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ESTUDIO DE LA SUSCEPTIBILIDAD A LA CISTICERCOSIS EXPERIMENTAL MURINA POR Taenia crassiceps

TESIS QUE PARA OBTENER EL GRADO DE
MAESTRA EN CIENCIAS QUIMICAS (BIOQUIMICA)

P R E S E N T A:

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CONTENIDO

RESUMEN

El trabajo presentado en esta tesis establece que la susceptibilidad a la cisticercosis murina causada por el metacéstodo de Taenia crassiceps está notablemente influenciada por los genes del Complejo Mayor de Histocompatibilidad (MHC) del ratón (H-2). Varias semanas después de provocar una infección intraperitoneal con larvas del metacéstodo las diferencias observadas en la carga parasitaria de ratones congénicos en H-2 con fondo genómico BALB, indican que las cepas de haplotipo $H\text{-}2^d$ son las más susceptibles mientras que las de haplotipo $H\text{-}2^k$ y $H\text{-}2^b$ son las más resistentes. Los genes del resto del genoma así como los niveles y especificidades de los anticuerpos inducidos por los antígenos parasitarios, no parecen tener un efecto tan importante e inmediato sobre la mayor o menor susceptibilidad de los ratones a esta cisticercosis experimental. En vista de estos hallazgos se propone que la mayor susceptibilidad de las especies hospederas puede deberse a una homología huésped-parásito a nivel de moléculas de $H\text{-}2^d$, de manera que, el grado de rechazo inmunológico del parásito podría estar influído por las similtudes entre los antígenos de histocompabilidad $H\text{-}2^d$ del ratón y algunas moléculas sintetizadas por el cisticerco.

En este trabajo de investigación se presentan varios resultados que consolidan esta propuesta:

1.- El hallazgo de antígenos similares a $H\text{-}2^d$ y $H\text{-}2^b$ en los parásitos que han crecido en ratones BALB con haplotipos $H\text{-}2^d$ y $H\text{-}2^b$, lo cual pudo ser confirmado utilizando un ensayo de inhibición de ELISA. 2.- La extensa reactividad cruzada , vista por Inmunoelectrotransferencias, entre los antígenos del parásito y un panel de anticuerpos anti H-2 de diferentes especificidades. 3.- Las aloinmunizaciones, previas

al desafío con parásitos, que mostraron que en la cepa susceptible BALB/c la inducción previa de una inmunidad de transplante no modifica la carga parasitaria respecto a los animales no inmunizados, en contraste con la cepa resistente BALB/B, en donde la inmunidad de transplante contra antígenos $H\text{-}2^d$ indujo resistencia al parásito. 4.- La homología genómica observada por hibridización positiva entre el genoma del parásito y la sonda ^{32}p-cDNA que codifica para antígenos MHC clase I del ratón (con el haplotipo $H\text{-}2K^d$), lo cual sugiere una capacidad potencial del parásito para sintetizar moléculas con secuencias similares a las de la cadena alfa del H-2.

Las conclusiones que se derivan de este trabajo contribuyen al conocimiento que se tiene de los mecanismos moleculares adaptativos que se desarrollan durante la convivencia biológica entre dos organismos. Esta nueva información podría ser de utilidad para comprender asociaciones hospedero-parásito más complejas.

ABSTRACT

The present work establishes that mice susceptibility to murine cysticercosis caused by Taenia crassiceps cysticerci is greatly influenced by MHC genes (H-2), as demostrated by the different parasite loads of H-2 congenic mice with BALB background, in which H-2^d mice (BALB/c) were the most susceptible while BABL/K (H-2^k) and BALB/B (H-2^b) were comparatively resistant. Furthermore, nor non-H-2 genes have an importante effect upon parasite load neither the greatest bulk of antibodies induced by parasite antigens are associated with resistance or susceptibility to this experimental cysticercosis. In view of these findings, we propose that the host susceptibility to cysticercosis is related to a homology between host and parasite in the context of H-2^d antigens, thus, as in regular tissue transplantation immunity, a correct matching in histocompatibility H-2 antigens of the host with some other parasite molecules may be involved in the observed susceptibility.

