIONES BIOMEDICAS
APARTADO POSTAL 70228
CIUDAD UNIVERSITARIA
04510 MEXICO, D. F.

VNIVERSIDAD NACIONAL
AVTENOMA DE
MEXICO

February 26, 1990.

DRS. ALUJA, CORREA, CRAIG, DUMAS, GEMMELL, HARRISON, LACLETTE, LARRALDE, LAWSON, LEID, LIGHTOWLERS, McMANUS, PARKHOUSE, RICKARD, SCHANTZ, TELLEZ-GIRON AND vanKAMPEN.

Dear Colleges:

Enclosed please find copy of a letter from David Overbosh indicating why the Proceedings of C-Now have not been published yet and when they will appear.

I am very sorry, but I myself did not know what the problem was, and thus could not do anything to solve it.

Please accept my personal apologies.

Yours sincerely,

Dra. Ana Flisser

var.

Dr. D. Overbosch, MD, PhD, Internist
Red Cross Hospital
Sportlaan 600
2566 MJ 's-Gravenhage
The Netherlands.
Telephone: (0)70-3614761
Telefax: (0)70-452216

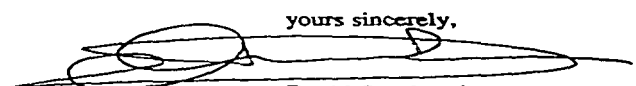
Tue, Jan 30, 1990

Mrs.Ana Flisser
Inst. de Investigationes
Biomedicas
P.O. Box 70228
04510 Mexico
Faxnr.: 09.525.3951435

Dear Ana,

Thank you for your letter, I received recently. I understand your worries concerning the C-Now! proceedings. I held up the publication because of the appalling quality of the therapeutic papers. I have forwarded them to the authors but have not received any reply. I tried to edit/rewrite their contributions but I am unable to include them in the issue of Acta Leidensia, concerning C-Now! which is completely ready now. This issue is printing at this very moment and will be forwarded to all participants early March 1990. The contributions of the therapeutic sessions in the C-Now! symposium will be printed in an issue that will appear in May/June 1990. I am sorry for this delay but could not prevent this unhappy outcome.
Concerning the data from our pigstudy, I have not yet received the results of the German laboratory. As soon as they are available I will discuss the results with you.
I still think about my stay with you in Mexico with very warm feelings.
Looking forward to meeting you again,

yours sincerely,



David Overbosch.

? to Hof Dam

Acta Leidensia

EXPERIMENTAL CYSTICERCOSIS BY *Taenis crassiceps* IN MICE: FACTORS INVOLVED IN SUSCEPTIBILITY*

Larralde, C., Sciutto, E., Huerta, L., Terrazas, I., Fragoso, G., Trueba, L., Lemus, D., Lomelí, C., Tapia, G., Montoya, R.M., Díaz, M.L. and Govezensky, T.

Instituto de Investigaciones Biomédicas, UNAM
Apartado Postal 70-228
México 04510, México, D. F.

* This project was supported by grants from Consejo Nacional de Ciencia y Tecnología de México, from Secretaría de Salud de México and from Química Hoechst de México.

The metacestode of Taenia solium is frequent cause of serious neurological illness of humans in Mexico and other countries of Latinamerica, Asia and Africa. Man is the only carrier of the adult worm and sole responsible of transmission to pigs and other humans via inadequate disposal of faeces. Recent years have seen advancement in therapy (1,2) diagnosis (3-5) and pathology of human and porcine diseases (6,7). Copious accounts of recent developments in cysticercosis are collected in (8), (9) is an exhaustive review of all literature on taeniasis/cysticercosis, while (10) is the most authorative and comprehensive account of human cysticercosis.

Factors determining the risk of humans contracting cysticercosis are thought to be mainly related to magnitude and frequency of exposure to eggs of T. solium. Evidence implicating biological factors in human susceptibility is tenuous. No impressive association of human cysticercosis with histocompatibility antigens was found in a doubtfully representative study performed recently (11), nor do the few terminal cases studied make a strong case for immunosupression determining human disease (12). However, inklings of biological factors being involved are present in a recent report of women showing more frequently than males severe inflammation in neurocysticercosis (13). Also suspicious of biological mediation is the lack of correlation between positive serology and social factors conventionally associated to high risk of infectious disease, such as low income and scholarity and defective personal hygiene (14). Further, the very heterogeneous clinical pictures

and forms of evolution of the disease, some curing spontaneously while others progress relentlessy to fatal outcomes or live-on essentially asymptomatic (10), together with the parasites'sensitivity to drugs, some resistant to praziquantel and others to albendazol (15), all argue for a complicated network of factors and events belonging to parasite (16), host and environment, concurring in the pathogenesis of cysticercosis.

Systematic exploration of the role of biological factors in susceptibility to cysticercosis is hardly possible studying man, and most laborious and costly in pigs. However, there are other tapeworms - T. crassiceps and T. taeniformis - whose metacestodes affect mice, that are most suitable for experimentation and have already provided with some evidence for the genetic background of the host influencing the outcome of infection with T. taeniaformis (17) and of immunity affecting installation of T. crassiceps (18). The latter is best suited as model of human disease. Mice harbor the cysticerci of T.crassiceps free in their peritoneal cavities as a chronic infection causing some inflammation in serose intestinal surfaces at late stages of disease, quite like that caused by T. solium in basal meninges of man. Furthermore, anatomically, the murine parasite resembles that of T. solium, although somewhat smaller, and shares the sociable behavior of the human parasite, not causing major damage to neighboring structures of the host, other than space occupation and scant inflammation, as evidenced by conventional light microscopy and NMR imaging (19). The biological cycle is also similar, in nature mice contract the

disease by ingesting eggs present in the environment contaminated with faeces from small carnivores -like foxes, cats, and perhaps others (20)- bearing the adult worm in their intestine. Experimental infection is simply attained by injecting metacestodes in the peritoneal cavity of mice, where they reproduce by budding, presumably asexually. Experiments yield quick and trustworty results as parasite load is easily assessed by simple parasite count, ranging in tens, hundreds and thousands, a couple of months after infection (21). If truly asexual, T. crassiceps' capability to multiply by budding, allows for control and uniformity of the parasite's genetic characteristics. To top it all, cysticerci of T. crassiceps fare well for weeks in conventional tissue culture conditions and for days in minimal media, facilitating evaluation of factors in simplified systems.

Thus, using experimental murine cysticercosis caused by T. crassiceps, we undertook the systematic exploration of factors involved in the host's susceptibility and the parasite's pathogenicity. Here we briefly inform of our major findings, as they stand today.

Parasite reproduction in the peritoneal cavity of mice experimentally infected with cysticerci of T. crassiceps (ORF strain) is under control of H2 locus, of gonads and of the immune system, acting as single factors as well as interacting systems:

a) H2d haplotype is more susceptible than H2b and H2k;

b) females are more susceptible than males;

c) vaccination induces protective immunity, seemingly independent of antibody;

d) sexual differences vary from strain to strain, and immune protection is most notable in male mice and in resistant strains than in females and susceptible strains, while antibody production is quantitatively and qualitatively independent of sex and strain variation;

e) the parasite's HYG strain grows less rapidly than ORF but is equally sensitive to the host's enviroment;

f) either strain of the parasite, when located subcutaneously are seemingly heedless of the host's histocompatibility, sexual and immune influences.

In view of these findings T. crassiceps cysticercosis appears as a host-parasite relationship controlled by a delicate network of factors from both parasite and host origin that, however, responds so drastically to experimental intervention, it may contribute to unfold the rules of conviviality between two biological entities in intimate contact. So far, the elements involved are sexual hormones, H2d histocompatibility antigens and -by exclusion - immune cells but not antibodies.

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V. ARTICULO ACEPTADO PARA SU PUBLICACION (como primer autor)

PARASITE IMMUNOLOGY

Edited by G.A.T. Targett, London School of Hygiene and Tropical Medicine and Bridget M. Ogilvie, Wellcome Trust, London

Please reply to:

25 JOHN STREET
LONDON
WC1N 2BL

10 January 1990

Dear Dr Larralde

Ms 1018:BMO - Cystercosis vaccine: ...

You have shown some interesting and potentially important cross-protection between T. solium and T. crassips. However I feel the paper is too long and one of our referees has raised a number of important points that must be answered.

I will reconsider it if you can make the paper about 60% of its present length, deal with the points raised, and greatly simplify Tables I and II.

I look forward to receiving a revised manuscript.

Yours sincerely

Professor G A T Targett
Editor

Dr C Larralde
Instituto de Investigaciones Biomedicas
UNAM
Ap 70228
Mexico
D F 04510
Mexico

Comments for transmission to the authors should be typed on these self duplicating sheets. Please return white and pink sheets to the Editorial Office and retain the yellow sheet for your own file. Do not sign.

PARASITE IMMUNOLOGY

TITLE: CYSTICEROCOSIS...

AUTHOR: MS 1018:BMO

COMMENTS:

This a potentially very interesting piece of work, but I would ask the authors to consider the following points:

1. Control animals received 10 µg of alum alone. Ideally each test group should have its own set of controls, receiving the equivalent ✓ amount of adjuvant. If this is logistically impossible (?) then the single control group should receive the highest dose of adjuvant experienced by any of the test groups ie $\frac{500}{30} \times 1 = 16.6$ µg. not 10 µg.
2. The number of mice per group and replicate experiments should be ✓ indicated in the Methods section.
3. In the Tables, it is not clear to me why the same number of infecting parasites gave such large variations in total load in control animals (Table 2 is 5× higher than Table 1), and whether this has been taken into account in the statistical analysis.
4. The control data presented in Table 3 is identical to that given in Tables 1 and 2. Does this mean that the experimental groups presented in Table 3 did not have a cohort group of controls?
5. The legend to Table IV does adequately describe the data, but the heading does not.
6. Table V – the value of data presented here is not tested by statistical analysis. This seems especially important in view of the fact that half of the control H-2b males were completely resistant without vaccination

PARASITE IMMUNOLOGY

Edited by G.A.T. Targett, London School of Hygiene and Tropical Medicine and Bridget M. Ogilvie, Wellcome Trust, London

Please reply to:
25 John Street,
London WC1N 2BL.

TELEPHONE 071-404 4101

09 May 1990

Dr C Larralde
Insituto de Investigaciones Biomedica UNAM
Ap 70228
Mexico DF 04510
Mexico

Dear Dr Larralde

Re: MS 1018 - Cysticercosis vaccine: cross ...

Thank you for submitting the above mentioned manuscript to the Journal.

I am pleased to say that your paper has been accepted for publication and the proofs will follow from the Publishers in due course. It will be appreciated if these are dealt with promptly and accurately.

Yours sincerely,

Dr B M Ogilvie
Editor

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CYSTICERCOSIS VACCINE: CROSS PROTECTING IMMUNITY WITH *T. solium* ANTIGENS AGAINST EXPERIMENTAL MURINE *T. crassiceps* CYSTICERCOSIS

Sciutto, E., Fragoso, G., Trueba, L., Lemus, D., Montoya, R.M., Diaz, M.L., Govezensky, T., Lomeli, C.*, Tapia, G. and Larralde, C.

Mailing address:

Dr. Carlos Larralde
Instituto de Investigaciones Biomédicas, UNAM
Ap 70228
México, D.F., 04510
México

Running Head

Cross immunity in *T. solium* and *T. crassiceps* cysticercosis

Keywords

Cysticercosis vaccine; *T. solium* and *T. crassiceps* cross immunity

SUMMARY

Vaccination of mice with an antigen extract from Taenia solium cysticerci induced protection against challenge with T. crassiceps cysticerci as successfully as did antigen extracts from T. crassiceps. Vaccination was more effective in male than in female mice and in the resistant strain (BALB/B) more so than in the susceptible strain (BALB/c). While only the resistant strain was completely protected by vaccination, the parasite load of the susceptible strain was significantly reduced by vaccination.

Cross immunity between the human and murine parasites establishes murine T. crassiceps cysticercosis as a convenient laboratory model in which to test promising T. solium antigens aimed at vaccine development against T. solium cysticercosis.

Further, results point to strong interactions of the immune system with sexual and histocompatibility factors in the host's dealing with cysticercosis.

INTRODUCTION

The metacestode of T. solium is a frequent cause of serious neurological illness of humans in Mexico and other countries of Latinoamerica, Asia and Africa (Gemmell et al, 1985). Man is the only carrier of the adult tapeworm and solely responsible for transmission to pigs and other humans via inadequate disposal of faeces. Recent years have seen advancement in therapy (Del Brutto and Sotelo, 1988; Escobedo et al., 1987), diagnosis (González et al., 1987 and Flisser et al., 1988), immunology (Flisser and Larralde, 1986) and pathology of human and porcine diseases (Rabiela, 1985). Control of transmission of T. solium cysticercosis in endemic areas has been less successful (Gemmell et al., 1985; Aluja, 1982). An effective vaccine in rustically reared pigs would decrease infection pressure for man and pigs by reducing the number of new tapeworm carriers while allowing continuation of rustic porcine rearing, which is the only form of porciculture economically accesible in endemic areas for a time to come.

That vaccination induces immunological protection against metacestode infections is very well documented in a variety of species of host and parasite, most effectively if vaccines comprise oncospheral antigens (Larralde, Flisser and Pérez-Montfort, 1982; Good et al., 1982; Rajasekariah, Rickard and Donnell, 1985). The literature on immunological protection to T. solium although encouraging is scanty and deals mainly with experimental challenges of a few pigs with eggs; a design of questionable realism on account of doubts about viability of eggs, effective dose and number of challenges, and

background immune state of the experimental host (Nascimiento et al., 1987; Molinari, et al., 1983, 1988). Optimism prevails, however, because of the extensive similarity among cestode infections in natural history, pathology and, most convincingly, in antigenic composition.

Experimentation leading to a vaccine against porcine cysticercosis is hampered by the high costs, genetic variability and slow data retrieval involved in testing pigs. Experimental murine cysticercosis, caused by T. crassiceps (Freeman, 1962; Smith, Esch and Kuhn, 1972; Larralde et al., 1990), could be a useful laboratory model in which to test promising antigens, provided some degree of cross-immunity between the pig and the mouse parasites could be demonstrated. Because a most recent account informs of dozens of crossreacting antigens in E. granulosus, T. solium and T. crassiceps (Larralde et al., 1989), an evaluation of cross protective immunity between T. solium and T. crassiceps was most pertinent.

MATERIAL AND METHODS

1. Parasites

Larval T. crassiceps utilized in this study come from a stock of the ORF strain kindly supplied by Dr. B. Enders (Behringwerke, Marburg, West Germany) in 1984. The parasites were maintained by serial intraperitoneal inoculation in young female BALB/c mice ever since. The parasites used to challenge control and vaccinated mice were obtained from donor mice - also BALB/c females - infected with 10 small (less than 2 mm) non-budding larvae, and allowed to reproduce for 1 to 4 months inside the donor host before harvesting.

2. Hosts

Male and female BALB/c (susceptible) and BALB/B (resistant) mice (Larralde et al., 1990) 4 to 6 weeks old, were used to test the effects of vaccination upon susceptibility to experimental infection.

3. Antigen Preparation

A total extract of the cysticerci was selected as vaccine, instead of purified fractions, there not being any information as to the specific antigens potentially useful in protecting against experimental metacestode infection in mice. Murine T. crassiceps cysticerci and porcine T. solium cysticerci were collected as described in detail elsewhere (Larralde, et al., 1986) and placed in cold .01 M phosphate buffered saline, pH 7.2 (PBS). The metacestodes were repeatedly washed in ice cold PBS, and then homogenized in a Polytron (Brinkmann Instruments); the final protein concentration was adjusted to 9 mg/ml in PBS, as estimated by the method of Lowry, and the preparations stored at -70 °C until used.

4. Vaccination

Groups of 8-10 male and female mice each were vaccinated by injecting intraperitoneally or subcutaneously 100, 200 or to 500 ug of vaccine protein per mouse, suspended in aluminum hydroxide (30 mg protein/1 mg of alum), once or twice, 4 weeks apart. Ten male and ten female non-vaccinated mice were injected with 10 ug of alum alone, as controls: a dose approaching the average dose of adjuvant received by the vaccinated mice. The experiments were designed so that many of the experimental groups were challenged simultaneously with the same harvest of parasites. Consequently, the same group of non-vaccinated mice are cohort controls of several experiments, and thus appear as reference of expected parasite loads in more than one table of results.

5. Challenge and measurement of parasite load

Vaccinated and non-vaccinated mice were each infected intraperitoneally (using a 26X16 needle on a disposable syringe), with 10 small (2-3 mm long), unbudding cysts, 30 or 60 days after vaccination. Mice were killed 30 or 60 days after infection. Parasite load was measured in each mouse after etherization, bleeding and killing by cervical dislocation. The larvae recovered in the peritoneal cavity from each mouse were placed in separate Petri dishes and counted one by one.

RESULTS

Table I shows the expected parasite load in non-vaccinated control mice (dose = 0) after 30 days of infection and those obtained in susceptible BALB/c mice vaccinated with differing doses of total antigen. Vaccination resulted in a slight increase in the number of totally protected mice - most clearly in males - as well as in a notable decrease in parasite load in those affected. Significant effects of vaccine dose were appreciated: the dose of 100 ug/mouse consistently induced higher levels of protection than larger doses. Very clearly, both vaccines, from T. crassiceps and from T. solium, induced significant levels of resistance to infection in vaccinated mice. Subcutaneous and intraperitoneal routes of vaccine administration produced similar levels of protection. Comparison of the results in Table I with those obtained in mice challenged 60 days after vaccination (Table II) indicated that protection declined as time elapsed between vaccination and challenge, more rapidly in females than in males. However, significant differences between vaccinated and control mice were also appreciable here, specially in male mice vaccinated with T. solium. Sexual differences in innate susceptibility, favoring males over females, were confirmed in these experiments, in ratios similar to those reported elsewhere (Larralde et al., 1990).

Statistical analysis of the results of the complete factorial design shown in Tables I and II requires careful consideration, there being many zeroes in individual parasite load and variances being so large and not normally distributed. These characteristics render

simple comparisons of means and standard errors in groups of doubtful meaning. We applied analysis of variance (ANOVA) to take advantage of the multifactorial design (Factors and Levels: dose of vaccine (0, 100, 200, 500 ug/mouse); source of vaccine (T. solium, T. crassiceps); sex (M,F); route of vaccine administration (SC, IP); interval between challenge and vaccination (30, 60 days); replicates mostly 10 per treatment combination with very few missing values. Thus, a measurement of the variation due to error and due to each factor and combination of factors was obtained. Because the model used (SAS, 1985) requires normal distribution of the response variable, several transformations of the individual parasite load were tried until $4\surd$ (Parasite load in each mouse + 1) was found to meet satisfactoriiy with the restriction of normality. Thus, we statistically confirmed that the single factors that most significantly affected parasite load were sex, vaccine/challenge time interval, source of antigen, and dose of antigens. A very significant interaction of source of antigen with dose indicated to significant dose differences for each antigen preparation, and that of sex and time interval to different evolution of the disease according to sex. No significant differences in route of administration of vaccine could be documented. The rest of the double and higher order interactions, although many were statistically significant, were of smaller magnitude or of obscure meaning.

The protective effect of vaccination is again discernible in Table III. Although protection is clearer in early infections (30 days vs 60 days) it is still discernible after 60 days of infection, specially in males, some females in long standing infections approaching the parasite loads of non-vaccinated animals. Table IV

also shows that secondary immunization 30 days after the first vaccine inoculation paradoxically resulted in a diminution of protection, more so in females and in longstanding infections than in males and early infections. Analysis of variance of results shown in Table IV, by the same procedure discussed above, indicated to highly significant differences between vaccinated and controls in all cases except the twice vaccinated females after 60 days of infection.

The results in Table IV refer to the role of MHC and vaccination in immune protection measured 30 days after infection. Results again show the significant protective effects of vaccination in duplicate experiments performed simultaneously by different coworkers. Also, Table IV indicates that complete immunity was induced in all animals only in the resistant BALB/B (H-2b) mouse strain (Larralde et al, 1990), in both males and females, while the congenic strain BALB/c, (H-2d), showed only reduced parasite load and occassional sterile immunity in males only.

DISCUSSION

Results support that vaccination with either T. crassiceps or T. solium antigens protects mice against experimental cysticercosis caused by T. crassiceps cysticerci placed in the peritoneal cavity. Cross-reacting immunity between the cestodes affecting human and porcine hosts with that of mice offers an expeditious and economic laboratory model of cysticercosis on which to test promising antigens in the development of a vaccine against T. solium cysticercosis. It also strengthens optimism about the eventual immunological protection of rustically reared pigs by similar procedures. In addition, murine cysticercosis by T. crassiceps would be capable of supporting the immediate industrial production of vaccine - instead of the unmanageable porcine source of antigens - while DNA recombinant or peptide synthesis technology develop alternative vaccines and they become accesible in endemic areas.

Our results also tell of the intricacies of the host-parasite relationship when hosts deal with as complex a parasite as cysticerci. Not only do the host's gender and histocompatibility genetic factors influence innate susceptibility (Larralde et al., 1990) but they also seem to regulate immunity, as the protection induced by vaccination was always greater in males than in females of either susceptible (BALB/c, H-2d) or resistant (BALB/B, H-2b) mouse strains. In fact, complete - sterile - immunity was obtained only in the resistant strain. Although sex-linked and histocompatibility-linked susceptibility to parasite infection have been recognized in a number of other parasitic diseaes (Blair and Campbell, 1976; Mitchell,

Rajasekariah and Rickard, 1980) no general pattern is recognizable. Some report females as more resistant than males and others indicate that haplotypes other than H-2d are related to susceptibility. It would seem that each species of parasite finds or sets it's own unique set of rules for convivial relationship with each host. We know of no formal report relating suceptibility with race in porcine T. solium cysticercosis, but the introduction of genetically resistant pigs in endemic areas would certainly be an important contribution to control of transmission.

Other interesting points on parasite immunology emerge from our experiments. That the differences in parasite load between vaccinated and control mice minimized as the infection was allowed to proceed would indicate that immunity operates most effectively at the early stages of diesease. However, it could also be a numerical artifact since, if exponential, the rate of growth of the parasite would only be stunted at the low parasite loads characteristic of early infections. The above possibilities are not disjunctive, of course, and there is still no good reason to favor either. That high doses of antigen in the vaccine, as well as boosting, induce lesser levels of protection than low doses and single inoculation, is consistent with a number of possibilities: from immunological paralysis to blocking antibodies, among other. The alleged endurance of cysticerci to immunological attack by the host was shown here to be conditioned by a number of factors - age of disease, as well as by sex, histocompatibility and immunological factors of the host - and not only due to a unique property of the developmental stage of the

parasite. Finally, the notable variation in parasite load within experimental groups deserves at least the comment that preliminary experiments indicate it comes from parasite diversification with respect to rate of growth in the challenge inoculum and not from uncontrolled breeding methods of the mice.

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LEGENDS OF TABLES

Table I. Immunization of susceptible BALB/c mice with different doses of T. crassiceps and T. solium antigens resulted in a considerable diminution of the individual parasite load and in some totally protected mice, after experimental challenge with 10 cysts placed intraperitoneally 30 days after vaccination and recounted 30 days after infection. Protective effects were most notable in males than in females and at the dose of 100 ug/mouse than in higher doses.

Table II. Immunization of susceptible mice with different doses of T. crassiceps and T. solium antigens, 60 days prior to challenge, also resulted in diminution of parasite load and in some totally protected animals, although the level of protection was not as high as that obtained when the interval between vaccination and challenge was of only 30 days (Table I). All mice were challenged with 10 cysts placed intraperitoneally in each mouse, and then recounted 30 days after infection. Protective effects were most notable in males than in females and at the dose of 100 ug/mouse than in higher doses.

Table III. Immunization of susceptible BALB/c mice with 100 ug/mouse of T. crassiceps or T. solium antigens once or twice, 30 days before challenge. Control and experimental animals were sacrificed and their parasites counted 30 and 60 days after infection with 10 cysts per mouse placed intraperitoneally. In all but one case (females twice vaccinated with T. crassiceps

antigens) vaccination resulted in a considerable and significant diminution of parasite load as well as in a notable increment in the number of totally protected mice (NTP). The protective effects of vaccination were most notable in early (30 days) than in late (60 days) infections and slightly more so with antigens from T. solium than from T. crassiceps.