The following results agree with our proposal:
1.- The finding of H-2^d and H-2^b-like antigens in parasites grown in BALB mice of H-2^d and H-2^b haplotypes, as identified by an inhibitory ELISA assay. 2.- The extensive crossreactivity in Western Blot between the parasite antigens and a panel of anti H-2 antibodies with different specificities. 3.- Allograft immunizations, previous to parasite challenge, did not modify the expected parasite load in BALB/c but in contrast, in BALB/B resistant mice transplantation immunity against H-2^d, significantly reduced the expected parasite load. 4.- The genomic homology by positive hybridization between the parasite genome and a ^{32}p-cDNA clone encoding one mouse class I MHC antigen (H-$2K^d$ haplotype), suggests a potential capability of the

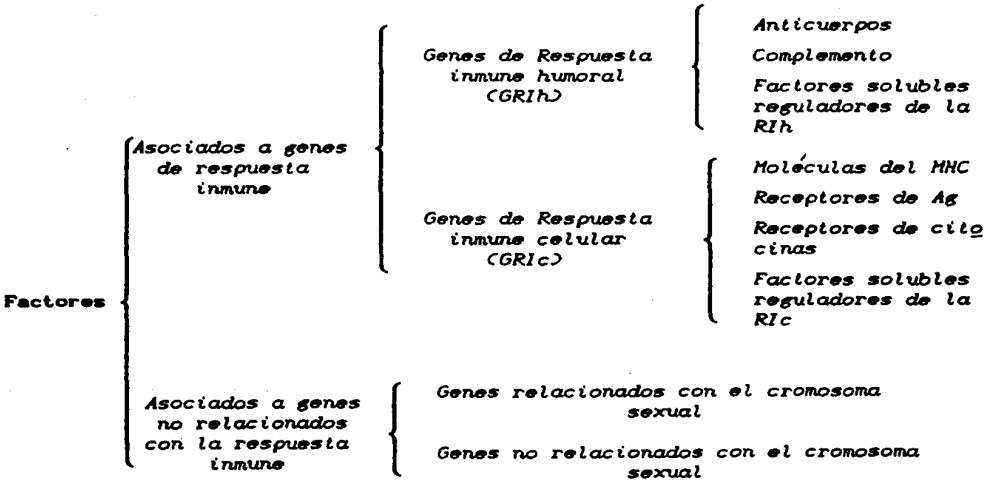
parasite to produce itself these H-2 like antigens.

The conclusions derived from this work, contribute to the knowledge of the adaptative molecular mechanims generated in host-parasite relationships.

INTRODUCCION

TABLA 1

FACTORES DEL HOSPEDERO QUE PUEDEN MODIFICAR LA RELACION HOSPEDERO-PARASITO



La persistencia de un parásito en un hospedero inmunológicamente competente ha constituido uno de los fenómenos biológicos más interesantes y de los que, paradójicamente, todavía se sabe poco. Esto se debe en parte al escaso conocimiento que se tiene sobre los mecanismos moleculares que controlan la relación hospedero-parásito, así como también a la gran complejidad y peculiaridad que los caracteriza. En este sentido, nos ha interesado conocer algunas de las estrategias de convivencia que se establecen en una relación hospedero-parásito así como también los fenómenos biológicos que determinan la mayor o menor susceptibilidad a ésta parasitosis. El presente trabajo de está enfocado a estudiar la posible homología de algunas de las moléculas del MHC del hospedero con otras del parásito, como una de las estrategias que participan durante la convivencia entre el cisticerco de Taenia crassiceps y su hospedero intermediario el ratón.

Un aspecto importante a considerar es la capacidad que tiene un parásito de poder infectar a organismos vivientes muy similares filogenéticamente con distintos grados de susceptibilidad. Al respecto es indudable que factores genéticos determinados tanto por el parásito como por el hospedero, así como factores ambientales, juegan un papel crucial en la habilidad que tenga este último para iniciar una respuesta inmunitaria duradera y sobre todo protectora que le permita controlar la infección.

Los factores genéticos asociados al hospedero capaces de modificar el curso de la relación hospedero-parásito pueden ser agrupados como se ilustra en la Tabla 1. Diferente expresión de los genes involucrados puede asociarse con distintos grados de resistencia tal y

TABLA 2
PROPIEDADES DEL PARASITO PARA MODIFICAR LA SUSCEPTIBILIDAD DEL HOSPEDERO

- *Modificación del hospedero*
  - *A nivel celular*
    - *Ambiente intramacrofágico*
    - *Expresión defectuosa de H-2*
  - *A nivel de los eventos que conforman la respuesta inmune*
    - *Inmunosupresión*
    - *Efectos anticomplementarios*
    - *Efectos antiinflamatorios*
    - *Degradación de anticuerpos*
- *Modulación de componentes del hospedero*
  - *Producción de anticuerpos bloqueantes*
  - *Producción de anticuerpos no específicos*
- *Modificacion del parasito*
  - *Variación antigénica*
  - *Modulación antigénica*
  - *Mimetismo molecular*

como se ha señalado detenidamente en el Apéndice 1.