Table IV. Vaccination with 100 ug/mouse of T. crassiceps antigens of congenic BALB mice differing only in H-2 haplotype resulted in significant diminution of parasite load in both strains, as measured 30 days after infection. However, vaccination resulted in complete sterile immunity of both sexes and of all animals only in the resistant BALB/B (H-2b) strain.

TABLE I

EARLY PROTECTIVE IMMUNITY AGAINST MURINE T. crassiceps CYSTICERCOSIS BY VACCINATION WITH T. crassiceps AND T. solium ANTIGENS

SOURCE OF VACCINE	SEX	DOSE OF PROTEIN ANTIGENS IN VACCINE (ug/mouse)						
		0 (CONTROL)	100 IP	100 SC	200 IP	200 SC	500 IP	500 SC
T. crassiceps	FEMALE	*138 ± 13 o(0)	14 ± 6'' (2)	30 ± 6'' (0)	68 ± 10'' (0)	43 ± 5'' (0)	89 ± 20'' (0)	58 ± 10'' (0)
	MALE	19 ± 5 (0)	0 ± 0'' (10)	2 ± 1'' (4)	3 ± 0'' (0)	12 ± 3' (0)	12 ± 4' (1)	23 ± 5 (0)
T. solium	FEMALE	As above	46 ± 10' (3)	12 ± 5'' (4)	48 ± 12'' (0)	15 ± 4'' (1)	82 ± 13' (0)	15 ± 3'' (0)
	MALE		1 ± 1'' (8)	0 ± 0'' (10)	1 ± 0'' (6)	4 ± 2'' (3)	22 ± 6'' (0)	5 ± 2'' (1)

* Mean ± Standard Error of individual parasite load in groups of ten mice each
' Significant difference with control values at 95% confidence level
'' Significant difference with control values at 99% confidence level
o Number of totally protected mice bearing zero cysts

TABLE II

LATE PROTECTIVE IMMUNITY AGAINST MURINE T. crassiceps CYSTICERCOSIS

BY VACCINATION WITH T. crassiceps AND T. solium ANTIGENS

SOURCE OF VACCINE	SEX	DOSE OF PROTEIN ANTIGENS IN VACCINE (ug/mouse)						
		0 (CONTROL)	100 IP	100 SC	200 IP	200 SC	500 IP	500 SC
T. crassiceps	FEMALE	*138 ± 13 o(1)	10 ± 4" (3)	51 ± 5" (0)	81 ± 8" (0)	94 ± 10' (0)	134 ± 7 (0)	136 ± 9 (0)
	MALE	19 ± 5 (0)	4 ± 2" (3)	10 ± 1" (0)	19 ± 3 (0)	19 ± 3 (0)	30 ± 2 (0)	25 ± 4 (0)
T. solium	FEMALE	As above	120 ± 23 (0)	112 ± 27 (0)	68 ± 21" (3)	43 ± 8" (1)	22 ± 13" (3)	12 ± 3" (1)
	MALE		1 ± 1" (7)	24 ± 2 (0)	7 ± 2' (3)	21 ± 2 (0)	2 ± 1" (7)	4 ± 1" (3)

* Mean ± Standard Error of individual parasite load in groups of ten mice each
' Significant difference with control values at 95% confidence level
" Significant difference with control values at 99% confidence level
o Number of totally protected mice bearing zero cysts

TABLE III

EFFECT OF BOOSTING AND SOURCE OF ANTIGENS UPON PROTECTIVE IMMUNITY AGAINST MURINE *T. crassiceps* CYSTICERCOSIS IN EARLY AND LATE INFECTIONS

SOURCE OF ANTIGENS	NUMBER OF VACCINATIONS (100 ug/mouse)	SEX	30 DAYS OF INFECTION *$\bar{X}$ ± SE	o	'NTP	60 DAYS OF INFECTION *$\bar{X}$ ± SE	P	NTP	Σ NTP
None	0	F	138 ± 1		0/10	658 ± 117		1/10	
		M	19 ± 5		0/10	306 ± 42		0/10	
			Σ NTP =		0/20	Σ NTP =		1/20	1/40 = .025
T. crassiceps	1	F	13 ± 6	.01	2/10	255 ± 46	.01	1/10	
		M	0 ± 0	.01	10/10	1 ± 1	.01	9/10	
	2	F	96 ± 25	.20	0/10	648 ± 40	.20	0/10	
		M	3 ± 2	.05	2/10	168 ± 46	.01	2/10	
			Σ NTP =		14/40	Σ NTP =		12/40	26/80 = .325
T. solium	1	F	46 ± 18	.05	3/10	287 ± 89	.01	5/10	
		M	1 ± 1	.01	9/10	103 ± 34	.01	4/10	
	2	F	12 ± 8	.01	5/10	48 ± 21	.01	2/10	
		M	2 ± 2	.01	7/10	24 ± 6	.01	5/10	
			Σ NTP =		24/40	Σ NTP =		16/40	40/80 = .500
	Σ NTP				38/80 = .475			28/80 = .350	

* Mean and standard error of individual parasite load in groups of ten mice each

o Probability of the null hypothesis of experimental group being equal to control

' NTP Number of totally protected mice bearing zero cysts divided by the number of mice in the group

TABLE IV

EFFECT OF VACCINATION WITH *T. crassiceps* ANTIGENS AGAINST MURINE *T. crassiceps* CYSTICERCOSIS IN BALB MICE DIFFERING IN H2 HAPLOTYPE

GROUPS	SEX	RESISTANT (BALB/B, H-2b)		SUSCEPTIBLE (BALB/c, H-2d)		Σ NTP
		*$\bar{X}$ ± SE	'NTP	*$\bar{X}$ ± SE	NTP	
CONTROLS	F	6 ± 1	0/10	78 ± 18	0/8	
	M	2 ± 1	5/10	21 ± 6	0/8	
		Σ NTP =	5/20	Σ NTP =	0/16	5/36
		o P		P		
VACCINATED (100 ug/mouse)	F(1)	0 ± 0 .01	10/10	6 ± 1 .01	0/10	
	F(2)	0 ± 0 .01	10/10	6 ± 1 .01	1/10	
	M(1)	0 ± 0 .05	10/10	2 ± 1 .01	4/10	
	M(2)	0 ± 0 .05	10/10	5 ± 1 .01	2/10	
		Σ NTP =	40/40	Σ NTP =	7/40	47/80

* Mean and standard error of individual parasite load in groups of ten mice each
' Number of totally protected mice bearing zero cysts divided by the number of animals in the group
o Probability of the null hypothesis of experimental group being equal to control

VI. ARTICULOS ENVIADOS PARA SU PUBLICACION (como primer autor)

MURINE *Taenia crassiceps* CYSTICERCOSIS: H-2 AND SEX INFLUENCE ON SUSCEPTIBILITY

Sciutto, E., Fragoso, G., Díaz, M.L., Valdez, F., Montoya, R.M., Govezensky, T., Lomelí C. and Larralde, C.

Mailing address:

Dr. Carlos Larralde,
Instituto de Investigaciones Biomédicas, UNAM.
Apdo. Postal 70228
04510 México, D.F.
Fax number: 550 00 48
Telephone number: 550-39-82

Running Head: H-2 and sex linked susceptibility in cysticercosis.

Keywords: Cysticercosis, *Taenia crassiceps*, H-2, sex, susceptibility.

ABSTRACT

Several inbred strains of mice were infected by intraperitoneal injection of ten *Taenia crassiceps* cysts per mouse. Genes linked with the major histocompatibility complex (H- 2) were found to greatly influence parasite growth, as demostrated by the different parasite loads of H-2 congenic mice with BALB background: BALB/c (H-2d) mice were the most susceptible while BALB/k (H-2k) and BALB/b (H-2b) were comparatively resistant. Non-H-2 genes had no significant effect on susceptibility in H-2d strains, as reflected by the similar parasite loads in BALB/c, DBA/2, and ([BALB/cxDBA/2]F1) mice. Using the H-2b (BALB/b, C57BL/6J) and H-2k (C3H/HeJ, BALB/k, and C3HeB/FeJ) strains, non-H-2 background genes were found to cause a small, but significant influence on parasite load. A recombinant mouse strain (K^k,I^k,S^d,D^d) was also susceptible, indicating that S or/and D regions of the H-2d complex are probably involved in the control of resistance to murine cysticercosis. Female mice of all strains were more susceptible than male mice. The same effects of H-2 genes were observed with two strains of *T. crassiceps* differing in rate of growth.

INTRODUCTION

Genetic differences in the immune response of mice to various parasitic infections are well documented (Vadas 1980; Wakelin 1978, 1985) and variation in their innate susceptibility has been found with most of the parasites tested. However, linkage of susceptibility with the major histocompatibility complex (MHC) has been successfully documented in only a few cases. Genes within H-2 have been shown to play an important role in resistance to *Trichinella spiralis* in mice (Wakelin and Donachie, 1983) as well as genes outside the MHC (Wasson, Brooks and Cypess, 1983); to *Trichuris muris* (Else and Wakelin, 1988); to *Schistosoma mansoni* (Class and Deeleder, 1979) and to *Plasmodium chabaudi* (Wunderlich *et al*, 1988). Also, differences in long-term immune response is controlled by MHC genes in the systemic form of murine leishmaniasis caused by *Leishmania donovani* (Blackwell, Freeman and Bradley, 1980). Finally, the immune response to secreted antigens of *Ascaris suum* is controlled as the level of H-2 in mice (Kennedy, Gordon, Tomlinson and Qureshi, 1986). Such correlations could have profound implications for diagnosis, treatment and control of human helminth infections (Parkhouse and Harrison, 1989).

Preliminary studies in experimental murine *Taenia crassiceps* cysticercosis pointed to significant genetic differences between mouse strains with variable levels of innate susceptibility

(Larralde *et al*, 1989). In these experiments the genetic basis of variable susceptibility was not mapped. Another hint of H-2 involvement comes from protection experiments, where vaccination with *T. crassiceps* was found to be more effective in H-2b than H-2d mice. (Sciutto *et al*, 1990).

We now present a more comprehensive study which strongly suggests that differences in susceptibility of mice to *T. crassiceps* are largely controlled by gene(s) within or closely adjacent to the major histocompatibility complex (H-2). In addition, female mice are significantly more susceptible to *T. crassiceps* infection than males, demonstrating an as yet unexplained role of gender in this host-parasite relationship.

MATERIAL AND METHODS

Parasites

The fast-growing ORF strain of *T. crassiceps* (Freeman, 1962) was supplied by Dr. B. Enders (Behringwerke. Marburg, West Germany), and the slow-growing HYG strain by Dr. J. Grun (Dept. of Biochemistry, Jefferson Medical College, Philadelphia) and were kept by serial passage in BALB/c female mice for five years (Sally, Chau and Freeman, 1976).

Parasites used in this study were harvested from the peritoneal cavity of the donor mice after one to four months of infection.

Mice

All male and female mice used were 5-7-week-old at the start of the experiments. They were bred in our animal facilities by the "single line breeding system" during 20 generations, starting with original stock from Jackson Labs in 1982 and fed Purina's Diet 5015 *ad libitum*. The strains of mice used in these studies were chosen because they either possesed common genetic backgrounds but different alleles at genes within the major histocompatibility complex (MHC) (BALB/c (H-2d), BALB/b (H-2b), BALB/k (H-2k)), or shared common MHC genes but differed in genetic backgrounds BALB/c AnN, DBA/2, BALB/c DBA/2 F1 (H-2d);

C57BL/6J, BALB/b (H- 2b); C3H/HeJ, C3HeB/FeJ AND BALB/k (H-2k). This allowed independent evaluation of the influence of MHC or non-MHC genes on susceptibility to cysticercosis.

Infections

Metacestodes used in challenge infections were removed from BALB/c female mice carrying *T. crassiceps* ORF or HYG strain of cysticercus. Ten small (aprox 2 mm diameter) non-budding larvae were suspended in 0.5 ml of PBS (0.15 M NaCl, 0.01 M sodium phosphate buffer, pH 7.2) and injected intraperitoneally into each mouse using .25 gauge needle. The infected mice were sacrificed and the cysts found inside the peritoneal cavity were counted. In this form of disease the parasites never migrate to another location in the host. In one experiment, where the kinetics of parasite growth in BALB/c as compared with BALB/b was of interest, mice were sacrificed at several times after infection, during an 150 days time period.

Statistical Analysis

The statistical significance of the effects of the experimental variables (H-2 genes, genetic background, sex of mice and strain of parasite) upon parasite load was analyzed by multifactorial analysis of variance (ANOVA) (SAS Institute Inc., 1985). Because individual parasite load, as measured by total number of parasites in each mouse, was not found to be normally distributed and the variances among the different groups were

not homogeneous, the response variable (parasite load = number of parasites in each mouse) required algebraic transformation to perform proper comparisions between groups. The transformed parasite load that successfully met with the requirements of normal distribution and homogeneous variances among groups was the fourth root of the number of parasites in each mouse plus one ($\sqrt[4]{[\text{Number parasites} + 1]}$). This transformation does not imply significant numeric changes in parasite load. Transforming response variables seeks to reduce variances and normalize distributions, while the addition of unity eliminates troublesome zeroes without greatly altering numeric scores. However, in keeping with the usual lack of concern with the restrictions of statistical analysis, the results were also analyzed without any transformation. Identical conclusions were reached with both procedures.

RESULTS

Mice of nine strains (BALB/c AnN, DBA/2, BALB/c X DBA/2 F1, BALB/b, C57BL/6J, C3H/HeJ, C3He B/FeJ, A/J) were infected intraperitoneally with ten *Taenia crassiceps* ORF cysticerci per mouse, sacrificed 30 days after infection and the parasite load in each mouse was counted. The mean number of larvae established in each strain is given in Table I. Strains of the H-2d haplotype carried the most numerous cysticerci, while parasite loads in H-2b and H-2k haplotypes were comparatively smaller. No significant differences were recorded among the three H-2d susceptible strains tested. However, among female mice carrying the resistant haplotypes (H-2b, H-2k) there were significant differences associated to each strain, but not so in males. The A/J natural recombinant strain H-2a haplotype carrying the K^k A^k E^k D^d S^d alleles harbored parasite loads essentially identical to the other H-2d strains.

Table I includes three H-2 congenic mice with a BALB genetic background in which two phenotype classes could be distinguished: the susceptible H-2d carrying large parasite loads and the resistant H-2b and H-2k carrying smaller parasite loads. The influence of H-2 complex was furthered studied infecting these three H-2 BALB congenic strains with cysticerci from *Taenia crassiceps* of HYG strain in addition to the ORF strain, and the parasite loads obtained in the different haplotypes are shown in Table II. The same pattern of susceptibility was observed

although the HYG strain grew slower than ORF. Parasite loads in mice with H-2d were significantly higher than those in H-2k or H-2b haplotypes, which carried essentially the same low number of parasites.

Female mice were more susceptible to intraperitoneal cysticercosis than males. This strong association between the host's sex and parasite growth was observed in all the strains of mice (Table I), although to different extents, and with the two strains of parasites (Table II).

Table III shows the rate of growth of *Taenia crassiceps* ORF in the peritoneal cavity of congenic BALB/c (susceptible, H-2d) and BALB/b (resistant, H-2b) male and female mice. In the first ten days after infection, no macroscopic parasites could be recovered from infected BALB/b mice while a different pattern of growth occurred on resistant male BALB/c mice, in which parasites could be recovered as soon as one day after infection.

DISCUSSION

In the present study several inbred strains of mice were infected with cysticercus from two strains of *Taenia crassiceps* (ORF, fast, and HYG, slow). Mice with the H-2d haplotype were the most susceptible whereas H-2b or H-2k strains were comparatively resistant. A gene(s) within or closely adjacent to H-2 must therefore control innate mechanisms responsible for susceptibility versus resistance to this form of infection. That the natural recombinant (k/d) A/J strain retained the susceptible phenotype indicates that MHC mechanisms affecting parasite growth are located in the S and /or D region of H-2 genome, since this strain is K^k, I^k, S^d, D^d (Klein, 1986).

The non-H-2 genetic background of the mouse strains tested had considerably less influence on parasite growth, with small differences in susceptibility only in the context of H-2b or H-2k in female mice. Thus non-H-2 determined factors only come into effect in mice carrying the H-2 haplotypes conferring resistance.

The relation between susceptibility and the major histocompatibility complex has been studied in a variety of experimental mouse infections (Wakelin, 1978, 1985; Vadas, 1980) However, H-2 involvement has been definitely established or excluded in comparatively few cases. H-2 linked genes influence the response of mice to infection with *T. spiralis* (Wakelin and

Donachie, 1983; Wasson, Krco and David, 1987), the response of mice to vaccination against schistosome infection (Sher, Hieny and James, 1984) and the long-term response in systemic leishmaniasis caused by *Leishmania donovani* (Blackwell, Freeman and Bradley, 1980). In all of these infections genes mapping outside the MHC are also of importance.

In murine cestode infections there is previous evidence for genetic involvement in innate susceptibility to *Taenia taeniaformis* (Mitchell, 1982), and *T. crassiceps* (Larralde *et al*, 1988). In these studies, we have now shown that innate susceptibility may be controlled at the level of the H-2 while non-H-2 background genes contribute a small or negligible level of control.

The mechanisms involved in innate and/or acquired susceptibility have yet to be determined but H-2 associated differences in antigen presentation would be the most immediate area to explore. Nevertheless, antigen recognition by T cells in the context of I-E is unlikely involved considering that H-2b mice, that fail in I-E expression, are as resistant as H-2k mice, in which I-E molecules express normally (Wasson, Krco and David, 1987). Considering the susceptibility of A/J strain, antigen presentation in the context of class I antigens can not be ruled out. Nor the activation of cytotoxic lymphocytes

neither the role of complement are discarded as phenomena critically involved in susceptibility, since the S region of H-2 is also probably involved. Another possibility would be the presence of H-2d epitopes in *T. crassiceps* from adsorption of host-epitopes from the BALB/c stock mice in which the parasites have been kept for several years now. We do not believe this is the case because mice infected with parasites grown in BALB/b had the same susceptibility pattern (data not shown).

The differences in susceptibility between male and female mice merit comment. It appears that these differences occur only in the peritoneal experimental infection with cysticerci while there were no sex differences are detected in oral egg challenge of common voles and other rodents (Delvalle, 1989). If these sex associated differences vary because of differential parasite stage susceptibility or tisular location is open to question. Sex associated differences in susceptibility consistently exist in all the strains of mice used, although to varying extents depending of the H-2 genome of the host. A possible interaction between H-2 functions and endocrine enviroment is envisaged (Besedovsky, Del Rey, Soskin, 1986; Blalock *et al*, 1985).

As judged by parasite growth curves, the H-2 dependent control of parasite growth appears to occur in the first thirty days of infection or not at all, since no macroscopic parasites could be recovered from H-2b mice early in the infection. In

contrast, in the sex associated control of parasite growth different mechanisms are probably involved considering that parasites could be recovered as soon as one day after infection. Thus, resistance determined by H-2 genes may depend in different mechanisms than resistance attributable to gender.

Clearly, HYG strain grew slower than ORF in all H-2 strains of mice and in both sexes. This difference in rates of growth, intrinsic to the parasite, could well be the outcome of selective pressure coming from experimentation: ORF having been kept in the laboratory for many more generations than HYG. So long in fact that it is thought to have lost it's ability to transform in a tapeworm and to reproduce sexually (Smith, Esch and Kuhn, 1972).

Finally, the finding of MHC related resistance to murine cysticercosis encourage efforts to find or develop and select pig strains resistant to cysticercosis as ways of controlling transmission of *T. solium* cysticercosis in endemic countries.

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LEGENDS OF TABLES

Table I. Mean and standard error of the number of parasites recovered from the peritoneal cavities of mice 30 days after infection with 10 cyst (ORF) per mouse.

Table II. Mean of the number of parasites recovered from peritoneal cavity of ten males and females congenic BALB mice 30 days after infection with 10 cyst (ORF or HYG) per mouse.

Table III. Mean parasite loads in resistant and susceptible mice infected with ten cysts per mouse and sacrificed from 1 to 150 days after infection.

TABLE I

SUSCEPTIBILITY TO *Taenia crassiceps* (ORF) CYSTICERCOSIS OF MICE WITH d,b, AND k H-2 HAPLOTYPES ON DIFFERENT BACKGROUNDS

H-2 HAPLOTYPE	STRAIN	FEMALE AVERAGE PARASITE LOAD ± *SE (n)	MALE AVERAGE PARASITE LOAD ± SE (n)
d	BALB/c	110.3 ± 4.6 (70)[a]	25.8 ± 1.9 (50)[b]
	DBA/2	83.2 ± 9.6 (9)[a]	N.D.
	(BALB/c x DBA/2)F1	123.6 ± 17.3 (7)[a]	34.0 ± 4.2 (16)[b]
a(k/d)	A/J	115.3 ± 5.4 (47)[a]	30 ± 2.5 (45)[b]
b	BALB/b	18.1 ± 2.3 (18)[b]	0.9 ± 0.4 (20)[d,c]
	C57 BL/6J	4.8 ± 1.9 (28)[c]	0.4 ± 0.2 (20)[d,c]
k	BALB/k	33.1 ± 8.0 (15)[b]	0 (15)[d]
	C3H/HeJ	21.8 ± 5.3 (15)[b]	3.7 ± 1.7 (15)[d,c]
	C3HeB/FeJ	6.0 ± 2.2 (10)[c]	2.7 ± 1.5 (21)[d,c]

* Standard error of the mean
n number of mice tested
a,b,c and d different literals with $P < 0.010$
Statistics labeled with the same literal are not significantly different from each other while those labeled with different literals are significantly different.

TABLE II

ROLE OF H-2 GENES IN THE CONTROL OF *Taenia crassiceps* (ORF AND HYG) CYSTICERCI GROWTH IN THE PERITONEUM OF INFECTED BALB MICE

STRAIN OF MICE	H-2 HAPLOTYPE	S E X	STRAIN OF PARASITE: ORF AVERAGE PARASITE LOAD ± * SE	STRAIN OF PARASITE: HYG AVERAGE PARASITE LOAD ± SE
BALB/c	d	F	138.3 ± 13.0	29.4 ± 9.5
		M	18.8 ± 4.6	2.4 ± 0.9
BALB/b	b	F	52.1 ± 17.8	4.0 ± 1.2
		M	0.0 ± 0.0	0.0 ± 0.0
BALB/k	k	F	54.2 ± 23.3	3.0 ± 0.6
		M	1.7 ± 0.9	0.0 ± 0.0

* Standard error of the mean

T A B L E III

GROWTH OF *Taenia crassiceps* (ORF) IN RESISTANT AND SUSCEPTIBLE MICE

		DAYS OF INFECTION: 1	3	5	7	10	20	30	60	150
BALB/c	F	*0.8	1.2	0.1	3.6	7.1	7.4	136.7	577.0	2663.3
	M	0.4	0.3	0.0	1.1	1.3	2.3	25.1	112.1	789
BALB/b	F	0.0	0.0	0.0	0.0	0.0	1.0	20.6	99.1	3221.4
	M	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.9	186.6

* Mean of the number of parasites recovered from peritoneal cavity of ten mice per group.
F, female; M, male

ROLE OF ANTIBODIES IN EXPERIMENTAL MURINE CYSTICERCOSIS CAUSED BY *Taenia crassiceps*.

Sciutto, E., Díaz, M.L., Fragoso, G., Govezensky, T., Montoya, R.M. and Larralde, C.

Mailing Address:

Dr. Carlos Larralde
Instituto de Investigaciones Biomédicas, UNAM
Apdo. Postal 70228
04510 México, D.F.
México

Running Title: Antibodies in *T. crassiceps* cysticercosis

Keywords: T. *crassiceps*, cysticercosis, antibodies, susceptibility.

SUMMARY

The specific antibody response of mice to experimental infection with *T. crassiceps* cysticerci was studied in naive and vaccinated male and female mice, from two H-2 congenic strains that differ in their relative resistance to infection with this parasite. The more susceptible conditions had higher antibody levels than the resistant conditions at equivalent parasite loads: H-2d (BALB/c) > H-2b (BALB/b); females > males; naive> vaccinated. Passive transfer of whole immune sera or total immunoglobulins from resistant and susceptible strains did not transfer protection to this parasite. In fact, whole sera from susceptible female mice increased susceptibility of naive females of the susceptible strain. Western Blot analysis of antigen recognition by circulating antibodies revealed extensive similarity between strains, sexes and immune status. However,resistance is clearly associated with the host's antibody response to three very conspicuous antigen bands, migrating between 80 - 93 Kd. Males of BALB/c susceptible strain mounted strong antibody responses to these antigens only if vaccinated prior to challenge, while males from BALB/b resistant strain did so in response to infection without need of previous vaccination. Females from either strains were very poorly reactive to the same antigens, even if vaccinated, although quite responsive to many others. In practical terms these findings point to metacestodes obtained by experimental murine *T. crassiceps* cysticercosis as a very abundant and manageable source of antigens potentially useful in a design of a vaccine.

INTRODUCTION

Taenia crassiceps is a larval cestode parasite of mice and rats that has proven useful as a laboratory model for *T. solium* cysticercosis in the development of potential vaccines and diagnostic reagents. (Sciutto *et al* 1990; Larralde *et al*, 1989, 1990; Prokopic, Kudrna and Vanova, 1988). In addition, murine cysticercosis is quite amenable for scientific inquiries dealing with the biological factors involved in the relationship between host and metacestodes (Rickard and Williams. 1982; Mitchell, 1989). In fact, we have recently shown that MHC, sex and immunological state of the host are factors that, acting individually and interactively, strongly influence the rate of parasite growth and alter the chances of cysticercus ever taking up residence in the experimentally infected mouse (Sciutto *et al*, 1990).

The dominant role played by serum antibodies in host protection against many rodent larval cestode infections is well known. In *Taenia taeniaeformis* cysticercosis, the evidence is compelling that antiparasite antibodies are necessary for expression of resistance (Mitchell *et al*, 1982; Soulsby and Lloyd, 1982). Also, an association between the differing abilities of individuals to cope with other parasitic infections and their humoral immune response is well documented (Gibbens, Harrison and Parkhouse, 1986; James and Cheveer, 1985; Else and

Wakelin, 1989). In *T. saginata*, *T. crassiceps* and *T. solium* cysticercosis, however, research in this area had established the notion that fully developed metacestodes are unharmed by antibodies, while early larvae are very susceptible (Rickard and Coman, 1977; Siebert and Good, 1980; Harrison and Parkhouse, 1985) However in view of the notorious antibody response of cysticercotic individuals -mice and humans- we felt this had to be reassessed through the use of immunochemical techniques and methods of analysis, in hope of identifying antigens involved in resistance and collect clues for the proper design of a vaccine.

In this study the humoral response to infection and vaccination with *T. crassiceps* of two congenic strains of mice of both sexes, (representing the extremes of resistance (BALB/b) and susceptibility (BALB/c) to primary infection) was asessed by use of quantitative enzyme linked immunosorbent assay (ELISA), Western Blotting and by passive transfer of whole immune serum or immunoglobulins to determine whether the quantitative antibody response and/or the specificity of the antibody response is associated with natural or acquired resistance to infection.

MATERIAL AND METHODS

Mice, Parasites and Sera

Males and females BALB/c and BALB/b mice aged 5 to 7 weeks, were used in these experiments. Each individual mouse was infected with ten *T. crassiceps* (ORF strain) cysticerci injected intraperitoneally in 0.3 ml of PBS. The infection was allowed to proceed from 1 to 150 days before mice were sacrificed by bleeding. Their sera were stored aliquoted at -20°C and the number of cysts recovered from each mouse was recorded. The ORF strain of *Taenia crassiceps* was supplied by Dr. B. Enders (Behringwerke, Germany) in 1984. The larvae were kept in our animal facilities by serial passaging (IP) in female BALB/c mice every 2 to 4 months. Cysticerci for experimental use were collected from the donor mice by rinsing their peritoneal cavity with PBS in sterile conditions. All cysticerci used in these experiments were 2.0 mm or less in diameter and had no visible buds on their surface. Immune mouse serum was obtained from vaccinated BALB/c and BALB/b mice, of both sexes, vaccinated 30 days before challenge with a total antigen extract of *T. crassiceps* cysticerci (100 µg/mice) and sacrificed by bleeding 30 days after intraperitoneal challenge with ten cysts per mouse as described elsewhere (Sciutto *et al*, 1990)

Antibodies Purification

Pooled sera from infected mice (immune sera) were precipitated at 50 and 33 percent of $(NH_4)_2SO_4$, the residual ammonium sulfate was eliminated by exahustive dialysis and protein concentration were determined using a method modified from Lowry *et al* (1951).

Preparation of Parasite Antigen Used in Vaccination ELISA and Western Blots

Cysticerci to be used in the vaccination experiments were collected from the peritoneal cavity of the BALB/c female mice used for serial passage, washed in four changes of cold PBS (9 volumes of solution to 1 volume of parasite). then ultracentrifuged at 100,000 x g for 60 min at 4°C. The protein content of the supernatant was adjusted to 3 mg/ml and stored aliquoted at -70°C until use. Total protein concentrations were determined using a method modified from Lowry *et al* (1951).

Enzyme-Linked-Immunosorbent Assay (ELISA)

Sera were processed in ELISA as previously reported (Larralde *et al*, 1986). Three replicates of each serum were always analyzed. Briefly, the plates were coated with 100 µl of a

10 µg/ml of *Taenia crassicceps*, antigens the mouse sera were diluted 1:200 in PBS-Tween (.01%), and Ab-Ag reactions were developed with biotinylated sheep antimouse IgG (Amersham). A solution of o-phenilendiamine (.04% in citrate phosphate buffer, pH 5) (Sigma) was used as the substrate.

Western Blotting

Electrophoresis and immunoblotting of antigens were performed as described elsewhere (Burnette, 1981; Larralde *et al*, 1986). Optimal results were obtained incubating each strip of paper containing the transfered antigens with 100 µl of the serum to be tested, diluted 1:10 in PBS-Tween (.01%) for 4 hrs at room temperature. Biotin conjugated polyclonal antimouse total Igs and Peroxidase - streptavidin (Amersham), diluted 1:400 were used to develop the paper strips. The substrate used was o-cloronaftol (0.05 mg/ml, H_2O_2 0.03%).

Passive Transfer of Humoral Immunity

Whole immune serum and purified immunoglobulins transfer experiments were designed to explore the role of the bulk of antibodies. BALB/c mice were used as the recipients of sera. Sera collected from BALB/b, BALB/c, males and females, previously infected or vaccinated with *T. crassiceps*, were used as donors of sera.

Males and females BALB/c mice were challenged intraperitoneally with 10 cysticerci per mouse, and a single intraperitoneal injection (0.5 ml per mouse) of control or immune sera or total Igs (2 mg per mouse) from BALB/c mice (15 days vaccinated or 30 days infected) or BALB/b mice (30 days infected) of both sexes was given intraperitoneally at the same time. Cysticerci were recovered 30 days after the challenge infection and counted in each mouse. Ten mice were included in each experimental group. The Student's t-test was used to analyze the statistical significance of differences between the means transfered and control groups. P values equal to 0.05 or smaller were considered significant.

RESULTS

Total antibody levels to vesicular fluid antigens in BALB/c and BALB/b mice of both sexes.

All ELISA tests were performed using individual sera from each mouse, and were done in triplicate. The values from each mouse were graphed against the individual parasite load (n) plus one, to avoid zero values. (Figure 1). Clearly, sera from the infected BALB/c mice (susceptible) had higher antibody levels than sera from the infected BALB/b mice (resistant). Also, in BALB/c mice the number of parasites correlates possitively with the level of antibodies, more clearly so than an BALB/b mice. Male and female of both strains did not show significant differences in antibody levels at the same level of parasite load.

Passive Transfer of Humoral Immunity

The data shown in Table II indicate that no protection was transfered by sera nor by immunoglobulins (data not shown). Contrary to expectations, transfer of sera from vaccinated female BALB/c mice to female BALB/c mice, rather than inhibit, favored the development of the parasites in the transfered host.

Antigen discrimination in Western Blots by antibodies of BALB/c and BALB/B mice of both sexes and of naive and vaccinated mice.

The Western Blots of *Taenia crassiceps* antigen reacting with sera from females and males BALB/c and BALB/b mice, infected vaccinated and non-vaccinated, as well as with sera from intact non-infected mice are shown in Figure 2. Antigen preparation share many antigens (as determined from the ink stained strips, at the left margin in Figure 2) most of which were similary recognized by susceptible and resistant mice. But, the most relevant finding is that resistance is clearly associated to three antigen bands migrating between 80-93 kd males of BALB/c susceptible strain mounted strong antibodies responses to these antigens only if vaccinated prior to challenge, while males from BALB/b resistant strain did so in response to infection wuthout need of previous vaccination. Females poorly reactive to the same antigens, even if vaccinated, although quite responsive to many others.

DISCUSSION

The role played by antibodies in susceptibility or resistance to experimental murine cysticercosis was quantitatively and qualitatively studied in three conditions differing in susceptibility: MHC differences (BALB/b, H-2b [(resistant); BALB/c, H-2d (susceptibility)], gender differences [females (susceptible) and males (resistant)] and differences in immune status [vaccinated (resistant) and naive (susceptible)]. Our results indicate that the greatest bulk of of antibody inducing antigens, are not clearly associated with resistance or susceptibility to experimental cysticercosis. This is well in keeping with most literature on cysticercosis, established cysts being heedless of antibody and complement possible mediated by the anticomplementary activity of the cysticerci, and early larvae being the most susceptible (Mitchell, 1989; Pond, Wassom and Hayes, 1988; Gansmuller *et al*, 1987; Harrison and Parkhouse, 1985). Because the form of experimental cysticercosis studied herein was produced by the injection of in the peritoneal cavity of recipient mice it's no wonder that antibody response in general was found irrelevant for parasite growth. In fact, the bulk of circulating antibody did not only fail to transfer protection from vaccinated mice to naive recipients but managed to favor parasite growth in BALB/c (susceptible) females in a manner reminiscent of a variety of antibody mediated forms of immune evasion (Gibbens, Harrison and Parkhouse, 1986; Grzych *et al*, 1984, Mitchell, 1989)

As our results shown the molecular mechanisms of the immune response to this parasite would be an intricate network of interactions that could involverate sexes, vaccination and H-2 dependent events as it has been reported for other parasitic infections (Wunderlich *et al*, 1988; Reiner and McMaster, 1987).

However, with the aid of Western Blots a set of three antigens migrates between 80-93 kd was found to be associated with resistance.

Antigens associated with susceptibility or resistance could be only a serological markers as is the case of Sj26 (antigen associated at resistance in mice to *Schistosoma japonicum* but is inefective in inducing high level of resistance in vaccination, Smith *et al*, 1986; Wakelin, 1985) or a critical responsible in the response to the infection.

Whether immune responses to these antigens are involved in same way in expression of resistance to *T. crassiceps* infection remains to be determined but the identification of these antigens may have considerable relevance to the development of effective vaccines via natural or synthetic procedures as well as means of probing deeper in the molecular events regulating host parasite transactions.

Finally, the most important finding in this report is the identification of a set of individual antigens associated with resistance, that are consistely present in a manageable source of antigens such as those obtained from *T. crassiceps* cysticerci and are potentially usefull in a vaccine design.

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FIGURES AND LEGENDS

Figure 1.

Serum antibody response (as measured by ELISA OD 492 values to vesicular fluid antigens of BALB/c and BALB/b mice before (□ ●) and after infection (▸) with 10 cysts per mouse, mice were sacrified at day 1, 3, 5, 7, 10, 20, 30, 60 and 150 in groups of ten for each sex and their antibody responses graphed against the logaritm of the parasite load (n) plus 1 (log n + 1) to discard zero values. Both susceptible and resistant strains of mice mounted antibody synthesis in response to infection by *T. crassiceps*; however BALB/c antibodies increased with parasite load whereas BALB/b remained constant. Antibody responses by males and females plotted randomly in the graph.

Figure 2.

Western blots showing the antigen/antibody reactions detected in vesicular fluid, overlaid with sera from vaccinated and non vaccinated mice and developed with anti-mouse-Ig. Each strip correspond to serum from individual mice bled 30 days after infection.

Table I.

[a]Mean ± standard error of the number of parasite recovered from 10 female BALB/c (susceptible) recipient mice transfered with 0.5 ml of immune sera from female or male BALB/b (resistant), or BALB/c (susceptible). [c]Serum from 30 days infected mice (ten parasites per mice). [d]Serum from 30 days vaccinated mice (100 μg per mice) Passive transfer of immune sera from donors mice did not transfer resistance to BALB/c mice.Transfered sera from vaccinated female donor mice significantly increased the susceptibility of females recipients.

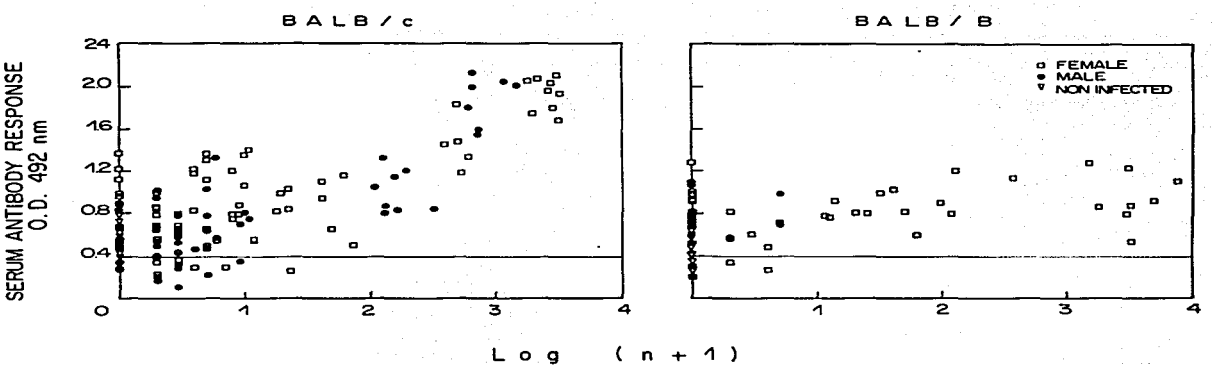
BALB/c
BALB/B
SERUM ANTIBODY RESPONSE
O.D. 492 nm
FEMALE
MALE
NON INFECTED
Log (n + 1)

FIGURE 1

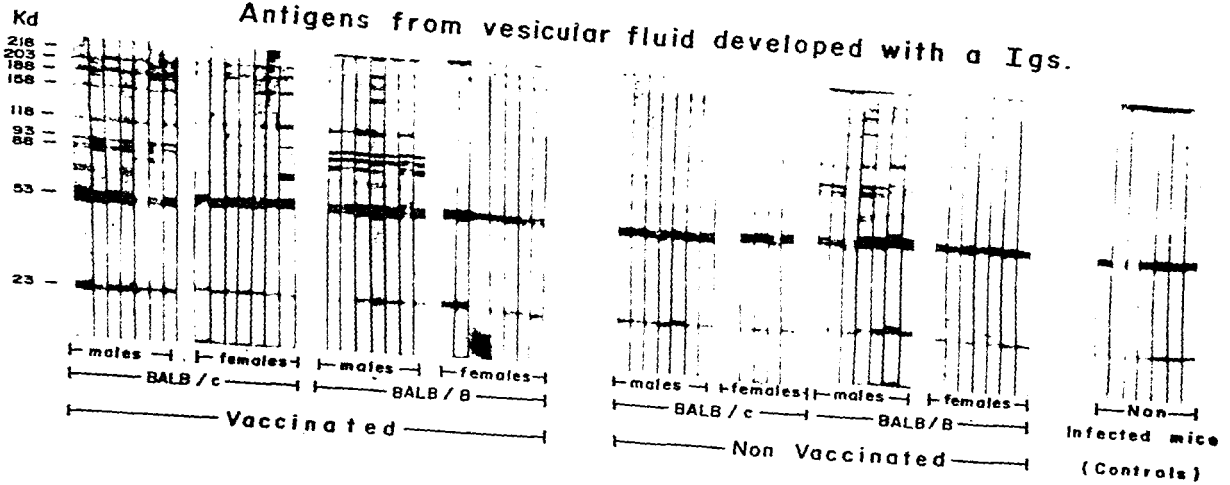
Kd
Antigens from vesicular fluid developed with a Igs.
218
203
188
158
118
93
68
53
23
males
females
males
females
BALB / c
BALB / B
Vaccinated
males
females
males
females
BALB / c
BALB/B
Non Vaccinated
Non-
Infected mice
(Controls)

FIGURE 2

TABLE I

SERUM TRANSFER FROM RESISTANT OR SUSCEPTIBLE MICE DOES NOT INDUCE HOST PROTECTION

	RECEPTORS OF SERUM (BALB/c)	
	Female	Male
Without serum transfered	150 ± 79	25 ± 22
With serum transfered from:		
BALB/c female		
°infected	°211 ± 106	N.D.
●vaccinacted	*311 ± 106	34 ± 15
BALB/c male		
°infected	156 ± 94	29 ± 23
●vaccinated	116 ± 108	20 ± 13
°BALB/b infected female	101 ± 49	N.D.
°BALB/b infected male	245 ± 113	40 ± 30

VII. ARTICULOS PUBLICADOS (en colaboración)

Am. J. Trop. Med. Hyg., 35(5), 1986, pp. 965–973

RELIABLE SEROLOGY OF *TAENIA SOLIUM* CYSTICERCOSIS WITH ANTIGENS FROM CYST VESICULAR FLUID: ELISA AND HEMAGGLUTINATION TESTS

CARLOS LARRALDE,* JUAN PEDRO LACLETTE,* CHARLES S. OWEN,** IGNACIO MADRAZO,† MIGUEL SANDOVAL,† RAFAEL BOJALIL,* EDDA SCIUTTO,* LUIS CONTRERAS,‡ JAVIER ARZATE,‡ MARIA LUISA DIAZ,* TZIPE GOVEZENSKY,* ROSA MARIA MONTOYA,* AND FEDERICO GOODSAID*

*Departamento de Immunologia, Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico, Mexico, **Department of Biochemistry, Jefferson Medical College, Philadelphia, Pennsylvania USA, †Departamento de Neurocirugia, Hospital de Especialidades, Centro Medico La Raza, Instituto Mexicano del Seguro Social, Mexico, and ‡Direccion de Inspeccion, Secretaria de Salubridad y Asistencia, Mexico

Abstract. New levels of reproducibility and sensitivity have been achieved in the detection of anticysticercus antibodies in human sera by using cysticercus vesicular fluid as the source of antigens for both ELISA and hemagglutination assays. Reproducibility both between tests on a serum and between similar sera was significantly improved over typical results using antigens from whole parasite extracts. Sera collected from uninfected individuals in endemic areas gave somewhat elevated values over those collected in nonendemic areas. This necessitated the use of a higher threshold in endemic areas to avoid false positives. With the threshold appropriate for a nonendemic area, both ELISA and hemagglutination were sensitive enough to detect infection in 95% of cases. With the threshold value for sera from an endemic area, these sensitivities were reduced to 80%–90%. A prominent 103-Kd protein of vesicular fluid, not related to antigen B, elicited the strongest antibody response in neurocysticercotic patients.

Cysticercosis caused by the metacestodes of *Taenia solium* is a serious health threat and economic burden in Latin America, Asia and Africa. A number of recent publications dealing with the parasite and the disease, including factors that propitiate transmission, testify to the growing concern for this parasitosis of humans and pigs.[1–3]

Epidemiological surveillance of human cysticercosis is probably only feasible by way of detecting circulating anticysticercus antibodies, due to numerous asymptomatic cases that would elude routine clinical examination. The cost of CT scan eliminates this powerful technology from the screening of populations. Likewise, lumbar tapping to identify soluble antigens in the CSF of neurocysticercoctic patients would probably meet with considerable resistance from the population under study.[4, 5]

Numerous techniques have been tried in search of anticysticercus antibodies but none has proven entirely satisfactory. Problems include poor reproducibility, cross-reactivity with other cestodes and a large fraction of false negative sera or questionable sensitivity because of inappropriate contrast with normal sera.[6–10]

To develop a serological test for the epidemiological surveillance of human cysticercosis we started by reevaluating all compartments of the parasite as sources of antigen. We obtained reliable serological results by employing soluble antigens from the vesicular fluid instead of the usual whole parasite extracts. Further, by comparing paired hemagglutination and ELISA methods on each serum, we show them to be roughly equal in sensitivity and specificity for neurocysticercosis. The unprecedented reproducibility of results is attributed to physical and chemical stability of the vesicular fluid antigens.

Accepted 7 April 1986.

MATERIALS AND METHODS

Antigens

Vesicular fluid from the cysticerci of *T. solium* was used as the only source of antigens. The cysticerci were dissected from skeletal muscle of pigs and collected in ice-chilled phosphate buff-

ered saline (PBS, pH 7.4). Immediately afterwards, the cysts were ruptured with scissors and vesicular fluid was collected. Calcium was removed by adding 50 μl of ammonium oxalate (0.3 M) and 25 μl of ammonia (1:3, v/v, in water) to each ml of vesicular fluid, centrifuging (2,000 × *g*, 4 min, at 20°C), and discarding all precipitated material. The supernatant, which contained all the initial proteins, was then collected and aliquoted in 100 μl samples and stored in liquid nitrogen. Minimizing the time between pig slaughter, fluid collection, and elimination of excess calcium, was critical for reproducible results. Final protein concentrations in the processed fluid varied somewhat between parasitized pigs, ranging from 3 to 6 mg/ml, but their qualitative protein compositions were quite constant.

Sera

Test sera were obtained through conventional procedures and grouped according to the likelihood of containing anticysticercus antibodies in the following groups: negative (10 sera from healthy people living in a nonendemic area—Federal Republic of Germany, provided by Dr. Enders, Behringwerke, Marburg, FRG); unlikely (12 sera from healthy people living in an endemic area—Mexico); suspicious (11 sera from neurologic patients in a health institution of Mexico); probable (14 sera from neurological patients with clinical and radiological pictures suggestive of cysticercosis—all Mexican); and confirmed (7 sera from patients from whose central nervous system a *T. solium* cysticercus had been surgically removed—all Mexican).

Serological tests

Sera were processed in 2 (ELISA) or 3 (hemagglutination) sessions; each session was performed the same day with the same batches of vesicular fluid and ELISA reagents and included 3 replicates of each serum, with some exceptions due to shortage of serum.

Optimal results in ELISA were obtained with Immulon (Dynatech) flat-bottom plates sensitized with antigen by incubating them with 100 μl of vesicular fluid, containing 10 μg of proteins, for 5 hr at 37°C and overnight at 4°C. After washing nonadherent proteins with PBS containing 0.05% Tween (PBS-T), plates were incubated with 100 μl of each serum diluted 1:15 in PBS for 3 hr at 37°C. The 1:15 serum dilution was established as the optimum by a set of preliminary experiments (not shown) which tested several dilutions for ability of the assay to distinguish between normal and cysticercotic human sera. The presence of anticysticercus antibodies attached to each well was detected by adding 100 μl of 1:400 biotinylated protein A (Amersham) in PBS-T for 1 hr at 37°C, followed by 100 μl of 1:400 biotinylated streptavidin-peroxidase (Amersham) in PBS-T for 30 min at 37°C. After washing, enzyme was detected on the plate by reaction with 100 μl of 0.4 mg/ml orthophenylenediamine (Sigma) and 0.03% H_2O_2 in citrate phosphate buffer (0.1 M, pH 5) for 30 min at 37°C. Plates were vigorously washed 5 times after each reaction step, washes lasting 5 min before flushing. The orthophenylenediamine reaction was stopped with 50 μl of 4 M sulfuric acid. Optical density readings at 492 nm were done in a Behring automatic ELISA processor.

Hemagglutination reactions were performed in v-shaped hemagglutination plates using sheep red blood cells (SRBC) sensitized with vesicular fluid antigens coupled via glutaraldehyde bridges.[11] SRBC were first washed several times in PBS and brought to a 50% suspension. To each 1 ml of the SRBC suspensions the following were added: 1.5 ml of 25% glutaraldehyde previously neutralized with 10% sodium carbonate, 5 ml of saline (0.15 M NaCl) and 1 ml of sodium biphosphate (0.015 M, pH 8). The mixture was stirred by rotation at 4°C for 24 hr. Afterwards, SRBC were washed 4 times with 30 times their volume of PBS and brought to a final 10% suspension in PBS. To each 1 ml of this glutaraldehyde treated SRBC suspension, 5 ml were added of 1:13 dilution of the cysticercus vesicular fluid in 0.1 M acetate buffer (pH 5). The mixture was gently stirred and the reaction allowed to proceed for about 16 hr at room temperature. SRBC were then washed 4 times in abundant PBS and separated by sedimentation or by gentle centrifugation and then resuspended in 0.1 M glycine in PBS to block the remaining free aldehyde groups.

Standardization of the hemagglutination reaction with hyperimmune rabbit serum against *T. solium* antigens indicated the critical importance of the serum diluent being PBS with 1% bovine serum albumin (BSA) in minimizing

nonspecific agglutination. Thus, all human sera were diluted with 1% BSA in PBS in a 2-fold serial fashion starting from a 1:10 dilution of the initial serum to reach 1:20,480. Each 50 μl of the final serum dilution was incubated with 25 μl of 3% sensitized SRBC, stirred by rotation for 10 min and left to sediment for about 2 hr at room temperature before scoring for agglutination. The serum titer was taken to be the inverse of the maximal dilution unmistakenly scored as positive.

Electrophoresis and immunoblotting

Polyacrylamide gel electrophoresis (PAGE) of the vesicular fluid contents, with sodium dodecylsulfate (SDS) and mercaptoethanol, was performed following the protocol of Laemmli using a Protean (BioRad) gel format at 10°C, 20–40 mA, for 3–4 hr.[12] The stacking gel was made with 3% acrylamide and 0.062% SDS, and measured 1.5 cm in length, 13 cm in width and 0.75 cm in thickness. The separating gel contained 7% or 10% acrylamide, 0.1% SDS and was 13 cm long, 13 cm wide and 0.75 mm thick. When required, staining of fixed gels was performed with 0.25% Coomassie brilliant blue R-250 in 45% methanol, 10% acetic acid in water (v/v) for 2 hr. Immunoblotting onto nitrocellulose paper was carried out by electrophoretic transfer at 1.4 A for 60 min in Tris-glycine buffer (pH 8.35), keeping the temperature below 37°C.[13] The paper containing the transferred antigens was then soaked overnight in 3% BSA in PBS-T and then cut into several 0.5-cm strips parallel to the axis of migration. Each strip was then incubated with 1 ml of the serum to be tested, diluted 1:15 in PBS-T for 6 hr at room temperature while stirring. Afterwards, the strips were washed by repeated immersions in 50 ml of PBS-T.

Binding of antibodies to the transferred paper strips was visualized by immersing each of the strips in a 1:400 dilution (in PBS) of the commercial preparation of biotinylated protein A for 1 hr at room temperature, followed by another immersion in a 1:400 dilution in PBS of biotinylated streptavidin-peroxidase for 30 min. Enzyme activity on the paper was developed by immersing the strips in a solution containing 30 mg of o-chloronaphthol (Sigma), 10 ml of methanol, 50 ml of PBS and 50 μl of H_2O_2 added immediately prior to use. Protein bands having bound antibodies stained black 5–10 min after immersion, contrasting with those not binding antibodies or binding a few nonspecific immunoglobulins, which stained light gray or not at all.

RESULTS

ELISA

Table 1 shows the results obtained with ELISA in 53 sera processed simultaneously in 1 session, grouped according to the likelihood of the serum sample coming from a donor having had previous exposure to the parasite. Entries for individual sera are the mean absorbance of 3 independent incubation mixtures followed by the standard deviation (SD), except in 7 cases where single determinations were performed due to lack of sample material. For each of the likelihood groups, the values for the individual sera were averaged and the mean and SD are given, as well as the coefficient of variation (mean/SD).

A significant increase in antibody concentrations, in going from the population with a small chance of infection to those in a confirmed cysticercotic state, was clearly shown by a 20-fold increase in mean values of absorbance. The variations within measurements on a given serum and between sera in each group increased even more rapidly than did the mean. This is indicated by the decreasing coefficient of variation of each group.

An important parameter in serological diagnosis is the threshold value of the response variable that, when exceeded by an individual determination on a sample, results in the donor being classified as serologically positive. These threshold values have been calculated separately using the nonendemic (negative) group and the endemic (unlikely) group as the negatives, with 95% confidence in each case. As shown in Table 1, these values (the mean + 2 SD) were 0.043 absorbance units for the nonendemic (negative) group and 0.085 for the endemic (unlikely) group.

Hemagglutination

Hemagglutination of SRBC coated with vesicular fluid antigens produced results very similar to those of the ELISA. Table 2 shows the results from a representative single session. The first

TABLE 1
Mean absorbance ± standard deviation by ELISA

Sera	Serum groups by likelihood of cysticercosis infection				
	Negative healthy nonendemic	Unlikely healthy endemic	Suspicious neurological patients	Probable neurological patients	Confirmed neurocysticercosis patients
1	0.030	0.037 ± 0.005	0.061 ± 0.007	0.894 ± 0.187	>2.200 ± 0.000
2	0.027	0.075 ± 0.019	2.140 ± 0.085	0.171 ± 0.041	0.637
3	0.038 ± 0.014	0.080	0.070 ± 0.017	0.028 ± 0.001	1.242 ± 0.251
4	0.027 ± 0.003	0.077 ± 0.020	0.071 ± 0.002	0.555 ± 0.180	0.080 ± 0.002
5	0.031 ± 0.001	0.054	1.405 ± 0.267	1.253 ± 0.293	0.074 ± 0.024
6	0.025 ± 0.001	0.037 ± 0.016	0.305 ± 0.111	1.197 ± 0.355	0.411 ± 0.012
7	0.027 ± 0.002	0.030	0.018 ± 0.001	0.395 ± 0.100	0.286 ± 0.099
8	0.025 ± 0.004	0.028 ± 0.004	0.035 ± 0.001	1.425 ± 0.441	
9	0.025 ± 0.003	0.042 ± 0.010	0.149 ± 0.038	0.231 ± 0.118	
10	0.028 ± 0.007	0.036 ± 0.014	0.562 ± 0.232	0.521	
11		0.031 ± 0.001		0.081 ± 0.016	
12		0.026 ± 0.004		1.397 ± 0.228	
13				1.170 ± 0.218	
14				0.778 ± 0.253	
Mean	0.029 ± 0.007	0.043 ± 0.021	0.482 ± 0.695	0.748 ± 0.550	0.746 ± 0.760
Coefficient of variation	4.41	2.04	0.69	1.36	0.98
Threshold	0.043	0.085			

well in each serial dilution contained a 1:10 dilution of each test serum, so that the numbers given in the table represent the $\log_2$ of one-fifth the effective titer. As before, the mean and SD are given for 3 independent replicate measurements of each serum, and the mean, SD and coefficient of variance are also given for each serum group.

In the 5 groups, both the mean titers and the degree of variation between samples within a group grew with increasing likelihood of previous contact with the cysticercus antigens. The 95% threshold values for the nonendemic (negative) group and the endemic (unlikely) group are shown in Table 2 along with the corrected threshold values, which are the same rounded up to the next integer. As with the ELISA, the threshold values using sera of donors from nonendemic areas were about half those which were found by using comparable sera from endemic areas.

Sensitivity

The sensitivities of both assays resulted from a combination of 2 factors: the strength of the mean detectable response to sample antibodies and the reproducibility of the assays. Both methods were sensitive enough to detect a difference between panels of samples from apparently uninfected individuals in nonendemic and endemic areas. This resulted in a higher threshold being required to avoid false positives in endemic areas. The number of false negatives which would result from use of either threshold can be estimated from the fraction of the samples in the probable and confirmed groups which lie below each threshold value. False positives would result from the use of the lower threshold in assays of samples from endemic areas.

These findings are summarized in Table 3. Both the ELISA and the hemagglutination assay reported positive results for 95% of the probable and confirmed samples when compared with nonendemic controls. The results with respect to the threshold for an endemic area dropped to 91% for the hemagglutination and 80% for the ELISA. Looking only at the suspicious group and using the endemic area threshold, the hemagglutination assay supported the diagnosis of neurocysticercosis in 70% of the cases and ELISA supported it in 50%.

Reproducibility

A major difference between our serological results and those using other procedures is the high degree of reproducibility which was found within

TABLE 2
Mean titer ± standard deviation by indirect hemagglutination

Sera	Serum groups by likelihood of cysticercosis infection				
	Negative healthy nonendemic	Unlikely healthy endemic	Suspicious neurological patients	Probable neurological patients	Confirmed neurocysticercosis patients
1	1.0 ± 0.0	1.3 ± 0.4	2.6 ± 0.4	10.0 ± 0.0	12.0 ± 0.0
2	1.0 ± 0.0	1.0 ± 0.0	12.0 ± 0.0	6.0 ± 0.8	10.6 ± 0.4
3	1.3 ± 0.4	5.0 ± 0.8	5.6 ± 0.4	2.6 ± 0.4	5.0 ± 0.0
4	1.0 ± 0.0	2.0 ± 0.8	3.0 ± 0.8	10.5 ± 0.5	7.0 ± 0.8
5	1.0 ± 0.0	1.0 ± 0.0	11.0 ± 1.4	9.3 ± 0.9	7.0 ± 0.8
6	1.0 ± 0.0	1.0 ± 0.0	7.3 ± 0.4	9.3 ± 0.4	9.0 ± 1.6
7	1.0 ± 0.0	1.0 ± 0.0	1.6 ± 0.9	9.0 ± 1.4	9.0 ± 1.6
8	1.0 ± 0.0	2.0 ± 0.8	2.0 ± 0.0	10.6 ± 0.4	12.0 ± 0.0
9	1.0 ± 0.0	1.0 ± 0.0	6.6 ± 1.2	10.3 ± 0.4	
10		1.0 ± 0.0	7.6 ± 1.2	10.0 ± 0.0	
11		1.0 ± 0.0		7.0 ± 0.0	
12		1.0 ± 0.0		11.3 ± 0.9	
13		1.5 ± 0.5		10.3 ± 2.3	
14		1.0 ± 0.0		8.3 ± 0.9	
15		1.0 ± 0.0		1.0 ± 0.0	
16		1.0 ± 0.0			
17		1.0 ± 0.0			
18		1.0 ± 0.0			
19		1.0 ± 0.0			
20		1.0 ± 0.0			
21		1.3 ± 0.4			
22		1.3 ± 0.4			
23		1.0 ± 0.0			
24		1.0 ± 0.0			
25		1.3 ± 0.4			
26		1.6 ± 0.4			
Mean	1.0	1.3	5.9	8.3	8.9
Standard deviation	0.1	0.7	3.4	2.9	2.5
Coefficient of variation	9.8	1.6	1.7	2.8	3.5
Threshold	1.2	2.9			
Corrected threshold	2.0 (1:20)	3.0 (1:40)			

measurements on each serum sample and between sera in each group. For example, in the ELISA, the mean for a given serum was greater than twice the SD in all but 1 case. Similarly, in the hemagglutination assay, only 1.4% of the sera showed SD exceeding half of the mean for that serum. Spearman's rank-order correlation coefficient (r_s) between the ELISA and hemagglutin-

TABLE 3
Effect of choice of threshold on fraction of sera above threshold

Threshold value	Fraction above threshold (specificity*)		Fraction above threshold (sensitivity*)
	Negative nonendemic	Unlikely endemic	Confirmed + probable sera
ELISA			
Nonendemic (0.043)	0/10 (1.0)	4/12 (0.7)	20/21 (0.95)
Endemic (0.85)	0/10 (1.0)	0/12 (1.0)	17/21 (0.80)
Hemagglutination			
Nonendemic (1:20)	0/10 (1.0)	3/26 (0.88)	22/23 (0.95)
Endemic (1:40)	0/10 (1.0)	1/26 (0.96)	21/23 (0.91)

* Specificity = fraction of confirmed and probable sera above threshold. Sensitivity = 1-fraction of healthy sera above threshold.

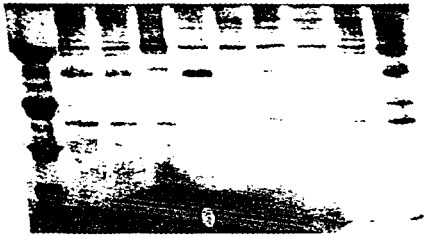

FIGURE 1. SDS-PAGE of proteins and peptides in solution in the vesicular fluid of 6 different collections of *T. solium* cysticerci, stored for different times in liquid nitrogen, frozen and thawed once or twice, and dissected immediately after the pigs' death or 1 day afterwards. Major bands located between molecular weights 200–38 Kd are quite constant from collection to collection and seem to withstand prolonged storage, freezing and thawing, and some delay in harvesting. Differences between collections localize in molecular weights <30,000 or >130,000 d. Molecular weight standard (far left) includes phosphorylase B (92,500 d), bovine serum albumin (68,000 d), ovalbumin (43,000 d) carbonic anhydrase (31,000 d), soybean trypsin inhibitor (21,500 d) and cytochrome C (14,400).

ation mean scores of the 51 sera that were processed with both procedures showed a very close association ($r_s = 0.9$, $t = 14.4$, $P < 0.0001$). Rank correlation was preferred over linear correlation because the hemagglutination variable is not a truly continuous variable.

Vesicular fluid antigens of T. solium

Figure 1 shows the similarity of vesicular fluid SDS-PAGE chromatograms for proteins and peptides extracted from different cysticerci, stored for different periods, frozen and thawed once or twice, or dissected immediately after death of the pig or 1 day later. Figure 2 shows the densitometry of a representative SDS-PAGE. There are about 15 distinct protein bands, with molecular weights ranging from 246 to 26 Kd and a cloud of assorted peptides in a broad region between 10 and 17 Kd. The most prominent are those located at 246, 149, 103, 65, 47 and 40 Kd, which will be referred to as P246, P149, P103, etc.

The immunoblots of the SDS-PAGE chromatograms with sera from cysticercotic patients (Fig. 3) showed antibody response in the naturally infected host to a number of the vesicular fluid proteins. The most distinct antigen band corresponds to P103, which is also the one most frequently recognized by the immune sera. Considerable antibody activity is also directed to the lower molecular weight proteins and to the cloud of assorted peptides but in a manner more variable between some. P103 did not react with conventional or monoclonal antibodies against antigen B (data not shown).[14–16] Further, antigen B could not be found in significant concentrations in the vesicular fluid (Figs. 1 and 2). In addition, it also should be mentioned that we could not detect any host or host-like proteins in unadulterated vesicular fluid when the immunoblots were reacted with mouse anti-pig serum.

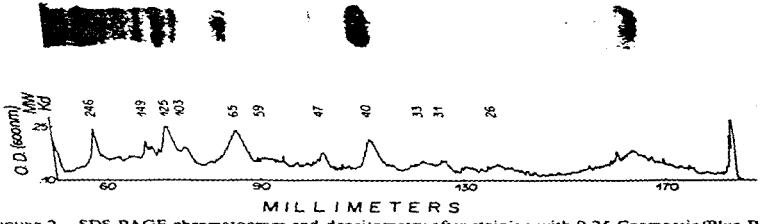

FIGURE 2. SDS-PAGE chromatogram and densitometry after staining with 0.25 Coomassie/Blue R-250 to better illustrate the protein and peptide composition of the vesicular fluid of the cysticercus of *T. solium*. Most notable protein bands localize at 246, 149, 103, 65, 47, 40, 33, 31 and 26 Kd. The last 3 bands are usually very faint. A cloud of stain, possibly indicating an assorted peptide mixture, is a common finding.

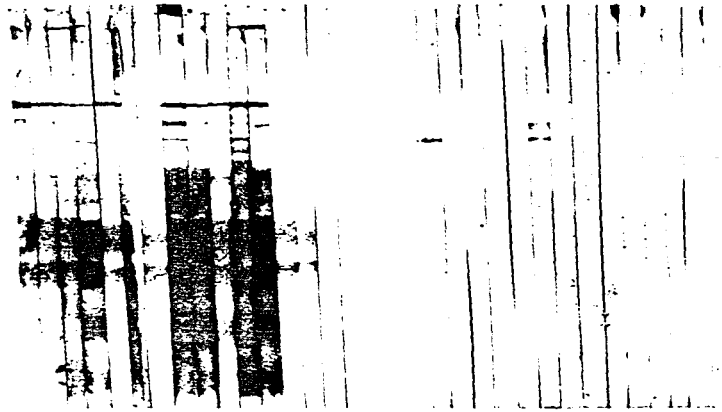

FIGURE 3. Immunoblots (biotinylated protein A, biotinylated streptavidin-peroxidase, o-chloronaphthol) after SDS-PAGE electrophoresis of the antigens in the vesicular fluid of *T. solium* cysticercus as detected by the sera of 16 different neurocysticercotic patients (first 16 strips from left to right) and of 15 different healthy donors residing in Mexico. The reaction patterns seem to differ somewhat between patients, 2 not reacting at all, but most have antibodies to the very conspicuous protein band located at 103 Kd (P103). There is also quite an active immune response in the patients against antigens located at molecular weights < 50 Kd but the reaction here is diffuse and does not resolve in clear-cut bands. A couple of the healthy donors have antibodies against proteins located between 90–60 Kd but none react with P103. Because of shortage of sera, we could not perform a complete design matching each serum reported in Tables 1 and 2 with an immunoblot pattern, only some were possible. However, we are under the impression that the most reproducible result in immunoblotting is the reaction of most of the patient's sera, with P103 and with the cloud of peptides of low molecular weights, the rest of the bands behaving somewhat capriciously. In fact, in our experience, immunoblotting proved to be so susceptible to variation that we find it useful in detecting interesting antigens but of little reliability as a diagnostic tool.

DISCUSSION

Two different immunoassay procedures, hemagglutination and ELISA, have been used with antigens from cysticercus vesicular fluid to detect serum antibodies in infected donors. The assays were striking in their reproducibility and performed very similarly when tested against 5 panels of sera in 3 different sessions. Both assays were sensitive enough to detect differences between sera from healthy donors in areas where cysticercosis is endemic and nonendemic. The ability to detect infection serologically is not limited by the inherent sensitivity of the assay, but only by the higher baseline of detectable antibody in normal samples from areas where cysticercosis is endemic. This difference presumably arises from previous exposure to parasites which share antigens with those of the assay.[19, 20]

Both ELISA and hemagglutination assays detected antibodies in samples from infected individuals with approximately 95% sensitivity in a nonendemic area, and 80%–90% in an endemic area. These percentages were estimated by taking results from individuals who were known to be, or who were very likely to be, infected, and comparing with threshold values drawn from the appropriate panel of uninfected controls.

The performance of the hemagglutination assay may be particularly helpful in the epidemi-

ological surveillance of taeniasis/cysticercosis since that technique is well suited for numerous samples at modest cost per sample. Likewise, it may well be the method of choice in medical institutions lacking access to the most sophisticated diagnostic procedures. Differences between hemagglutination and ELISA were minimal, although we noted that the former supported the diagnosis of neurocysticercosis in 70% of the suspicious sample group while ELISA supported it in only 50%. Evaluation of the significance of this finding must await the collection of more clinical, surgical or anatomical data from this group with which to compare these results.

We attribute the reproducibility of results to the use of vesicular fluid as a source of stable antigen instead of the usual homogenates of cysticercal tissues. It is in marked contrast with previous work using homogenates or partially purified homogenates of whole parasite in double immunodiffusion, immunoelectrophoresis, complement fixation and ELISA assays.[14–16, 19–22] Possible explanations for the stability of vesicular fluid antigens over those from homogenates include the presence of intracellular enzymes in homogenates and the natural tendency of materials extracted by acid and or strong salt solutions to aggregate when placed in neutral isotonic media. Even purified or partially purified molecules, like antigen B, from the solid parts of the parasite have been quite unstable in solution, tending to gelatinize or precipitate forming fibrils.[16]

Immunoblots of vesicular fluid SDS-PAGE patterns confirmed the heterogeneity of the immune response by humans to this parasite, as detected with less sensitive techniques.[14, 15, 20] One antigen in particular, P103, was strongly favored. Since P103 did not share antigenic determinants with antigen B from crude extracts, it is anticipated that the effectiveness of immunoassays can be further improved if a controlled and stable mixture of P103 and antigen B can be devised.

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DECIPHERING WESTERN BLOTS OF TAPEWORM ANTIGENS (*TAENIA SOLIUM, ECHINOCOCCUS GRANULOSUS,* AND *TAENIA CRASSICEPS*) REACTING WITH SERA FROM NEUROCYSTICERCOSIS AND HYDATID DISEASE PATIENTS

C. LARRALDE, R. M. MONTOYA, E. SCIUTTO, M. L. DIAZ, T. GOVEZENSKY, AND E. COLTORTI

Departamento de Immunologia, Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico, and Centro Panamericano de Zoonosis, Buenos Aires, Argentina

Abstract. Complex antigen mixtures displayed in Western blots may be immediately and quantitatively categorized with respect to specificity and immunogenicity by immunoplotting. This involves plotting the frequency with which each antigen band reacts with a set of immune sera against the frequency of the same band when reacted with another set of immune sera. Immunoplotting has proven to be a powerful method of analyzing Western blots of reactions between vesicular fluids from the metacestodes of *Taenia solium, E. granulosus,* and *T. crassiceps,* and sera from human cases of neurocysticercosis and hydatid disease. Immunoplotting readily sorts out those antigens useful for discriminative immunodiagnosis from the multitude of bands in the sera of sick and healthy people. It aids in assessing the antigenic similarity between the human parasites and the murine parasite *T. crassiceps,* validating the latter as an alternative source of antigens for immunodiagnosis of cysticercosis and hydatid disease.

Western blotting is of great value in the analysis of antigen mixtures because it combines the fine resolution of polyacrylamide gel electrophoresis with the high sensitivity of antibodies coupled to tracers, enzymes, or radionuclides.[1] However, in cases of complex antigen mixtures, especially when contrasts with other equally complex mixtures are performed, unaided visual inspection of the blots usually misses the details of the antigen profile. Thus, to fully realize its potential, Western blotting requires a systematic yet operative procedure of analysis commensurate with it's biochemical virtues.

To this end, we have developed the immunoplot. This simple graphic procedure of contrasting blots was developed while studying the antigen composition of some cestodes causing serious human disease, *Taenia solium* and *Echinococcus granulosus,* and a related murine parasite, *Taenia crassiceps.*

We expected these parasites would pose a challenge to antigen analysis because of the variety of specific and shared antigens,[2] and because of Western blotting's great sensitivity. Immunological diagnosis of neurocysticercosis and hydatid disease requires a thorough analysis of antigen composition of involved parasites. Antigen identification would certainly help immunodiagnosis in places where CT and NMR imaging are unavailable[3–5] and aid in recognizing risk areas and factors involved in transmission. Furthermore, careful antigen discrimination could advance our understanding of the parasites causing chronic disease, and why some produce severe and even fatal damage while other progress benignly or go unnoticed.[6] *T. crassiceps* was included because it was hoped extensive antigen similarity in this murine parasite would provide a less variable source of antigen than usual the sources, which are *T. solium* antigens from cysticercotic pigs[7] and *E. granulosus* antigens from parasitized ovines, bovines, humans, and camels.[8]

MATERIAL AND METHODS

Immunoplot

The simplest immunoplot is the plot of the frequency with which each individual antigen

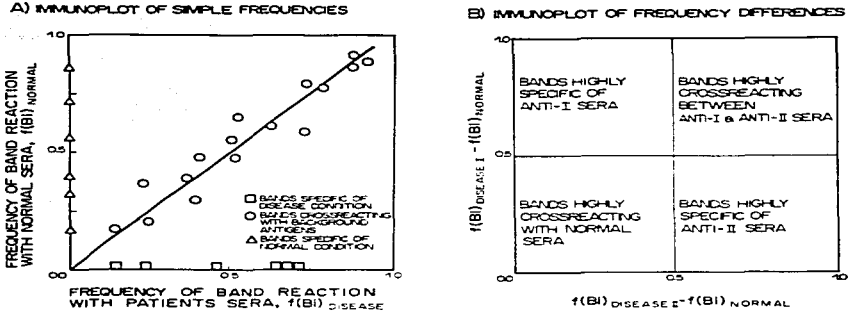

FIGURE 1. Plotting the frequency of reaction of each antigen band in the set of sera from a given disease condition (fBi) against that found in normal sera (A) or against that found in the sera of a different disease condition after subtracting background reactivity (B) places each antigen band in regions of the plot with distinct immunological meaning.

band in a Western blot reacts with a set of sera collected from patients of a certain disease against the frequency of that same antigen band when reacted with sera from normal donors. This immunoplot of simple frequencies (Fig. 1A) allows immediate identification of troublesome antigens reacting with normal sera because they plot in the plane between the axes, while those reacting only with patients' sera plot on the corresponding axis at levels related with the frequency of reaction.

A more elaborate but most informative immunoplot results from increasing contrast with background by subtracting the frequency of each band obtained with normal sera from that in infected sera. Thus, plotting these frequency differences in sera from patients with a certain disease against those in sera from patients with a contrasting disease defines a plane wherein the position of each antigen band has an immediate immunological meaning (Fig. 1B). Any band having zero or negative frequency values is of doubtful interest for the immunology of the diseases involved. Any band having a low frequency value in 1 axis but a high frequency value in the other is a candidate for discriminating immunodiagnosis. Any band having high frequency values in both axes is favorable for nondiscriminating diagnosis and is of tentative interest in cross-reacting immunity. The spread in the cluster indicates heterogeneity in immunogenicity among the antigens in the mixture. Linearity of the cluster points to antigens of related immunogenicity in both antigen extracts, their ordering from low to high frequency values reflecting their increasing immunogenicity. The linear slope of the plot, if any, would point to the overall tendency of the antigen mixture to favor specific reaction with I or the other category of sera (slope near unity meaning equal tendencies).

Collecting the frequency values for each band is done by first constructing a Western blot master pattern that includes all different bands in the antigen mixture (that is, all bands appearing at least once in the blots when reacted with all sera) ordered by molecular weight, and then scoring for each band's presence or absence in each serum. The frequency value of each individual band is the sum of all sera in the category that reacted with that band divided by the total number of sera in the category.

The decision of whether a given serum reacts or not with a certain band is crucial. Scoring is not a major problem when the blots are exceptionally clean, but this is seldom the case with whole parasite extracts reacting with many different sera in a single session. Furthermore, as there is quite a range in reactions, scoring may

be uncertain in the case of tenuous bands located in blurred sectors of the blots. We have found that band detection by visual inspection performed by an experienced technician is as reliable as the currently available computer technology in decisions on peak detection in densitograms. There is no question as to the need to improve this phase of the procedure. Meanwhile, it is safe to work only with clearly visible bands, or to categorize them according to conspicuousness before attaching much meaning to conclusions derived from their observed positions in the immunoplot.

Antigens and antisera

The antigens of all parasites were those in solution in the vesicular fluid of the respective metacestodes. These antigens were selected because they provide the most reliable serological results.[9, 10] *T. solium* cysticerci were dissected from skeletal muscle of naturally infected pigs slaughtered in Mexico City's abbatoire Ferreria. The vesicular fluid was collected by slicing the cyst walls and letting the fluid drain into sterile vessels immersed in crushed ice. Metacestodes of *T. crassiceps* were collected from the peritoneal cavity of BALB/c mice infected experimentally with strain ORF of *T. crassiceps* as described by Kuhn,[11] and their vesicular fluid obtained after rupturing the cysts by centrifugation (35,000 × *g*) and collecting the supernatant. Immediately after collection, the vesicular fluids were treated to remove calcium by adding 50 μl of ammonium oxalate (0.3 M) and 25 μl of ammonia (1:3 v/v in water) to each ml of vesicular fluid. This was then centrifuged at 100,000 × *g* for 60 min at 4°C to discard particulated material. The supernatant was then divided into 100 μl aliquots and stored in liquid nitrogen until use. Final protein concentrations in the processed fluids varied somewhat between parasitized pigs and mice (3–6 mg/ml), but their qualitative protein compositions were quite constant. Vesicular fluid was collected from hydatid cysts found in the liver of infected sheep and then lyophilized; later, just before use, it was resuspended in PBS and its concentration adjusted to 3 mg·ml total protein (PBS donated by Centro Panamericano de Zoonosis, Buenos Aires, Argentina).

Sera were obtained through conventional procedures from donors with different parasitic conditions: 30 sera came from asymptomatic adult patients living in Mexico; 21 cysticercotic sera were collected from patients with confirmed neurocysticercosis hospitalized in different health institutions in Mexico (Instituto Mexicano del Seguro Social, Instituto de Salubridad y Seguridad Social de Trabajadores del Estado and Instituto Nacional de Neurologia y Neurocirugia); and 27 sera from patients with hydatid disease were donated by E. Ziegelmeyer (Behringwerke, Marburg, FRG), P. Schantz (Communicable Disease Center, Atlanta, GA) and by Centro Panamericano de Zoonosis, Buenos Aires, Argentina.

Western blotting

Electrophoresis and immunoblotting of the vesicular fluid of all parasites was performed as described elsewhere.[9, 12]

RESULTS

Western blots

Illustrative Western blots of the 3 parasite antigens reacting with sera from neurocysticercosis and hydatid disease patients as well as with sera from normal donors are shown in Figure 2. Although the photographs show less detail than the original paper strips, several things are immediately apparent. To begin with, there are numerous antigen bands in the 3 different antigen extracts with molecular weights of >300–~20 kDa. Also, the sera of neurocysticercosis patients, as well as those of hydatid disease patients, react with antigens from *T. solium*, *E. granulosus*, and *T. crassiceps*. Furthermore, normal sera have less intense reactions than infected sera, but they do react with several antigens in all 3 extracts. The use of *T. crassiceps* antigens result in more well-defined reaction patterns than those with the other parasites' vesicular fluids. Finally, in spite of the great similarity between patients' blots, there are also differences, some outstand-

FIGURE 2. Illustrative Western blots of sera from neurocysticercotic and hydatid disease patients and normal donors reacting with vesicular fluid antigens of *T. solium*, *E. granulosus*, and *T. crassiceps*. The Western blot master pattern of each antigen preparation is shown on the left margin of each panel of blots.

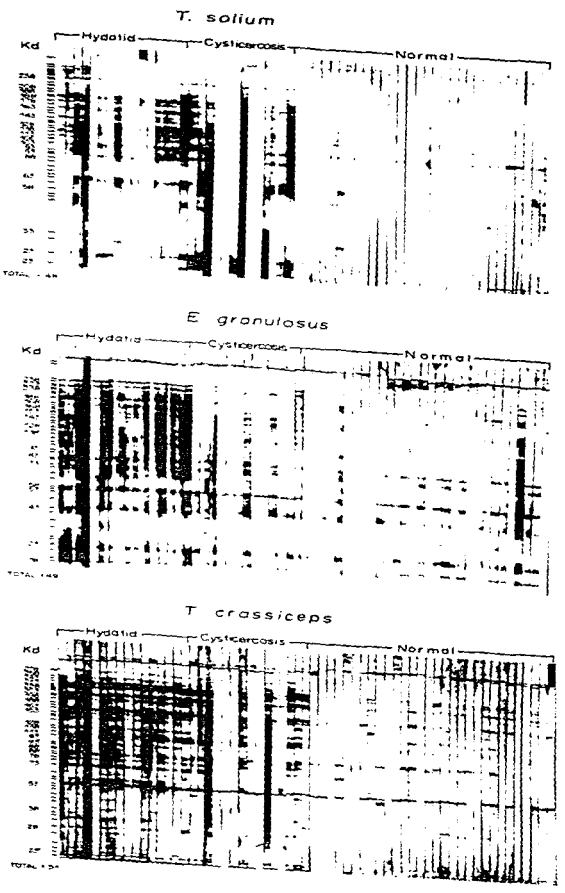
T. solium
Hydatid
Cysticercosis
Normal
Kd
E. granulosus
Hydatid
Cysticercosis
Normal
Kd
T. crassiceps
Hydatid
Cysticercosis
Normal
Kd

TABLE 1

Listing of all different antigen bands found in the vesicular fluid of T. solium, E. granulosus, *and* T. crassiceps *by molecular weight ranking order (inner columns), by molecular weight estimates (leftmost column), and by average kDa used in immunoplots (rightmost column)*

kD interval of band location	*T. solium*	*E. granulosus*	*T. crassiceps*	Band identification by mean kD
311–315	1			313
306–310		1		308
301–305		2		303
296–300				
291–295				
286–290	2			288
281–285				283
276–280	3		1	278
271–275				
266–270	4			268
261–265	5			263
256–260	6		2	258
251–255	7			253
246–250	8		3	248
241–245		3	4	243
236–240	9	4		235
231–235	10		5	233
226–230		5	6	228
221–225	11	6	7	223
216–220	12	7		218
211–215		8	8	213
206–210	13		9	208
201–205		9	10	203
196–200	14	10	11	198
191–195				193
186–190	15, 16	11	12	188, 188'
176–180	17		13	178
171–175		12	14	173
166–170	18	13, 14		168, 168'
161–165	19	15	15	163
156–160	20	16		158
151–155	21	17, 18	16	153, 153'
146–150			17	148
141–145	22	19	18	143
136–140	23	20	19	138
131–135	24	21		133
126–130	25	22	20	128
121–125	26		21	123, 123'
116–120	27	23, 24	22	118, 118'
111–115	28		23	113
106–110	29	25		108
101–105	30	26		103
96–100		27	24, 25	98, 98'
91–95	31, 32	28	26, 27	93, 93'
86–90	33	29	28, 29	88, 88'
81–85	34	30, 31		83, 83'
76–80	35		30, 31	78, 78'
71–75	36, 37	32, 33	32	73, 73'
66–70	38	34		68, 68'
61–65	39	35	33	63
56–60	40, 41	36	34, 35	58, 58'
51–55	42	37, 38	36, 37, 38	53, 53', 53"
46–50	43, 44	39	39, 40	48, 48'
41–45		40	41	43
36–40		41	42, 43	38, 38'
31–35	45	42, 43	44, 45	33, 33'
26–30	46	44, 45	46, 47	28, 28'
21–25	47, 48	46, 47, 48	48, 49, 50	23, 23', 23"
16–20		49	51	18

ing and others subtle, which shall be dealt with later on in the text.

Figure 2 also shows, on the left margin of each panel of blots, the Western blot master pattern of each antigen extract; that is, the collection of all bands in a mixture that were developed by at least 1 serum, and the molecular weight of the most conspicuous or interesting bands (vide infra). Intended for scoring, the master patterns were drawn without attempting to reproduce the intensity or width of the bands. Forty-eight bands were found in *T. solium*, 49 in *E. granulosus*, and 51 in *T. crassiceps*. A complete list of all bands found, as well as the precise molecular weight of each band, are given in Table 1. For further data handling, bands were labeled with the mean kDa of the molecular weight interval in which they occurred, as shown in the rightmost column of Table 1. Because molecular weight assessments had a 2.5 kDa standard deviation between blots (data not shown), bands differing by <5 kDa were not deemed as truly different bands unless they occurred in the same antigen extract in the same blot, in which case they were additionally labeled with quotation marks. After this correction for variation among blots, the total number of different bands detected by all sera on all antigen preparations were only 76, instead of the 120 that would result from a simple summation of all bands differing in molecular weight, however negligibly.

Immunoplots

Figure 3 shows all immunoplots, simple, and frequency differences plots with the 3 sources of antigen (*T. solium*, *E. granulosus*, and *T. crassiceps*) reacting with the 3 types of sera (cysticercotic, hydatid, and normal. Simple Immunoplots immediately identified troublesome bands frequently reacting with normal sera. By arbitrarily selecting threshold values in both axes, one may define antigens of interest at precise levels of specificity (background reactivity with normal sera) and sensitivity (reactivity with patients' sera). For example, if threshold values were $X > 0.70$ and $Y < 0.1$, then one would select in *T. solium* extracts bands kDa 23, 23', 93', 128, 188, and 208 as capable of discriminating cysticercotic from normal sera and band kDa 208 for hydatid disease (Fig. 3A). When employing antigens from *E. granulosus*, none seemed useful in detecting cysticercosis; however, bands kDa 83, 118', and 143 were best for discriminating between hydatid disease and background (Fig. 3B). *T. crassiceps* had 1 antigen, kDa 78, with very low reactivity with normal sera and very high reactivity with sera from both cysticercotic and hydatid patients (Fig. 3C).

Immunoplots using frequency differences best organized the data in each antigen extract. *T. solium*'s band kDa 208 scored highest for both diseases, while kDa 108 was the most specific for cysticercosis. Many more bands seemed to be specific of hydatid disease in *E. granulosus*, but bands kDa 143 and 118' were best. Band kDa 88 of *T. crassiceps* was highly specific for cysticercosis, while bands kDa 23', 38, 148, 198, 203, and others favored hydatid disease. Bands kDa 118, 113, 88', 78, and 28 of *T. crassiceps* reacted frequently with sera from both diseases and rarely with normal sera.

Linear correlation of the frequency differences immunoplots revealed highly significant correlation coefficients in all 3 plots. All slopes were smaller than unity and only the intercept of the plot using *E. granulosus* was significantly >0 (see legend of Fig. 3 for numerical values). Regression coefficients of the plots did not differ significantly from each other, thus pointing to essentially similar dispersion of the data about the regression lines in the 3 antigen preparations.

DISCUSSION

Simple inspection of Western blots revealed many cross-reacting antigens in *T. solium* and *E. granulosus*, which affect humans, and in *T. crassiceps*, which affects mice. This is an important, and not totally unexpected, finding. Earlier reports point in this direction;[13–16] here we document the great cross-reactivity among these parasites, including the fraction of antigens localized to the vesicular fluid. We have found vast cross-reactivity, which hints that it may extend to other cestodes frequently affecting humans, livestock, and domestic animals.[17–19] Such extensive antigen sharing is ominous for immunodiagnostic technology based on whole or partially purified parasite extracts,[2, 9, 10, 14] especially if oriented towards seroepidemiology.

The sera of healthy individuals contains antibodies to many different bands in all antigen preparations. This may explain the higher overall reactivity in ELISA of people living in endemic areas[9] and may reflect higher levels of endemia

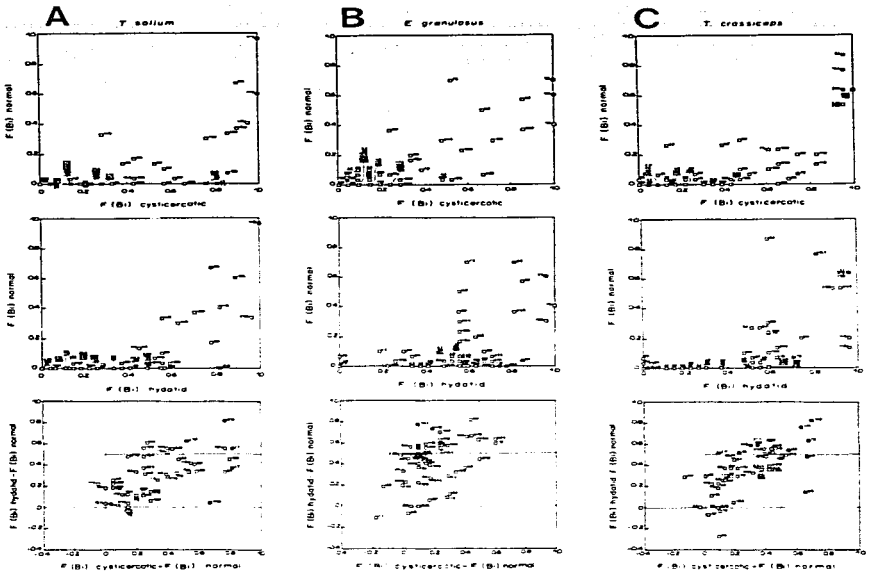

FIGURE 3. Simple and frequency differences immunoplots of neurocysticercosis and hydatid disease sera as they reacted with the vesicular fluid antigens of *T. solium*, *E. granulosus*, and *T. crassiceps*. Correlation Statistics for frequency differences immunoplots were: for *T. solium*, r = 0.571, df = 46, slope = 0.470 and intercept = 0.141; for *E. granulosus*, r = 0.461, df = 47, slope = 0.605 and intercept = 0.304; for *T. crassiceps*, r = 0.664, df = 49, slope = 0.717 and intercept = 0.151.

or closer contact with tapeworms than usually conceded. The ostensive presence of many antigens of human parasites in the murine parasite supports *T. crassiceps* as an alternative source of antigens for immunodiagnosis and, possibly, for the design of cross-reacting vaccines.

Immunoplotting, in particular that of frequency differences, greatly simplifies the task of sorting out pertinent antigens from complex antigen mixtures. Because the plot shows frequencies of reaction, one may arbitrarily set the limits of discrimination (sensitivity and specificity) to fit different problems. As a first approximation towards identification of likely candidates for specific immunodiagnosis of cysticercosis and hydatid disease, sensitivity and specificity were set at $X > 0.5$ and $Y < 0.5$ for cysticercosis and $X < 0.5$ and $Y > 0.5$ for hydatid disease in all 3 frequency differences immunoplots (Fig. 3A–C). Within these limits, *T. solium* contains 8, *E. granulosus* none, and *T. crassiceps* 4 different promissory antigens for specific immunodiagnosis of cysticercosis. Of those, only *T. solium*'s kDa 108 and *T. crassiceps*' kDa 88 seem capable of more stringent demands on sensitivity and specificity. Searching for these 2 antigens in

their respective Western blots finds *T. solium*'s kDa 108 to be rather inconspicuous, while *T. crassiceps*' kDa 88 is clearer, running very near kDa 88', a band that cross-reacts with hydatid sera and kDa 93 and 93', bands that show high reactivity with normal sera.

We found 1 *T. solium* antigen favoring cysticercosis, kDa 23', inside the interval kDa = 25 ± 5, where others[15] have found reactivity highly specific for cysticercosis. However, if we are dealing with the same antigen, we found a higher level of cross-reactivity with hydatid sera than previously reported. *T. solium*'s kDa 103 band, which we had previously identified as potentially useful in immunodiagnosis,[9] was rendered worthless by immunoplot because of it's high reactivity with normal sera collected from the endemic area.

Specific diagnosis of hydatid disease looks a little more promising, as there are many more bands in that region of the immunoplots: 16 in *E. granulosus*, 5 in *T. solium*, and 10 in *T. crassiceps*. Of these, none are very conspicuous or isolated in *E. granulosus*' nor in *T. solium*'s vesicular fluids. However, *T. crassiceps*' kDa 198, 38, and 23' are easily identifiable in the corresponding Western blot and are not crowded by contaminants. We are not certain which *E. granulosus* antigens in our Western blots correspond to arc 5, which is the antigen of choice in the immunodiagnosis of hydatid disease with reactions involving precipitating antigen-antibody complexes,[2, 20, 21] To judge from the reported molecular weight in reduced conditions[20, 22] and it's alleged high specificity, it could correspond to *E. granulosus*' kDa 38 and *T. crassiceps*' kDa 38, which score in the quadrant specific for hydatid disease, although not at the highest level of specificity.

It would seem that nondiscriminating immunodiagnosis of either cysticercosis or hydatid disease can be tried with a number of antigens present in the vesicular fluids of *T. solium* and *T. crassiceps*. This is not true of *E. granulosus*, which has only 2 antigens in this sector of the immunoplot, and they are not very conspicuous in Western blot. However, almost any of those in *T. solium* and *crassiceps* are prominent and fairly free of other antigens of similar molecular weight, especially *T. solium*'s kDa 238 and kDa 23, as well as *T. crassiceps*' kDa 118, 113, and 78. Antigen B, a prominent antigen in the diagnosis of cysticercosis[7] known to cross-react with antibodies against other tapeworms,[23] is probably not present in the vesicular fluid of these parasites since no band of similar molecular weight (kDa 95)[24] was found in the quadrant of high frequency cross-reacting antigens.

TABLE 2

Antigen bands most likely useful in the immunodiagnosis of hydatid disease and cysticercosis as sorted by immunoplot from antigens found in Western blots of vesicular fluids of related parasites

Source of antigens	kD's of Antigens to use in serology of cysticercosis and hydatid disease (kDa)		
	Nondiscriminating	Discriminating of	
		Cysticercosis	Hydatidosis
T. solium	238, 23	108, 23'	
E. granulosus			143, 148', 38
T. crassiceps	118, 113, 78	88	198, 38, 23'

In short, after checking the antigens sorted by immunoplot for conspicuousness and nearby contaminants in Western blots, it appears that *T. solium*'s kDA 238 and 23, and *T. crassiceps*' kDa 118, 113, and 78 would be best for immunodiagnosis not discriminating between cysticercosis and hydatid disease. For specific diagnosis of hydatid disease, only *T. crassiceps*' kDa 198, 38, and 23' meet the stringent conditions of conspicuousness and high sensitivity and specificity. We found no antigen promissory of specific immunodiagnosis of cysticercosis in the vesicular fluid of neither *T. solium*, *T. crassiceps*, or *E. granulosus*, the closest ones being *T. solium*'s kDa 108 and 23' and *T. crassiceps*' kDa 88 (Table 2).

Because of similar dispersion of the points in the frequency differences immunoplots, it would seem that immune responses of humans to *T. solium* and *E. granulosus* are equally heterogeneous. Variation is too large to give credence to the slope obtained with *T. crassiceps*' antigens; its being smaller than unity would indicate the murine parasite's greater similarity with *T. solium* than with *E. granulosus*. However, because all 3 slopes were smaller than unity, we believe that sera of neurocysticercotic patients react more frequently with all bands, or show smaller background reactivity, than sera from hydatid disease patients.

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Authors' addresses: C. Larralde, R. M. Montoya, E. Sciutto, M. L. Diaz, and T. Govezensky, Departamento de Immunologia, Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico. E. Coltorti, Centro Panamericano de Zoonosis, Buenos Aires, Argentina.

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ADDRESS REPLY TO:
William W. McLendon, MD, *Editor*
Department of Pathology
CB# 7525
University of North Carolina
Chapel Hill, NC 27599-7525
USA
(919) 966-5902

April 11, 1990

Dr. Carlos Larralde
Instituto de Investigaciones
Biomedicas, UNAM
AP 70228
Mexico DF 04510
Mexico

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Running Title

Murine T. crassiceps antigens in diagnosis of T. solium neurocysticercosis.

Title

Immunodiagnosis of human neurocysticercosis: Antigens from murine Taenia crassiceps effectively substitute those from porcine Taenia solium in ELISA and hemagglutination tests of cerebrospinal fluid and serum.

Authors

Larralde, C., +Sotelo, J., Montoya, R.M., +Palencia, G., Padilla, A., *Govezensky, T., *Diaz, M.L., and *Sciutto, E.

Institutions

*Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico; +Instituto Nacional de Neurologia y Neurocirugia de Mexico

Mailing Address

Dr. Carlos Larralde; Instituto de Investigaciones Biomedicas, UNAM; AP70228; Mexico DF 04510; Mexico

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Abstract

Antigens from T. crassiceps performed as well as antigens from T. solium in ELISA detection of anticysticercus antibodies in 96 CSF samples from neurocysticercotic patients and 96 CSF from other varied neurological ailments. Likewise, both sources of cysticercus antigens gave essentially similar results with control positive and negative human sera in both ELISA and hemagglutination tests. Thus, the very manageable murine model of experimental cysticercosis alleviates the problem of antigen inaccesibility in performing immunodiagnosis of cysticercosis for both clinical and epidemiological applications, and hints to effective means of industrial production for world distribution and standarization of diagnostic reagents for cysticercosis and possibly of other cestode human diseases.

Introduction

Increased awareness of human neurocysticercosis as a severe, often fatal disease, frequently seen in developing countries and their migrating workers, has prompted many requests of T. solium's antigens from research and diagnostic laboratories from all over the world. [1-3] Unfortunately, most petitions must go unrequited because the procurement of cysticercotic pigs, the usual source of T. solium's antigens, is being increasingly difficult in the endemic areas by the very same disease-awareness that rather than help to curb transmission has instead furthered clandestine slaughtering and marketing of parasitized pigs. World distribution of antigens is also hampered by sanitary regulations in customs. The recent documentation of extensive sharing of antigens among T. crassiceps, T. solium and E. granulosus [4] incited a thorough evaluation of the possibility of substituting T. solium's antigens with those from the laboratory adapted murine cestode, T. crassiceps ORF strain [5-6] in immunodiagnosis of neurocysticercosis in cerebrospinal fluid (CSF) and in seroepidemiological surveys.

Material And Methods

We studied the correlation in anticysticercus antibody reactivity of antigens obtained from T. solium with those obtained from T. crassiceps in samples - CSF or serum - from cysticercotic patients and controls. Immunological tests were performed blindly in two different but experienced laboratories (INNN and IIBM), each following their standard procedures upon each sample. [7,8] Afterwards, the code was revealed and the results subjected to correlation analysis; namely, that between antigen source within each laboratory and that between

laboratories within each antigen source.

192 CSF samples were chosen from the CSF bank of the Instituto Nacional de Neurologia y Neurocirugia de Mexico (INNN). 96 CSF belonged to confirmed cases of neurocysticercosis - as diagnosed by clinical picture, positive ELISA and complement fixation tests in CSF, and CAT scans, and in some cases by surgery. CSF was collected upon the patients' first visits for consultation, prior to extensive treatment. In addition, 96 control CSF belonged to roughly contemporaneous cases of other neurological patients in whom the diagnosis of cysticercosis had been discarded.

Sera were chosen from the Sera Bank of the Instituto de Investigaciones Biomedicas, UNAM (IIBM), assembled over several years by serum donations from diverse neurological and sanitary institutions. 24 positive sera -persistently and repeatedly positive over several months - and 12 negative sera were employed in the study.

CSF samples were processed by ELISA in the two laboratories, each following their own established technical variants previously published elsewhere. $7,8/ The main divergence between laboratories being that one - INNN - utilizes an antigen extract from the solid portions of the cysticerci, runs their tests in duplicate and detects both IgG and IgM antibodies, while the other - IIBM - prefers the antigens in solution in the vesicular fluid, detects only IgG antibodies and assays each sample in triplicate. The major technical features of the ELISA protocols followed by the two laboratories on the CSF samples are: a) INNN used carbonated buffer (.1M, pH 9.6) in the sensitization of plaques by T. solium and T. crassiceps antigens,

at a total protein concentration of .74 ug/well, 1:100 goat anti human IgG and IgM coupled to alkaline phosphatase (Sigma) as second antibody, and 1:30 dilution of CSF in PBS-Tween .1% ; b) IIBM dealt with T. solium and T. crassiceps antigen sensitization of plaques in different buffers, Tris-HCl (.01M, pH 7.5) and phospate (.01M, pH 7.5) respectively, it use a .1 uf of total sensitizing protein per well, second antibody was 1:1000 goat anti human IgG coupled to phosphatase alkaline (Boheringer), and CSF dilution was 1:10 in PBS-Tween .05%. Serum samples were analyzed by IIBM only following procedures previously published $8/ but with some changes in ELISA and none in hemagglutination, namely: sensitization antigen concentration were .1 ug/well and 1 ug/well, for T. solium and T. crassiceps respectively; second antibody was goat antihuman IgG coupled to alkaline phosphatase (Boehringer) diluted 1:4000 in PBS-Tween .05%; and serum dilution was 1:1000 in PBS-Tween .05%.

Results

In CSF samples the ELISA values obtained using antigens from T. solium and from T. crassiceps showed a high positive correlation (Figure 1). Correlations were essentially similar in both laboratories; if anything, the one from INNN (Figure 1A) was tighter than that from IIBM (Figure 1B), which showed greater variation with T. crassiceps at the high OD values of T. solium. In both laboratories the slopes of the fitted linear regressions were consistently, and significantly, smaller than unity; thus pointing to higher OD values when using antigens from T. solium than those obtained with T. crassiceps. Correlations of results between both laboratories were strikingly high with both antigens (Figure 2A and 2B).

Table 1 shows the degree of diagnostic concordance of results from the two laboratories with the definitive medical diagnosis, each laboratory using slightly different immunological procedures and cut-off ELISA values to score the samples as positive or negative. However, in spite of differences in procedure, degrees of concordance were very high (93-97 %) for both antigen preparations and for both laboratory techniques, although, again, INNN did somewhat better than IIBM.

The correlations between ELISA and hemagglutination results obtained in sera with antigens from T. solium and from T. crassiceps were also very high. (Figure 3A and 3B). The degree of concordance of serological results with the original serum category (control positive or control negative) was 100%, for both tests and both antigen sources (Table 1).

Discussion

Results establish that antigens from T. crassiceps may confidently substitute those from T. solium in immunodiagnosis of neurocysticercosis, as currently performed in CSF $7/ with minor modifications, as well as in serological analysis. $8/ Correlations between antigens and between laboratories were consistingly significant in both CSF and control positive and negative sera. Likewise, the degree of concordance between immunodiagnosis and definitive clinical diagnosis was very high in CSF and 100% in the case of immunological discrimination of control positive and negative sera. The rare inconsistencies encountered in CSF may result from degradation of the sample after prolongued storage and/or following

repeated freezing and thawing. The consistently higher ELISA values associated to T. solium antigens is probably only a question of technique standardization since these cestodes share tens of antigen bands in their vesicular fluids, and perhaps more in the solid parts of the parasite. $4/ In fact, the results reported here point to crossreactivity between the two species of taenia extending further into the parasite's tissues than just their vesicular fluids, since INNN laboratory uses solid parts of the parasite to extract antigens while IIBM uses vesicular fluid, and yet both labs obtained essentially similar results.

Substitution of T. solium antigens with those from T. crassiceps in immunodiagnosis of neurocysticercosis alleviates the problem of antigen access to many laboratories, particularly those situated outside the endemic areas. Experimental infection of mice is easily accomplished by injecting a few live metacestodes in the peritoneal cavity of recipient mice $5,6/, and yields grams of antigens in a few weeks, from which tens of mg of protein antigens are easily obtained by standard and uncomplicated procedures. $8 Even if antigens specific for different cestode diseases were deemed necessary, the murine parasite offers several of them in quantitites amenable for purification. $9,10,4/ Whether T. crassiceps also contains the glycoproteins of T. solium, recently reported to produce 98% sensitive and 100% specific serological results for neurocysticercosis, remains to be elucidated. $11/ In any case, itself an ingtriguing parasitic disease, deeply influenced by immunological, histocompatibility and sexual factors $12,13/, experimental murine cysticercosis is also a manageable source of cysticercal antigens for experimental and

industrial applications in immunodiagnosis of T. solium cysticercosis and possibly for vaccination.

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Legends Of Figures And Tables

Figure 1

Correlation in average ELISA readings between different antigen sources in detecting anticysticercus antibodies in 184 CSF samples, as performed in two different laboratories: INN (A) and IIBM (B). Regression analysis results: for (A), r$2/ = .90, intercept = .006, slope (std error of slope) = .87 (.02), df = 184; and for (B), r$2/ = .78, intercept = .016, slope (std error of slope) = .78(.02), df = 184. Note the very significant positive correlation between immunodiagnostic results obtained with the different parasites. The slopes consistently smaller than unity indicate to higher OD readings with T. solium antigens: and the larger variation of OD readings obtained with T. crassiceps at the high T. solium OD readings in IIBM could result from excessive sensitivity of this laboratory's technical variant (B).

Figure 2

Correlation between laboratories in average ELISA readings for anticysticercus antibodies in 184 CSF samples when using antigens from T. solium (A) and from T. crassiceps (B). Regression analysis results: for (A), r$2/ = .89, intercept = .100, slope (std error of slope) = 1.21(.03), df 184; and for (B), r$2/ = .96, intercept = 0, slope (std error of slope) = 1.21(.01), df =

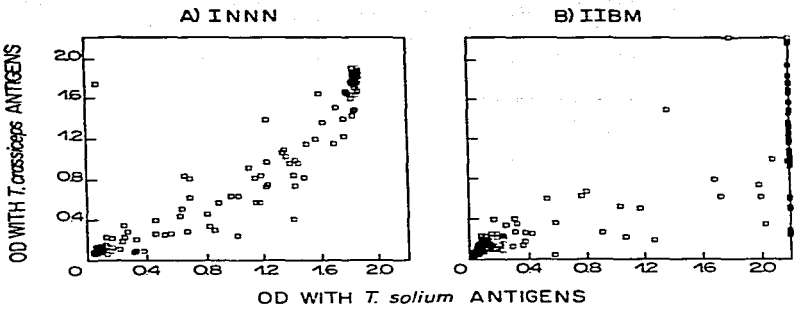
184. Note the very significant correlation of results between laboratories with both antigens, more so with T. crassiceps than with T. solium.

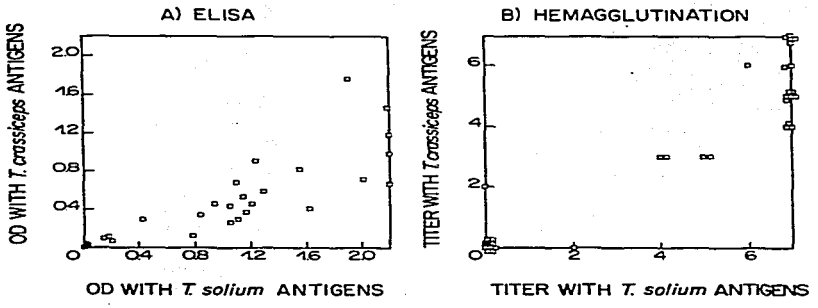
Figure 3

Correlation in average ELISA readings (A) and hemaglutination titers (B) between different antigen sources in detecting anticysticercus antibodies in 24 control positive and 12 negative sera. Regression analysis results: for (A), rS2/ = .76, intercept = -.01, slope (std error of slope) = .49(.04), df = 34; and for (B), rS2/ = .87, intercept = .03, slope (std error of slope) = .78(.05). Note the very significant positive correlation of serological results obtained with the different parasites.

Table 1

Very high degrees of concordance were noted between the immunodiagnostic results in CSF and sera with respect to the original clinical diagnosis or serological category of sera with either source of parasite antigens. (Concordance is simply a measurement of agreement in diagnosis between two assessments of each case; in this instance it was calculated as the sum of agreements divided by the total number of cases in each cell of the table).


A) INNN
B) IIBM
OD WITH T. crassiceps ANTIGENS
0
0.4
0.8
1.2
1.6
2.0
OD WITH T. solium ANTIGENS


A) ELISA
B) HEMAGGLUTINATION
OD WITH T. crassiceps ANTIGENS
TITER WITH T.crassiceps ANTIGENS
0
0.4
0.8
1.2
1.6
2.0
2
4
6
OD WITH T. solium ANTIGENS
TITER WITH T. solium ANTIGENS

SOURCE OF ANTIGEN	CONCORDANCE WITH ORIGINAL DIAGNOSIS			
	CSF		SERUM	
	ELISA			
	[illegible]	IIIBM	ELISA	HEMAG
T. solium	96 %	96 %	100 %	100 %
T. crassiceps	97 %	94 %	100 %	100 %

SOURCE OF ANTIGEN	CONCORDANCE WITH ORIGINAL DIAGNOSIS			
	CSF		SERUM	
	ELISA			
	[illegible]	IIIBM	ELISA	HEMAG
T. solium	96 %	96 %	100 %	100 %
T. crassiceps	97 %	94 %	100 %	100 %

SOURCE OF ANTIGEN	CONCORDANCE WITH ORIGINAL DIAGNOSIS			
	CSF		SERUM	
	ELISA			
	[illegible]	IIIBM	ELISA	HEMAG
T. solium	96 %	96 %	100 %	100 %
T. crassiceps	97 %	94 %	100 %	100 %

SOURCE OF ANTIGEN	CONCORDANCE WITH ORIGINAL DIAGNOSIS			
	CSF		SERUM	
	ELISA INNN	ELISA IIBM	ELISA	HEMAG
T. solium	96 %	96 %	100 %	100 %
T. crassiceps	97 %	94 %	100 %	100 %

SOURCE OF ANTIGEN	CONCORDANCE WITH ORIGINAL DIAGNOSIS			
	CSF		SERUM	
	ELISA INNN	ELISA IIBM	ELISA	HEMAG
T. solium	96 %	96 %	100 %	100 %
T. crassiceps	97 %	94 %	100 %	100 %

VIII. REVISION BIBLIOGRAFICA PUBLICADA

El Complejo Mayor de Histocompatibilidad del Ratón y la Respuesta Inmune

Edda Sciutto. Facultad de Medicina

El complejo Mayor de Histocompatibilidad (CMH) es un conjunto de genes ubicados en serie, que codifican proteínas de superficies celulares y plasmáticas las que tienen capacidad de interactuar a distintos niveles con el sistema inmune.

En 1936, Peter Gorer publicó las bases de lo que es hoy el CMH. Usando un suero de conejo anti-ratón demostró en ratones de una cepa A la presencia de un antígeno de superficie de eritrocitos que no estaba presente en otra cepa de ratones C57B1. Al año siguiente publicó otro artículo en el que demostró que el locus que determina ese antígeno de superficie también determina la susceptibilidad y la resistencia a transplantes en la cepa A. Los estudios de Gorer no produjeron respuesta en el ambiente científico sino hasta una década más tarde con los primeros intentos de transplantes de tejidos y la asociación de estos antígenos con el rechazo rápido de transplantes. El progresivo descubrimiento de la participación del CMH en la respuesta inmune contra muchos antígenos intensificó su estudio. Así, hasta la fecha se continúa explorando el CMH en muchos vertebrados (conejo, hombre, caballo, rata, pollo, y otros) y en especial en el ratón donde la posibilidad de utilizar cepas estables de endocría permitió identificar casi todos los loci y establecer sus posiciones, asociándolos con sus productos fenotípicos.

I. Organización Genética

El complejo H-2 del ratón se localiza en el cromosoma 17, donde ocupa de 0.3 a 1.5 centimorgans* de longitud dependiendo de qué loci se consideren dentro del H-2. El criterio más reciente, considera el límite el extremo centromérico marcado por la región K y el límite del extremo telomérico a 1.5. centimorgans del Tla (Fig. 1).

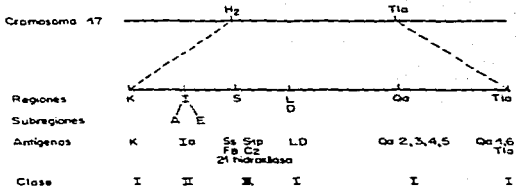


Fig 1 Representacion esquematica del complejo mayor de histocompatibilidad en raton

Experimentos de recombinación del cromosoma 17, proveniente de padres heterocigotos, permitieron conocer la posición de los loci del H-2. Podemos observar (Fig. 1) la representación esquemática del complejo H-2, que hasta la fecha está constituido por las regiones: K, I, S, D, L, Q y T. La región I está formada por dos subregiones: A y E. Cada región consiste en un segmento cromosómico ocupado al menos por un locus y separado de otros segmentos por un entrecruzamiento de cada lado. Los loci del CMH son extraordinariamente polimórficos, y se identificaron más de 50 formas posibles de un mismo gen. A la combinación particular de alelos en loci H-2 individuales se les designa como haplotipo; así la cepa de ratones singénica C57B/10 (B10) es de haplotipo b y todos sus alelos se designan con la misma letra K b, Ib, Sb, Db, Lb, Qb, Tb).

Los loci H-2 se agrupan en tres clases según la homología probada o presunta entre sus productos fenotípicos. Las

*1 centimorgan es una unidad de distancia de mapa equivalente 1% de probabilidad de entrecruzamiento entre dos genes.

clases se designaron arbitrariamente con números romanos del I al III.

II. Clasificación de los loci H-2 según su expresión fenotípica.

A.a. LOCI CLASE I
1.1. Organización Genética.

Existen tres **loci** clase I bien caracterizados: K, D y L, los cuales están localizados en los extremos opuestos del mapa del H-2. Estos **loci** son funcionalmente indistinguibles. El **locus** L se localiza en la vecindad de la región D, y se considera que deben estar muy cerca uno del otro, quizás adyacentes, porque no se encuentra ninguna recombinante entre ellos. Dentro de los **loci** de clase I se incluyen también los loci Qa y Tla. Estos **loci** se localizan en el extremo telomérico del cromosoma 17 y ocupan una región dos o tres veces más larga que el intervalo K-D. Los datos de genética molecular indican que la mayor parte de los genes de clase I se localizan en la región Qa-Tla y comparativamente pocos en la región K-D-L. Así, en BALB cJ (haplotipo H-2 d) de aproximadamente 35 genes de clase I, 30 se localizan en Qa-Tla; en B10 (haplotipo H-2b), de un total de 26 genes de clase I, 23 se localizan en Qa-Tla. Los genes Qa-Tla se incluyeron dentro de los genes de clase I porque forman complejos híbridos con fragmentos de DNA complementarios a la región K, y la D; sin embargo, existen tendencias a incluirlos en una cuarta clase, debido a que sus productos fenotípicos presentan diferencias en polimorfismo expresión, y funcionalidad respecto a los productos K, D, L.

2. Productos fenotípicos

Los **loci** clase I (K-D-L) codifican para glicoproteínas de membrana de un peso molecular aproximado de 45 kDa. En la figura 2 observamos un esquema de antígeno de clase I (Mallissen B, 1986), constituido por una cadena polipeptídica de tres dominios (cadena pesada), cada uno de los cuales tiene asociado covalentemente un resto hidrocarbonado (Coligan *et al*, 1978). Los primeros dos dominios $\alpha 1$ y $\alpha 2$ parecen estar involucrados en el reconocimiento de los linfocitos T y el tercer dominio es muy conservado y presenta gran homología con el dominio constante CH3 de inmunoglobulinas. A esta cadena pesada se asocia, por interacciones no covalentes, un polipéptido más corto (PM 12 kDa), la $\beta 2$ microglobulina, que constituye la cadena liviana del antígeno de

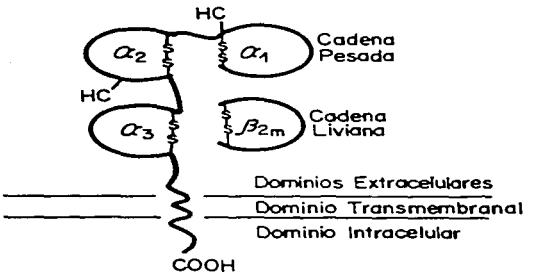


Fig. 2 Esquema de antígeno de clase I

clase I y que se codifica fuera del complejo H-2 (cromosoma 2) (Ploegh *et al*, 1979). La antigenicidad y las funciones biológicas de la molécula residen en su cadena pesada, la cual se extiende desde el compartimiento extracelular hasta el citoplásmico. La inserción en la membrana no depende de la β 2 microglobulina, la cual se asocia después de la glicosilación de la cadena pesada. Los estudios realizados por Ploegh *et al*., (1981) indican que la β2 microglobulina juega un papel importante en la eficacia del transporte de la cadena pesada desde el sitio de glicosilación (retículo endoplasmático) hasta la membrana plasmática.

Si bien, las regiones Qa y Tla contienen la mayoría de los genes de clase I encontrados en el genoma del ratón, sólo unos pocos de los productos de estos genes se han identificado. Algunos autores afirman que muchos de estos presuntos genes son en realidad pseudogenes, otros, que sus productos son serológicamente no detectables, o bien que podría tratarse de productos de diferenciación con una expresión tisular limitada. Las moléculas Tla y Qa descritas están formadas por dos cadenas de peso molecular de aproximadamente 40 kDa, asociadas de manera no covalente a una cadena de 12 kDa que se parece a la β2 microglobulina.

3. Detección Serológica e Histogenética de los Productos

Las moléculas de clase I pueden ser identificadas por medio de anticuerpos capaces de reaccionar específicamente contra estos antígenos. Actualmente se identifican más de cien determinantes antigénicos clase I (K-D-L). En las moléculas mejor caracterizadas se detectan hasta

30 determinantes antigénicos distintos. Estos determinantes individuales tienen frecuencias características para los alelos K o D, que pueden variar desde determinantes restringidos a un solo alelo (determinantes privados) o compartidos por hasta el 75% de los alelos probados (determinantes públicos). Pueden además estimular la inmunidad celular, y se identifican por ensayos de citotoxicidad linfocitaria.

4. Polimorfismo

Los antígenos de Clase I (K-D-L) presentan un elevado polimorfismo que reside en la cadena pesada. Se encuentran 12 alelos en el **locus** K y 10 alelos en el **locus** D. La β 2 microglobulina, en cambio, es muy conservada entre especies. Las moléculas de los **loci** Qa y Tla, si bien muy similares estructuralmente a las K-D-L, son muchos menos polimórficas.

5. Distribución Celular e Interacción con el Sistema

Los antígenos de clase I (K-D-L) están presentes en casi todos los tipos celulares. Abundan en macrófagos y linfocitos, escasean en la mayoría de las células somáticas (células musculares, fibroblastos, células de tejido nervioso) y pueden detectarse algunas veces en células germinales, trofoblásticas y células cebadas.

Los antígenos de clase I (K-D-L) son los antígenos clásicos involucrados en el rechazo rápido de transplantes de tejidos. Por su extensa distribución celular, sirven como marcadores celulares de lo propio. Estos antígenos permiten el reconocimiento de células propias cuya superficie se encuentra alterada. Así, cuando una célula normal modifica su fenotipo por una infección viral, química o de otra etiología, el individuo reconoce el fenotipo modificado como no propio y se activan las células T citotóxicas eliminando la célula normal modificada. En este fenómeno de reconocimiento, la célula T identifica a la célula propia modificada a través de los antígenos de clase I (Zinkernagel, R., 1978). Las reacciones a través de las células T estimulan preferencial pero no exclusivamente a linfocitos Lyt23+ y respecto a su participación en la respuesta humoral, estimulan y controlan la producción de anticuerpos.

Los antígenos Qa-Tla están distribuidos en células hematopoyéticas y en ciertas poblaciones linfocitarias y aunque no se conoce su papel fisiológico no parecen funcionar como mediadores de respuestas citotóxicas como los antígenos K y D.

B. LOCI CLASE II

1. Organización Genética

Murphy *et al.* (1980) obtuvieron las primeras pruebas sobre la región I, y propusieron, con base en análisis serológicos, la existencia de 5 **loci**: A, B, J, E, C. Sin embargo, los primeros estudios de clonación molecular (Steinmetz *et al.* 1982) identificaron sólo dos de estas regiones I-A e I-E. Estos resultados dieron lugar a grandes controversias que no llegaron a su fin sino hasta 1985, en que se confirmó que la región I estaba constituida sólo por las regiones A y E. Con respecto a las otras subregiones Hayes *et al* 1984 encontraron que las determinantes de la I-J parecen ser productos de genes fuera del H-2, los genes I-C o sus productos no se identificaron y los I-B parecen el resultado de un efecto fenotípico de los **loci** I-A e I-E.

Actualmente se sabe que existen ocho genes clase II en la región A y E en el ratón, Aβ3, Aβ2, Aβ1, Aα, Eβ1, Eβ2, Eα y Eβ3 (Larhammer *et al.*, (1984). El Aβ3 es el más próximo al extremo centromérico y el Eβ3 el más alejado y su existencia sólo se han confirmado en BALB c (Moller, G., 1985). De estos genes sólo se conocen como funcionales A 1, A , E 1, E .

2. Productos Fenotípicos

Las moléculas de clase II, A y E, están compuestas por dos cadenas polipeptídicas asociadas por interacciones no covalentes, α(33-35 kDa) y β(26-29 kDa). Estas cadenas polipeptídicas presentan 2 dominios globulares extracelulares polimórficos, α1 y β1, un dominio extracelular muy conservado α2, y β2, que es homólogo a los dominios constantes de inmunoglobulinas, un dominio transmembranal, y un carboxilo terminal en la porción citoplasmática (Fig. 3).

3. Detección Serológica e Histogénica de los productos

Hasta la fecha se han identificado más de 40 determinantes de clase I, agrupados en dos series, una de cerca de 30 determinantes controlados por los **loci** I-A y otra serie más pequeña controlados por los I-E.

4. Polimorfismo

El **locus** A es tan polimórfico como los **loci** de clase I, se han estimado alrededor de 50 alelos entre los ratones

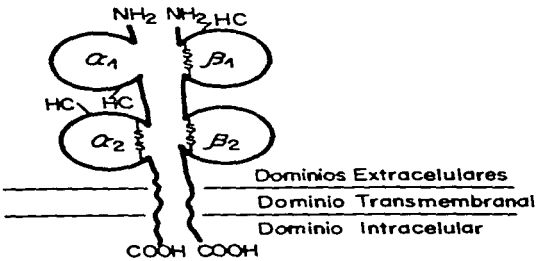


Fig. 3 Esquema de antígeno de clase II

silvestres (Bonoist *et al.*, 1983). La variabilidad entre estos alelos reside fundamentalmente en el dominio globular más alejado de la membrana plasmática, y se observan en este dominio tres zonas de hipervariabilidad (Benoist *et al.*, 1983). Los determinantes de locus E son menos polimórficos y se estiman en ratos silvestres aproximadamente 10 alelos (Mengle-Gae y McDevitt, 1983).

5. Distribución Celular e Interacción con el Sistema Inmune

Las moléculas de clase II (Ia) están expresadas selectivamente en la superficie de células del sistema inmune, linfocitos B, células presentadoras de antígeno como los macrófagos, células de Kupfer en el hígado, y células de Langerhans en la piel (Moller, 1985); además, es inducible su producción y expresión en superficie de otros tipos celulares no inmunes (Barclay and Mason, 1984), como en células epiteliales.

Respecto a su participación en la respuesta inmune, los linfocitos T ayudadores reconocen a un antígeno en el contexto de los productos de los genes clase II, y permiten que los linfocitos B se activen y produzcan anticuerpos contra ese antígeno.

Si bien, estas moléculas son polimórficas en una especie, son invariables en un individuo y se requieren células T y moléculas de clase II del mismo individuo para inducir la respuesta contra un antígeno extraño (Weis *et al.*, 1984, Pease *et al.*, 1983). Este complejo ternario: célula T (receptor), moléculas clase II en superficie de célula presentadora de antígenos y de antígeno, es de naturaleza desconocida; sin embargo, se propone a la célula T como estabilizadora de las interacciones entre las moléculas Ia y el antígeno. El interfon (IFN) puede inducir la expresión de los antígenos Ia (Steeg *et al*) en macrófagos así como en otros tipos celulares. Esta expresión inducida presenta distinta sensibilidad, dependiendo del tipo celular, lo que indica que posiblemente participen múltiples mecanismos de regulación en la expresión de genes de clase II.

C. LOCI CLASE III

1. Organización Genética

Los genes de clase III incluyen una región (S), que codifica los componentes del sistema de complemento C2, C4 y factor B y los genes de una enzima esteroidea (21 hidroxilasa) que no se encuentra involucrada con el sistema inmune. Esta región se localiza entre la región I y la D. Una segunda región intercalada entre la región D y Qa codifica para una proteína reguladora de C4 (Rodríguez de Córdoba *et al.*, 1985) y una tercera región codifica para C3, la consideración de esta tercera región como parte del H-2 es aún muy discutida. En el ratón, la región que codifica para C3 está localizada a una distancia aproximada de 11 unidades mapa H-2, en el extremo telomérico a la derecha de D (Da Silva *et al.*, 1978) y por esto se debate si debe ser o no incluido dentro del H-2.

2. Productos Fenotípicos

Los productos de clase III codificados por la región S son: los componentes de complemento C4, C2, el factor B, y una proteína no hemolítica similar a C4 llamada Slp.

Screffler y Owen describieron en 1963 la existencia de una región S que contiene un locus que determina la cantidad de una proteína sérica (Ss). Estudios posteriores encontraron que se trataba de dos proteínas diferentes (Curman *et al.*, 1975); una era el componente C4 del complemento (Meo *et al.*, 1975), para el que se han identificado dos alelos, la otra una proteína limitada al sexo Slp, que muestra gran homología con C4 pero no tiene actividad hemolítica y su función es aún desconocida.

La molécula de C4 (18 a 20 K Da) consiste en tres cadenas polipeptídicas unidas covalentemente, α (90 kDa), β (70 kDa) y γ (30 kDa). El locus Ss codifica para un precursor de una sola cadena de 185 kDa, el cual es dividido después de la transcripción en las cadenas α, β y γ. El locus Slp parece ser distinto de Ss aunque no se han encontrado recombinantes que separen a los dos

loci, el locus Slp codifica para una proteína que se parece a la molécula de Ss pero difiere de ella en el tamaño de las cadenas individuales: la cadena alfa es ligeramente más grande y las cadenas y más pequeñas. El componente de complemento C2 es una glicoproteína de una sola cadena (100 kDa), química y funcionalmente semejante al factor B de la vía alterna. Ambas circulan en plasma como zimógenos y tienen actividad de proteasa cuando son activadas. El componente C3 (185 kDa) consiste en dos cadenas eslabonadas covalentemente: (11,000 d) y (75,000 d).

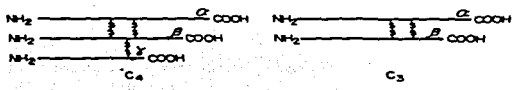


Fig. 4. Representación esquemática de los componentes de complemento C4 y C3

3. Polimorfismo

El polimorfismo en los genes de clase I y II es notable y también para C4 es excepcionalmente grande. Se han identificado dos loci para el componente C4; el C4A y el C4B. Con base en diferencias de antigenicidad y carga pueden identificarse 35 alelos (Mauff *et al.*, 1983); sin embargo, estudios serológicos y de secuencia nucleotídica sugieren un número mucho mayor (Belt *et al.*, 1985, Whitehead *et al.*, 1984). En C2 existe un alelo común y muy raramente dos; en el factor B dos alelos comunes y muy raramente 4 (Alper, 1976); para el locus C3 se han identificado cuatro alelos. Para la proteína Slp no se ha identificado polimorfismo serológicamente detectable.

4. Distribución Celular e Interacción con el Sistema Inmune

Entre las células productoras de componentes de clase III se ha reportado que hepatocitos y macrófagos producen C4 (Newell *et al.*, 1983) y el factor B es producido por linfocitos y macrófagos.

Las proteínas del sistema de complemento regulan una gran variedad de eventos biológicos y tienen un papel central en el proceso de inmunidad humoral. La activación del complemento en cascada, tanto por la vía clásica como por la alterna, resulta de interacciones específicas entre varios componentes individuales. El proceso de activación de complemento por vía clásica se inicia por la reacción antígeno anticuerpo que induce cambios conformacionales en el fragmento Fc de la inmunoglobulina favoreciendo la fijación de Fc y a Clq, una de las tres proteínas que forman el complejo Cl; esto induce la activación de Cl que degrada a C4 y C2 en C2a C2b, C4a, C4b. Las moléculas de C4b y C2a, que se unen a la superficie celular, forman la C3 convertasa que actúa sobre C3, fragmentándolo en C3a y C3b. El C3b se une junto con C4b y C2a formando la C5 convertasa. Esta última actúa sobre C5, produciendo un fragmento de C5b que inicia la formación del complejo CAM (Complejo de Ataque a la Membrana).

La reacción antígeno anticuerpo (IgE, IgA, IgG_4H), así como lipopolisacáridos y veneno de cobra activan la vía alterna de complemento, se inicia con la unión de C3 con el factor B (FB), formando un complejo reversible que puede ser activado por el factor D en presencia de Mg++, liberando un fragmento Ba, con la generación del complejo C3bFB con actividad de C3 convertasa. Las moléculas de C3 producidas por esta convertasa son capaces de unirse a más factores B y D, originando un mecanismo de amplificación. Esta C3 convertasa con mayor número de moléculas de C3 modifica su especificidad, adquiriendo capacidad de escindir a C5 en C5a y C5b continuando el mismo camino que la vía clásica. En el H-2 son codificados algunos componentes críticos en la activación y amplificación de ambas vías. En la figura 5 se muestra la relación entre C2, C3, C4 y factor B, en la vía clásica y alterna.

La proteína Slp se encuentra en el plasma de solo ciertas cepas de ratones y en algunas cepas se halla regulada por los niveles de hormonas androgénicas, mientras en otras son independientes. La proteína reguladora de C4 es el cofactor proteico de la proteína inactivadora de C4 que hidroliza a C4 a nivel de la cadena

Conclusiones y Perspectivas

Uno de los más importantes eventos de la inmunología en las últimas décadas fue establecer el concepto de que

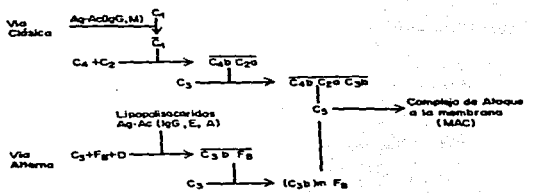


Fig. 5. Representación esquemática del proceso de activación de complemento por la vía clásica y la vía alterna.

los productos del CMH influyen en la Respuesta Inmune. Los avances tecnológicos de la genética molecular están permitiendo conocer la estructura y organización genética del H-2. Podemos observar en esta revisión lo reciente de los hallazgos en este tema y la falta aún de consolidación de los conocimientos adquiridos. Se ha profundizado el estudio descriptivo del genoma y tratado de definir los productos fenotípicos; ese profundo nivel de exploración no se extiende todavía al estudio de la regulación de la expresión genética, ni al papel fisiológico que los productos fenotípicos del H-2 tienen en la respuesta inmune. Queda así mucho por conocer en esta área y seguramente, en los próximos años, el estudio del H-2 continuará siendo uno de los temas fundamentales a elucidar tanto para los genetistas moleculares como para lc · inmunólogos.

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IX. REVISION BIBLIOGRAFICA ENVIADA PARA SU PUBLICACION

SUSCEPTIBILIDAD GENETICA A INFECCIONES PARASITARIAS MURINAS

Fragoso, G., Sciutto, E., Estrada, I. y Larralde, C.

Correspondencia a:

M. en C. Edda Sciutto
Instituto de Investigaciones Biomédicas, UNAM
Apartado Postal 70-228
04510 México, D.F. MEXICO

En esta revisión se considerará el determinismo genético de las infecciones parasitarias en el ratón concentrando la atención en las variaciones entre individuos de una misma especie determinadas genéticamente.

El ratón es la especie mejor conocida genéticamente e inmunológicamente y por lo tanto es ideal como modelo experimental de parasitosis por helmintos y protozoarios. Empleando cepas singénicas, congénicas y recombinantes se ha podido identificar aquellas que son resistentes y susceptibles en cada parasitosis (Tabla I). Un siguiente nivel de análisis consiste en la identificación de la cantidad de genes involucrados en la determinación de la susceptibilidad. Las comparaciones se dificultan por criterios arbitrario de susceptibilidad que van desde asociar la susceptibilidad al índice de mortalidad hasta el número de parásitos recuperados en fases no sintomatológicas de la parasitosis y en consecuencia siempre quedan dudas de la validez de las generalizaciones.

1.a Susceptibilidad innata del hospedero al parásito.

La diferencias en la susceptibilidad innata de los individuos de una misma especie a un parásito, se puede considerar según si la región del genoma involucrada está relacionada con la respuesta inmune (genes de respuesta inmune, GRI) o no (genes de no respuesta inmune, GNRI).

Dentro de los GRI consideraremos aquellos que codifican para productos fenotipicos participantes de la respuesta inmune del hospedero contra el parásito, a través de los cuales controla su crecimiento (ie, anticuerpos). Mientras que los productos fenotipicos de los GNRI identificados, determinan lo propicio del nicho ecológico en el que el parásito desarrolla su mediación inmunológica (ie, hormonas).

Susceptibilidad innata asociada a genes relacionados con la respuesta inmune.

Consideraremos como genes relacionados con la respuesta inmune a aquellos que codifican para proteinas y factores solubles asi como para proteinas de superficies celulares.

Dentro de los genes que codifican para proteinas solubles y factores solubles se encuentran los de inmunoglobulinas -moléculas efectoras de la inmunidad humoral - los de interleucinas, -polipéptidos que modulan mecanismos de defensa localizados y sistémicos-(43) y otros como moléculas del sistema del complemento, interferón, protaglandinas y linfotoxinas.

Dentro de las proteinas de superficies celulares se encuentran un grupo de glicoproteinas que pertenecen al Complejo Mayor de Histocompatibilidad (MHC), a las que se les ha asociado entre otras funciones las de presentación de antigenos a células immunocompetentes (35,74) asi como la codificación de algunos de los factores que intervienen en el Sistema del Complemento. Otras moléculas de superficie, que median fenómenos de activación y proliferación, son los receptores de antigenos (9) los receptores de moléculas liberadas por las propias células linfoides (interleucinas) presentes en linfocitos T, B y en macrófagos (79).

Susceptibilidad asociada a proteinas solubles codificadas por GRI.

Anticuerpos:

La resistencia o susceptibilidad mediada por anticuerpos puede estar relacionada con la especificidad, afinidad, isotipos de inmunoglobulinas asi como con la cantidad de anticuerpos totales.

La susceptibilidad a diversas enfermedades parasitarias está asociada a la especificidad y al isotipo de algunos inmunoglobulinas. Asi, por ejemplo, en la triquinosis experimental murina un factor critico en el desarrollo de la infección lo constituye la destrucción de larvas tempranas por citólisis mediada por anticuerpos (19). Los complejos larva-anticuerpos se fijan a células responsables de la destrucción larvaria (macrófagos y/o eosinófilos). Esta reacción incluye la asociacion antigeno-anticuerpo (afectada por la especificidad y afinidad de los anticuerpos) y la asociación de este complejo a receptores celulares (determinada por el isotipo de las inmunoglobulinas). Asi, las cepas resistentes presentan altos titulos de IgE e IgG especificas, inmunoglobulinas para los que existen receptores celulares en las células efectoras (61).

Respecto a una asociación entre susceptibilidad y cantidad de anticuerpos presentes, existen algunos reportes que la documentan. En la trichuriasis experimental murina se ha

encontrado que las cepas más susceptibles presentan mayores titulos de anticuerpos de clase IgM contra antigenos de secreción - excreción. (16). Otro ejemplo lo constituye Taenia taeniaeformis para la que se ha reportado mayores titulos de anticuerpos en la cepa resistente que en la cepa susceptible y su fase critica de acción parece residir en los primeros dias de infección ya que son las larvas tempranas las susceptibles a anticuerpos. (26,47,54).

Respecto a la asociación entre susceptibilidad y repertorio de antigenos reconocidos, en el modelo de infección murina con Schistosoma japonicum, la cepa resistente 129/J reconoce un antigeno de 26 kDa, no reconocido por la cepa susceptible (49).

De las asociaciones encontradas entre anticuerpos y susceptibilidad, queda aún por dilucidar si estas asociaciones son responsables total o parcialmente de los diferentes patrones de enfermedad. Cabe senalar que en otras parasitosis no se ha encontrado asociación entre la especificidad y/o niveles de anticuerpos con diferentes patrones de enfermedad como en el caso de la leishmaniasis causada por Leishmania tropica (48).

Sistema del complemento:

El sistema del complemento es un conjunto de proteinas cuya activación resulta en la lisis celular. Una de las estrategias utilizadas para estudiar su influencia en el determinismo genético de la susceptibilidad a parásitos ha sido el empleo de cepas singénicas de ratones deficientes en alguno de sus

componentes. Por ejemplo en cisticercosis murina por T. taeniaeformis se ha encontrado que el conjunto de cepas más susceptibles son deficientes en C4 y/o C5 (C3H/He, C3H, AKR/J, A/J) (46).

Son pocas las asociaciones entre los niveles de los componentes del complemento y la susceptibilidad innata del hospedero a la parasitosis, si bien existen muchos reportes acerca de la capacidad del parásito para evitar o disminuir el daño por complemento (factores anti-complementarios, enzimas proteolíticas, enmascaramiento con antigenos del hospedero).

Factores solubles reguladores de la R.I.:

En la Tabla 2 se mencionan las principales moléculas solubles reguladoras, descritas a la fecha así como sus funciones más relevantes. Aún hay mucho por explorar sobre la capacidad del parásito para modificar los niveles de proteínas reguladoras producidas por el hospedero. Uno de los casos reportados lo constituye la inmunosupresión inducida por Trypanosoma cruzi a través de la disminución de los niveles de IL-2 (77). Otro caso es el de la esquistosomiasis en la que estudios in vitro sugieren que los antigenos de huevecillos de Schistosoma japonicum disminuyen la actividad de la IL-2, a través de un mecanismos mediado por células T supresoras (73).

Susceptibilidad asociada a proteinas celulares codificadas por GRI.

Entre las proteinas de superficies celulares codificadas por GRI se encuentran las moléculas receptoras para antigenos, para interleucinas y para moléculas codificadas por el MHC. Estas moléculas son las mediadoras de interacciones entre células y entre células y factores solubles; asociaciones que tienen como consecuencia fenómenos de activación y/o proliferación celular (Tabla 3).

Dentro de este conjunto de proteinas solo se han descrito asociaciones entre la susceptibilidad a infecciones y los genes que codifican para los antigenos de histocompatibilidad.

El efecto de los productos del MHC en la susceptibilidad a infecciones parasitarias se ha explorado extensamente en muchos modelos experimentales. Sin embargo no son muchos los casos en los que se ha encontrado una asociación. Los ejemplos más estudiados son los modelos murinos de infección con nemátodos: Trichinella spiralis y Nematosporidius dubis. En ambas parasitosis las cepas resistentes ($H\text{-}2^{s}$, $H\text{-}2^{b}$, $H\text{-}2^{q}$, $H\text{-}2^{f}$) presentan como caracteristica común ser $I\text{-}E^{-}$ y las más susceptibles ($H\text{-}2^{k}$) ser $I\text{-}E^{+}$. Se ha propuesto que la respuesta inmune en el contexto de antigenos I-E es fundamentalmente supresora (56), en tanto que en el contexto de I-A se induce una respuesta proliferativa lo que podria justificar las diferencias de susceptibilidad en estas parasitosis (83). También en el modelo experimental de paludismo a Plasmodium chabaudi se ha

identificado la resistencia asociada a la presencia de $I\text{-}A^{b}$ en la superficie de células presentadoras de antigeno (87). Sólo en estos casos se ha podido proponer asociación entre el genoma y sus productos fenotipicos; la mayor parte de la información se limita a reportar la asociación entre la susceptibilidad-resistencia con los haplotipos de histocompatibilidad (Tabla 1).

Susceptibilidad asociada a genes no relacionados con la respuesta inmune.

Los genes no relacionados con la respuesta inmune serán considerados asociados o no al cromosoma sexual.

Siendo inmediata las diferencias sexuales entre individuos de una misma especie, la sexualidad ha sido una de las primeras caracteristicas que se ha intentado asociar a diferencias en la susceptibilidad a infecciones.

GNRI relacionados con el cromosoma sexual

En la Tabla 4 se senalan las enfermedades parasitarias cuya susceptibilidad se asocia al sexo. Como puede apreciarse en todos los modelos experimentales murinos los machos son los más susceptibles, con excepción de la cisticercosis murina por Taenia crassiceps y la tricomoniasis por Trichomonas vaginalis. Estas diferencias pueden estar determinadas genéticamente en el cromosoma "Y" o bien resultar de la influencia del sistema neuroendócrino, ya sea modificando el compartimiento en el que el parásito se desarrolla o modificando la respuesta inmunológica que el hospedero genera contra el parásito. La mayor

susceptibilidad de los machos parece ser independiente del fondo genómico, ya que el patrón de susceptibilidad se presenta en todas las cepas de ratones singénicos utilizadas, y coincide con los reportes de respuestas inmunes humorales y celulares disminuidas en los machos respecto a las hembras, aparentemente reguladas por el sistema neuroendócrino (2,25)

GNRI no relacionados con el cromosoma sexual

Un ejemplo tipico de esta clase de susceptibilidad es el paludismo humano para el cual la resistencia del hospedero esta asociada a la clase de hemoglobina de sus eritrocitos. Asi los individuos con anemia hemolitica congénica, (cuya hemoglobina tiene una mutuación puntual en la cadena de la hemoglobina) tienen menor capacidad para acarrear oxigeno, lo cual los hace más resistentes a la infección por Plasmodiun falciparum. (18,59). En el paludismo por Plasmodium vivax y P. knowlesi, los individuos Duffy (-) son más resistentes a la enfermedad debido a que su grupo sanguineo se encuentra asociado con la incapacidad del parásito para penetrar la célula del hospedero (45).

En el caso de Leishmania donovani (parásito intracelular obligado) responsable de la leishmaniasis visceral en el ratón, se ha identificado que la resistencia innata esta determinada por uno o un grupo de genes localizados en el cromosoma 1 murino, designados como genes Lsh. (6,13). Estos genes determinan que un individuo se enferme o no dado un contacto con el parásito y, una vez enfermo, la gravedad de

la enfermedad dependera del haplotipo de histocompatibilidad de la cepa de ratón infectada. (5,66). Estos genes Lsh parecen ser los mismos que determinan la susceptibilidad natural a Salmonella typhimurium, denominados Ity (63).

1.b Susceptibilidad innata del parásito al hospedero.

Si bien el estudio de la genética de vertebrados ha permitido identificar regiones genómicas que determinan la susceptibilidad del hospedero al parásito, en el parásito no se han estudiado regiones genómicas equivalentes. Esto se debe en parte, a que es más reciente la aplicación de técnicas de Biología Molecular en parásitos. Los primeros reportes de aislamiento de material genético de parásitos, aparecieron en 1974 para Leishmania (11); 1980 para Plasmodium (15) y en 1982 para Schistosoma mansoni (70). Desde entonces se ha venido intentando identificar regiones con fines diagnósticos, filogenéticos y taxonómicos fundamentalmente.

Para fines diagnósticos, como estrategia del estudio del genoma, se han identificado zonas de DNA muy repetidas en el parásito. Esta búsqueda ha proporcionado información colateral que resulta de interés respecto a la influencia del genoma del parásito en la susceptibilidad a la infección. Así, en Plasmodium berghei, la cepa NK65 productora de gametocitos viables, presenta en la fase intraeritrocitaria un 18% de secuencias altamente repetidas, mientras que la cepa ISTISAN, que ha perdido en pasajes de laboratorio la capacidad de dar gametocitos viables, presenta solo un 3% de

secuencias repetidas (15). Estudios posteriores por Birago, C. et al., (4) confirman estos resultados encontrando una clara asociación entre la pérdida de infectividad de clonas puras de parásitos NK65 y la cantidad de DNA repetido. Estos resultados sugieren que el DNA altamente repetido se encuentra asociado a gametogénesis por lo que podemos tomarlos como ejemplos de diferencias de susceptibilidad innata del parásito, para desarrollarse en su hospedero.

Otro aspecto que puede considerarse en esta clase de susceptibilidad son las infecciones virales de los parásitos reportadas desde 1960 para algunos protozoarios como Entamoeba histolytica. Es factible que la búsqueda de virus en parásitos más complejos resulte exitoso y sea un agente causante de heterogeneidad en la población. Así podríamos encontrar formas parasitarias no infectadas con virus extracromosomales de RNA o DNA o integradas al cromosoma del hospedero.

Es razonable suponer que la presencia de virus en estos parásitos modifiquen diferencialmente su expresión genética dependiendo del virus infectante y por lo tanto su capacidad de sobrevivir en el hospedero, lo que implicaría diferencias intrínsicas de susceptibilidad del parásito al hospedero.

1.c Susceptibilidad innata asociada a interacciones entre parásito y hospedero a nivel genómico.

Modificación del genoma del hospedero por el parásito

Es posible que el genoma del parásito y del hospedero resulten de un proceso de selección de la interacción más apta entre las especies parasitarias y las hospederas, o bien que se haya modificado como consecuencia de su interacción.

Estudios de traducción in vitro del genoma de Babesia rodhany han revelado secuencias de aminoácidos antigénicas comunes entre el parásito y el hospedero. Esta homologia proteica podría ser el resultado de la interacción entre el genoma del parásito y del hospedero. (71).

Un ejemplo de que los parásitos pueden modificar la expresión genómica del hospedero se presenta en la esquistosomiasis (producido por Schistosoma mansoni) en la cual el parásito disminuye la traducción de proteinas a través de la disminución de los niveles de andrógenos (30). Este parásito ademas induce cambios en la expresión del receptor de IL-2, (27,77) así como en los niveles de IL-2 (33), dando como resultado el patrón de inmunosupresión característico de esta parasitosis. Cambios de este tipo podrían ser inducidos por el parásito a fin de generar un ambiente más apropiado para su sobreviviencia.

Conclusiones

Definiendo un individuo como hospedero y otro como su parásito, la especie hospedera ofrece ambientes heterogéneos respecto a lo propicio para el crecimiento del parásito también el parásito será heterogéneo potencialmente respecto a su patogenicidad o a su capacidad de reproducción en el hospedero. Una vez en contacto, el convivio de ambas especies puede generar en ambas, modificaciones que cambien el destino de la relación hospedero-parásito modificándose la susceptibilidad inicial. Según estos criterios las diferencias de susceptibilidad del hospedero al parásito pueden ser directamente proporcionales a la heterogeneidad inicial en la población hospedera y parásita y la heterogeneidad generada en ambas poblaciones como consecuencia de esta interacción. A la fecha se han identificado algunas regiones genómicas cuyas formas alternativas modifican los patrones de susceptibilidad, sin embargo falta mucho por conocer acerca de las heterogeneidades poblacionales relevantes para modificar la susceptibilidad en la relación hospedero-parásito.

Otro aspecto por explorar son las funciones de los productos fenotípicos que se han asociado a diferencias de susceptibilidad a las parasitosis. Los avances en este aspecto permitirán conocer la dinámica de la interacción hospedero-parásito. Es muy probable que el manejo de nuevas técnicas de Biología Molecular como la creación de ratones transgénicos sea de gran utilidad a fin de dilucidar la relación especie-especificidad de las prasitosis. La adaptación de los modelos experimentales en condiciones experimentales de infección permitirá explorar la relevancia de

los factores genéticos en la infección y transmisión de las parasitosis.

TABLA 1

DETERMINISMO GENETICO DE LA SUSCEPTIBILIDAD A INFECCIONES PARASITARIAS MURINAS:

PROTOZOARIOS

PARASITO	RESISTENTE Cepa	Haplotipo	SUSCEPTIBLE Cepa	Haplotipo	Heredabilidad	Criterio de medición de la infección	Ref
Trypanosoma cruzi (Protozoario hemático)	B10	b	A	a	Resistencia dominante (Poligénico)	Variaciones en los niveles de parasitemia en sangre.	77
	BALB/c	d	A.BY,C3H.SW	b			
	C3A/B10.BR	k	DBA/2	d			
	DBA/1.SJL	q	AKR.C3H	k			
	B6	b					
Trypanosoma brucei (Protozoario hemático)	B6	b	C3H/He	k	Resistencia dominante o codominante (Poligénico)	Variaciones en los niveles de parasitemia en sangre.	12
Trypanosoma congolense (Protozoario.	B6	b	AJ			Variaciones en los niveles de parasitemia en sangre y muerte.	52,53
Leishmania enigma (Protozoario intracrofágico)	C3H, AKR	k	BALB/c	d	Susceptibilidad dominante	Desarrollo de lesión cutés persistente.	58
	A	a	B6	b			31
			DBA/2, B10.D2	d	Un solo gene autosómico.		
L. donovani (Protozoario intracrofágico)	A	a	BALB/c, B10.D2	d		No. de parásitos presentes en núcleos de células hepáticas.	5,6,7
	DBA/2	d	B6	b			
	C3H/He	k					
L. brasiliensis (Protozoario intracrofágico)			A	a		Desarrollo de lesión cutánea persistente.	37
			B6	b			
			BALB/c	d			
			C3H	k			
			SWR	q			
L. mexicana (Protozoario intracrofágico)	C3H, AKR	k	C3A	k	Resistencia dominante o codominante.	Desarrollo de lesión cutánea persistente.	60
	B6	b	BALB/c	d			
Giardia muris (Protozoario intestinal)	BALB/c	d	C3H/He	k		Desarrollo de infección crónica.	67
Toxoplasma gondii multigénica (Protozoario de células endoteliales y leucocitos mononucleares)	B6	b	BALB/c	d		Indice de mortalidad	3,86
	B10.D2	d					
	C3H	k					
	DBA/1	q					
Plasmodium berghei (Protozoario intraeritrocítico)	B6	b	A	a	Resistencia dominante multigénica	Variaciones en niveles de parasitemia y muerte.	23,45

HELMINTOS

PARASITO	RESISTENCIA Cepa	Haplotipo	SUSCEPTIBLE Cepa	Haplotipo	Heredabilidad	Criterio de medición de la infección	Ref
Echinococcus multilocularis (Helminto larvario hepático)	A	a	AKR	k		Cambios hematológicos y alteraciones gruesas en el sistema linforeticular.	67
	B10	b					
	BALB/c, DBA/2	d					
	C3H/C3A	k					
	DBA/1	q					
Heterocyprinoides subtilis (Nematodo intestinal)	B6	b	C3H	k	Resistencia dominante (poligénica)	No. de larvas eliminadas antes del día "n". Resistencia a reinfección.	42,64
	BALB/c	d	A	a			
Ancylostoma suum (Nematodo pulmonar)	C3A	k	B6	b	Resistencia dominante	No. de arvas en pulmón.	37
	C3H	k	C3A/N, B10.Br	k			
Schistosoma mansoni (Trematodo hemático)	A, B10.A	a	C3H, B10	b		No. de gusanos recuperados.	75
	B6, B10	b	BALB/B	b			
	DBA/2, B10.D2	d	BALB/c	d			
	C3H	k					
	S.SL	s					
S. japonicum (Trematodo hemático)	129/J		B6	b		No. de gusanos recuperados.	49
Taenia taeniaeformis (Cestodo larvario hepático)	B6	b	C3H, AKR	k		No. de cisticercos en hígado.	57,40
	BALB/c, DBA/2	d	C3A/H				
Trichinella spiralis (Nematodo intestinal)	A	a	B10.M(17R)	sq1	Resistencia dominante	No. de larvas expulsadas antes del día "n" o No. de larvas en tejido.	76,80
	BALB/c	d	B6	b			81,82
	C3H, AKR	k	B10.A(1R)	b1			
	DBA/1, B10.Q	q	B10.BM	b3			
	B10.S	s	B10.A(4R)	b4			
			B10.A(5R)	b5			
			AKR.B10.K	k			
			B10.A2M	m			
			B10.P	p			
			B10.RIII	r			
			S.SL	s			
			B10.HTT	t3			
Trichuris muris (Nematodo intestinal)	BALB/c	d	B10	b	Resistencia dominante	No. de larvas eliminadas antes del día "n"	78
	BALB/B	b	B10.D2/n	d			
	BALB/T, C3A	k	B10.BR	k			
	KIH		B10.6	Q			
			C3A/2				
Taenia crassiceps (Cestodo larvario)	BALB/K	k	BALB/c	d		No. de cisticercos recuperados en cavidad peritoneal	68
	BALB/B	b					
	B6	b					

TABLA 2

PROTEINAS REGULADORAS SECRETADAS POR EL SISTEMA LINFOHEMATICO

	Funciones principales	Sitio de codificación en Murinos	
IL-1	Acción sinérgica con IL-2. Inducción de producción de citocinas y linfocinas. Promotor de diferenciación de células T.		41
IL-2	Factor de crecimiento de linfocitos		32
IL-3	Funciones hematopoyéticas.		69,29
IL-4	Factor estimulatorio de linfocitos B. Inducción de MHC Clase II.		34
IL-5	Factor de crecimiento de linfocitos B.		34
IL-6	Factor de diferenciación de linfocitos B. Inducción del receptor de IL-2 en células T.	Ch 5 (ratón)	34,51
IL-7	Factor de crecimiento de células B.		20
IFN-α	Incrementa la expresión moléculas Clase I del MHC en linfocitos. Modula respuestas de Ac's. Incrementa actividad de células NK.	Ch 4 (ratón)	10,39,62
IFN-γ	Induce moléculas del MHC clase I y clase II. Potente activador de la función de macrófagos.	Ch 10 (ratón)	39,62

TABLA 3

PROTEINAS DE SUPERFICIE CELULAR CODIFICADAS POR GRI

		Sitio de codificación en Murinos	Funciones principales	Ref
Receptores para Antígenos	LB (Ig's)	Cκ Ch 12 Cλk Ch 16 Cλ1 Ch 6	Reconocimiento de antígeno soluble o libre y en superficies celulares de células presentadoras de antígeno	55
	LT	RCT Ch 13 RCT Ch 6 RCT Ch 14	Reconocimiento de antígeno en superficies celulares asociados a productos codificados por genes del MHC.	9
Receptores para Interleucinas	IL-1 IL-2 IL-3 IL-4 IL-5 IL-6 IL-7	Ch 10	Reconocimiento de la interleucina especica y participación como elementos dinícos en la secuencia de eventos que llevan al efecto final de la interleucina	72 39
Moléculas del MHC	K/D/L Qa Tla	Ch 17	Moléculas mediadoras de lin focitolisis y rechazo de transplantes.	35
	I-A/I-E	Ch 17	Moléculas presentadoras de antígeno, mediadoras de fun ciones supresoras y cooperadoras.	36

TABLA 4

PATRONES DE SUSCEPTIBILIDAD A ENFERMEDADES PARASITARIAS MURINAS ASOCIADAS A SEXO

Parasito	Susceptible	Resistente	Cepa	Ref.
P R O T O Z O A R I O S				
Lesishmania mexicana	M	H	DBA/2	1
Leishmania major	M	H	BALB/c DBA/2N DBA/2J	50
Leishmania donovani	M	H		21
Trypanosoma rhodesiense	M	H	C576B1/6J	24
Trypanosoma cruzi			CBA/N C3H A	28
Plasmodium berghei	M	H	DBA	22
Plasmodium chabaudi	M	H	B10	87
Trichomonas vaginalis	M	H		2
Brugua pahangi	M	H	C57BL/6	88
H E L M I N T O S				
Trichinella spiralis	M	H	CD-18 wiss	65
Nematospiroides dubius	M	H	C3H	14
Echinococcus granulosus	M	H	NAMRU-3	17
Taenia crassiceps	H	M	BALB/c BALB/B BALB/K	68
Taenia taenieaeformis	M	H	C3H/He	47

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XI. DISCUSION GENERAL Y PERSPECTIVAS

La cisticercosis causada por T. solium es un serio problema de salud cuando afecta el humano y económico cuando afecta al cerdo. En México se reporta una alta incidencia de esta enfermedad asi como en varios paises de Latinoamérica, Asia y Africa. La forma grave de cisticercosis es la neurocisticercosis, y su incidencia es alarmante según los reportes de los servicios de neurologia y neurocirugia del pais.

En cisticercosis porcina se ha intentado estimar las pérdidas económicas que genera esta enfermedad, calculando los porcentajes de cerdos cisticercosos reportados por los ratros oficiales. Estos resultados son probablemente muy bajos comparados con la frecuencia de la enfermedad en varios estados, donde el número de cerdos sin inspección por parte de las las instituciones oficiales alcanza hasta el 50% o más del total de cerdos sacrificados.

Siendo un problema de salud y económico en México, el estudio de esta enfermedad resulta de gran interés práctico, además del biológico implicito en la convivencia intima de dos organismos muy bien diferenciados biológicamente.

En esta tesis se incluyen un grupo de trabajos que intentan ahondar en el conocimiento de la cisticercosis. La estrategia utilizada ha sido el empleo de otro cestodo: Taenia crassiceps, cuya fase de cisticerco es capaz de reproducirse en ratones. El modelo ha permitido contribuir en distintos aspectos de la cisticercosis que discutiré separadamente:

1. DIAGNOSTICO Y EPIDEMIOLOGIA
2. INMUNOPROFILAXIS
3. SUSCEPTIBILIDAD A LA CISTICERCOSIS

Diagnóstico y Epidemiologia

El cisticerco de Taenia crassiceps comparte gran cantidad de antigenos con el cisticerco de Taenia solium, y puede reemplazar a los antigenos de T. solium sin detrimento en la sensibilidad y especificidad en la serologia diagnóstica de cisticercosis, con la gran ventaja de contar con una mezcla antigénica de heterogenidad controlada. Este hallazgo, aunado a la facilidad en la obtención de antigenos a partir de los ratones infectados, constituye un avance significativo en el diagnóstico de la cisticercosis. Del conjunto de antigenos de Taenia crassiceps hemos identificado aquellos reconocidos con alta frecuencia por individuos cisticercosos. Esta información podria ser de utilidad en la selección de antigenos a ser clonados para utilizarse con fines diagnósticos. A la fecha ya se han comenzando a utilizar los antigenos de T. crassiceps en proyectos epidemiológicos que incluyen tanto la determinación de cantidad de anticuerpos (por hemaglutinación y ELISA) como la determinación del repertorio antigénico reconocido en el liquido vesicular del cisticerco por inmunoelectrotransferencia.

Las discusiones sobre el inmunodiagnóstico de la cisticercosis no han terminado; sobreviven gracias a la heterogeneidad en los procedimientos y a confusión conceptual. El uso de antigenos de T. crassiceps podria favorecer la uniformización de los antigenos

utilizados en las pruebas diagnósticas reduciendo posibles discrepancias.

A pesar del avance y simplificación del inmunodiagnóstico con el uso de estos antigenos, quedan aún aspectos importantes por resolver como es la distinción entre los distintos tipos de contacto con el cisticerco: a) contacto sin infección; b) contacto con infección resuelta; c) contacto con infección no localizada al SNC; d) contacto con infección localizada al SNC, interpretando el valor predictivo de la serologia positiva. La resolución de estos aspectos seria de enorme valor en la interpretación de los resultados epidemiológicos.

En este sentido estamos estudiando la respuesta inmune en los distintos tipos de contacto utilizando inmunoelectrotransferencias. La senal obtenida por esta técnica es muy rica en sus posibilidades discriminativas. En un primer nivel descriptivo nos permite identificar la respuesta o no contra un conjunto de antigenos, en un siguiente nivel es susceptible de agregar una tercera dimensión al fenómeno de reconocimiento: la intensidad de la respuesta. El análisis integro de la senal obtenida nos permitirá obtener perfiles de un sujeto a manera de huellas dactilares con las que mejor discriminar los múltiples facetas de la cisticercosis.

Otro camino alternativo para avanzar en el inmunodiagnóstico es la utilización de antigenos o péptidos preparados en base a procedimientos bioquímicos y/o técnicas de biología molecular o de sintesis química. Seria demasiado optimista suponer que el uso de antigenos purificados aislados pudiera satisfacer la interpretación de

la relación tan heterogénea entre hospedero y parásito. Es posible que aún purificados se requiera la mezcla de varios de estos antigenos para cubrir el amplio espectro de formas de contacto en esta parasitosis.

Inmunoprofilaxis

Otro aspecto de gran interés en cisticercosis es el control de la transmisión de la enfermedad a través de medidas efectivas, accesibles a nuestras capacidades cientificas y técnicas, y adecuadas a las circunstancias de la crianza rústica de cerdos. En este sentido debe considerarse que la supervivencia de este parásito subyace en problemas socioeconomicos muy dificiles de modificar, que favorecen las condiciones que mantienen el ciclo biológico. Del hecho que la respuesta inmune media en la relación hospedero parásito de varias teniasis - cisticercosis se desprende la esperanza de reducir el riesgo o la intensidad de la infección en cerdos, en especial considerando que el tiempo de protección efectiva se reduce a aproximadamente un ano, tiempo después del cual los cerdos entran al consumo. Parece entonces una medida realista de control la posibilidad de reducir parcial o totalmente la cisticercosis porcina por vacunación interfiriendo en el ciclo de la teniasis/cisticercosis.

El modelo de cisticercosis murina ofrece un modelo experimental adecuado para obtener un primer estimado de las posibilidades reales de una vacuna.

En esta tesis se describe en detalle la evaluación experimental de una vacuna contra la cisticercosis, utilizando el modelo de cisticercosis murina y antigenos provenientes de cisticercos de Taenia solium asi como de cisticercos de Taenia crassiceps. Los resultados indicaron que ambos antigenos resultan igualmente eficaces en inducir protección parcial o total contra el desafio experimental.

Esta fase experimental ha producido la suficiente información para proseguir con bases objetivas y optimistas la evaluación de una vacuna en cerdos expuestos al desafio natural utilizando antigenos de T. crassiceps. Cabe remarcar que la producción masiva de la vacuna no presenta dificultades considerando la capacidad de crecimiento de Taenia crassiceps. Sin embargo, es de considerar que podriamos aumentar la eficiencia de la vacunación y reducir el costo de la vacuna a través de la clonación de antigenos, la sintesis de péptidos y/o purificación de antigenos. En este sentido el análisis del repertorio antigénico reconocido por animales vacunados y protegidos senalará a los mejores candidatos a ser clonados o secuenciados a fin de ser sintetizados.

Los estudios de la respuesta inmune humoral realizados con animales vacunados y no vacunados senalan algunos antigenos que parecen ser más frecuentemente reconocidos en los grupos de ratones protegidos por vacunación. Sin embargo, ante las evidencias recopiladas en este estudio de que los anticuerpos no desempenan un papel protector importante al menos de una manera sencilla, quizás para la identificación de antigenos relevantes asociados a protección deban agregarse estudios de la capacidad de antigenos para inducir inmunidad celular.

Susceptibilidad a la cisticercosis

La cisticercosis experimental en ratones ofrece la posibilidad de conocer la intimidad de la relación hospedero parásito, y esta relación permite explorar los mecanismos moleculares que el hospedero genera en respuesta al parásito así como la capacidad del parásito para modificarlos. En la búsqueda de este objetivo, se realizaron los primeros diseños a fin de identificar los determinantes biológicos que afectan la susceptibilidad del hospedero al parásito.

Estos diseños permitieron identificar como variables biológicas de la susceptibilidad al sexo, al complejo mayor de histocompatibilidad, al estado inmunológico y al compartimento en el que se desarrolla la infección.

La asociación de la susceptibilidad con el H-2 resulta de especial interés considerando que las moléculas codificadas por el Complejo Mayor de Histocompatibilidad (H-2) están involucradas en los mecanismos de presentación antigénica, en los fenómenos inmunológicos resultantes de esta presentación, y en el conjunto de eventos inmunológicos que determinan el destino de la relación hospedero y parásito.

El modelo de cisticercosis murina podría ser una herramienta muy útil para estudiar estos eventos considerando: la marcada asociación entre la susceptibilidad a la infección y el complejo mayor de histocompatibilidad; la accesibilidad del peritoneo, compartimento en el que se desarrolla óptimamente la infección; la disponibilidad de

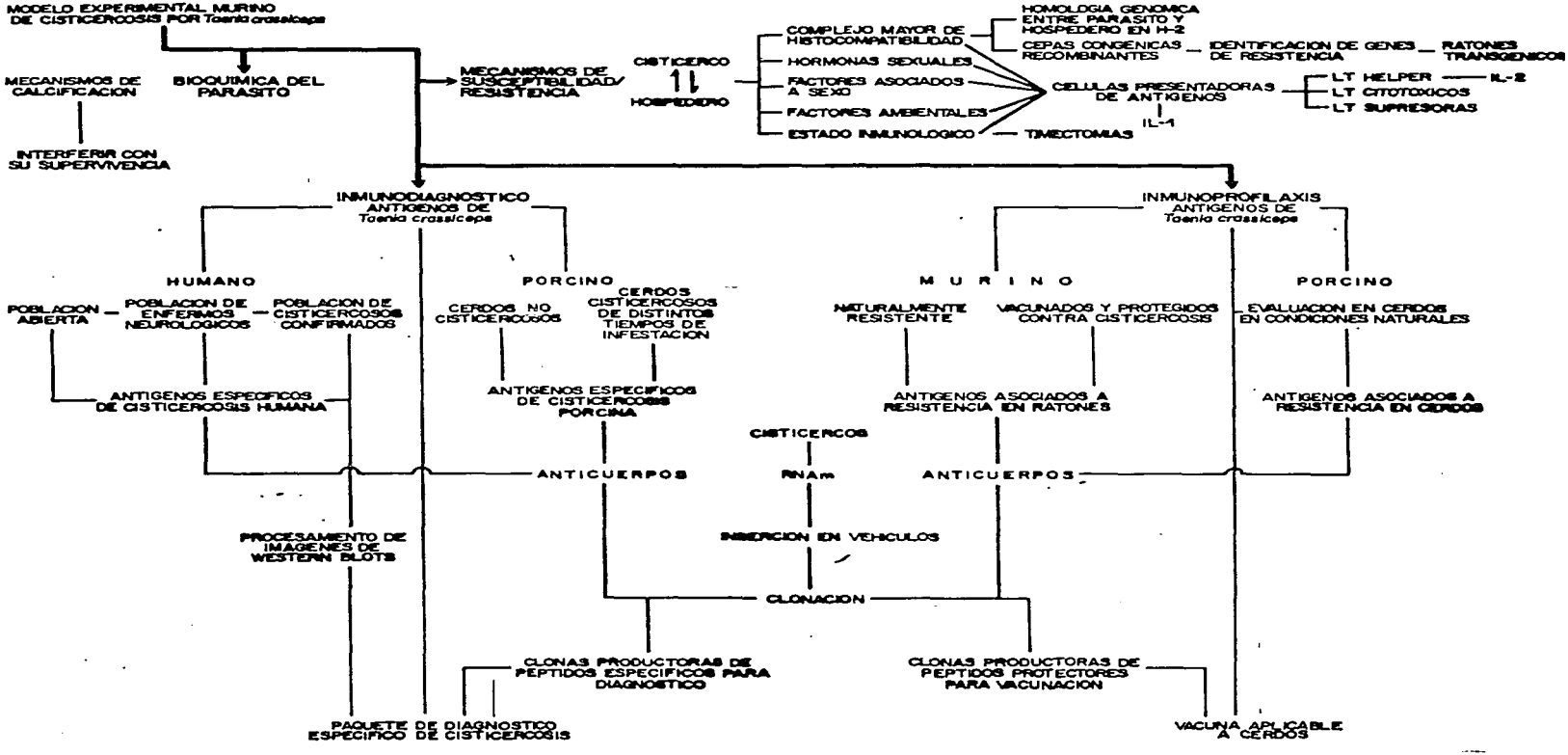
reactivos biológicos (anticuerpos especificos contra marcadores celulares y productos de H-2; sondas para regiones genómicas de H-2, de inmunoglobulinas, de receptores de linfocitos T) asi como la posibilidad de modificar el genoma murino introduciéndole genes de resistencia o genes del propio parásito a través de técnicas de transfección de genes.

Los resultados en la evaluación de la respuesta inmune humoral no indican funciones protectoras sencillas asociadas a los anticuerpos séricos. Esto sugiere que la respuesta celular constituye probablemente el componente crítico que media esta relación. Por otro lado la marcada diferencia de susceptibilidad asociada al sexo agrega a este panorama otro aspecto muy interesante ante la expectativa de que esta diferencia entre sexos esté mediada inmunológicamente. Es posible que las variables sexo y H-2 no sean independientes, considerando los reportes recientes de modulación de la expresión de H-2 por hormonas sexuales.

Desde un punto de vista aplicativo este trabajo establece las bases necesarias para especular sobre posibles influencias genéticas en la susceptibilidad de cerdos a cisticercosis por T. solium. En este sentido y en el intento de disminuir la incidencia de la cisticercosis porcina, podría considerarse la identificación o desarrollo de razas de cerdos resistentes a la cisticercosis y favorecer su crianza.

Es mi esperanza que la cisticercosis experimental de los ratones señale caminos de exploración más probablemente fructíferos en la identificación de factores biológicos asociados a la susceptibilidad en cisticercosis por T. solium

En el esquema que anexo a continuación intento sintetizar las áreas de investigación que aparecen como especialmente relevantes a la vista de los resultados reportados en este trabajo de tesis.


MODELO EXPERIMENTAL MURINO DE CISTICERCOSIS POR Taenia crassiceps
MECANISMOS DE CALCIFICACION
INTERFERIR CON SU SUPERVIVENCIA
BIOQUIMICA DEL PARASITO
MECANISMOS DE SUSCEPTIBILIDAD/ RESISTENCIA
CISTICERCO
HOSPEDERO
COMPLEJO MAYOR DE HISTOCOMPATIBILIDAD
HORMONAS SEXUALES
FACTORES ASOCIADOS A SEXO
FACTORES AMBIENTALES
ESTADO INMUNOLOGICO
HOMOLOGIA GENOMICA ENTRE PARASITO Y HOSPEDERO EN H-2
CEPAS CONGENICAS RECOMBINANTES
IDENTIFICACION DE GENES DE RESISTENCIA
RATONES TRANSGENICOS
CELULAS PRESENTADORAS DE ANTIGENOS
IL-1
LT HELPER
IL-2
LT CITOTOXICOS
LT SUPRESORAS
TIMECTOMIAS
INMUNODIAGNOSTICO ANTIGENOS DE Taenia crassiceps
INMUNOPROFILAXIS ANTIGENOS DE Taenia crassiceps
HUMANO
POBLACION ABIERTA
POBLACION DE ENFERMOS NEUROLOGICOS
POBLACION DE CISTICERCOSOS CONFIRMADOS
ANTIGENOS ESPECIFICOS DE CISTICERCOSIS HUMANA
PROCESAMIENTO DE IMAGENES DE WESTERN BLOTS
PORCINO
CERDOS NO CISTICERCOSOS
CERDOS CISTICERCOSOS DE DISTINTOS TIEMPOS DE INFESTACION
ANTIGENOS ESPECIFICOS DE CISTICERCOSIS PORCINA
CLONAS PRODUCTORAS DE PEPTIDOS ESPECIFICOS PARA DIAGNOSTICO
PAQUETE DE DIAGNOSTICO ESPECIFICO DE CISTICERCOSIS
ANTICUERPOS
CISTICERCOS
RNAm
INSERCION EN VEHICULOS
CLONACION
MURINO
NATURALMENTE RESISTENTE
VACUNADOS Y PROTEGIDOS CONTRA CISTICERCOSIS
ANTIGENOS ASOCIADOS A RESISTENCIA EN RATONES
ANTICUERPOS
CLONAS PRODUCTORAS DE PEPTIDOS PROTECTORES PARA VACUNACION
PORCINO
EVALUACION EN CERDOS EN CONDICIONES NATURALES
ANTIGENOS ASOCIADOS A RESISTENCIA EN CERDOS
VACUNA APLICABLE A CERDOS