El parásito a su vez posee facultades de afectar la susceptibilidad del hospedero. Como se aprecia en la Tabla 2 la organización de estas propiedades, bien sea que hayan sido demostradas o estén postuladas, sigue tres líneas generales: modulación de los componentes del hospedero, modificación del hospedero y modificación del parásito.

La modificación de los macrófagos es uno de los mecanismos que más frecuentemente ejercen algunos parásitos. No obstante que los macrófagos son células cuya función más importante reside en su capacidad de matar, degradar y fagocitar a una amplia variedad de agentes infecciosos, existen diversos microorganismos como Mycobacterium tuberculosis, Mycobacterium leprae y algunos protistas como Toxoplasma, Leishmania (El-On et al. 1990) y Trypanosoma cruzi que pueden subsistir y crecer dentro de ellos. Se han reconocido al menos tres mecanismos que pueden ser empleados por algunos de estos parásitos para evadir la acción citocida del macrófago. Característico de Toxoplasma gondii es su capacidad de inhibir la fusión de los lisosomas macrofágicos con las vesículas fagocíticas que lo contienen (Jones & Hirsh 1972). Algunos parásitos como Leishmania tropica y Leishmania enrielti permiten la formación del fagolisosoma y, sin embargo, pueden subsistir dentro de él, probablemente por su capacidad de inhibir enzimas lisosomales (Behin et al. 1975). El tercer mecanismo observado, principalmente en Trypanosoma cruzi, consiste en la habilidad del parásito para mantenerse en el citoplasma de la célula, evadiendo así su contacto con las enzimas lisosomales (Kress et al. 1975, Nogueira & Cohn 1976). Otra modificación que pueden sufrir los macrófagos como

consecuencia de la presencia del parásito es una reducción en la expresión de antígenos de H-2, lo cual ha sido propuesto por Handman et al. (1979) como mecanismo para explicar las diferencias de susceptibilidad a Leishmania tropica entre cepas singénicas de ratones.

Además, se ha reportado que los parásitos pueden modular la respuesta inmunológica del hospedero ejerciendo efectos inmunosupresores. La inmunosupresión se ha sugerido en muchos parásitos entre los que podemos citar a Echinococcus granulosus (Ali 1978, Annen et al. 1981) a Trichinella spiralis (Barriga 1980, Faubert and Tanner 1975) y a Plasmodium falciparum (Hviid et al. 1990). Estos estados de inmunosupresión se han definido en función de que se presenta un aumento en la capacidad de los animales para retener aloinjertos así como porque disminuyen las cantidades de células formadoras de placa, debido posiblemente a la liberación de sustancias linfocitotóxicas.. Han sido descritos varios estados de inmunosupresión generalizada que parecen asociarse a infecciones por Schistosoma mansoni (Ottesen and Poindexter 1980), Trypanosoma brucei (Jayawardena et al. 1978), Toxoplasma gondii y Plasmodium falciparum (Bloom 1979, Wedderbrun and Dracott 1977). Es importante considerar que los estados de inmunosupresión que se han observado en muchas parasitosis pueden ser una consecuencia de la infección o bien pueden ser un proceso que se inicia antes de la misma y que de alguna manera determina la susceptibilidad del individuo a la parasitosis.

Respecto a la capacidad del parásito para modificar algunos de los eventos que conforman la respuesta inmune del hospedero podemos considerar las siguientes estrategias: degradación de anticuerpos IgG por

proteasas liberadas por Schistosoma mansoni (Auriault 1980); efectos anticomplementarios en T. taeniaeformis determinados por la presencia de sustancias en la superficie del parásito que bloquen la cascada del complemento (Hammerberg 1980).; efectos antiinflamatorios de T. spiralis manifestados por la capacidad de la larva para impedir la formación del granuloma en grados similares a los inducidos por esteroides adrenocorticales (Castro et al. 1980).

El segundo y tercer mecanismos generales que emplean los parásitos para aumentar la susceptibilidad a la infección consiste en la modulación de los componentes del hospedero y la modificación del propio parásito a fin de reducir la antigenicidad de sus componentes.

La antigenicidad reducida puede apreciarse en distintas formas: por generación de anticuerpos bloqueantes en la cisticercosis murina causada por Taenia taeniaeformis (Mitchell 1980); por producción de anticuerpos no especificos en la malaria humana (Greenwood 1975); por modulación de antígenos en Leishmania donovani y L. enrielti (Dwyer 1976, Doyle et al. 1974); por variación antigénica como se observa en Fasciola hepatica (Hanna 1980), Trypanosoma brucei (Seed 1974), Nippostrongylus brasilensis (Ogilvie 1974) y Giardia lamblia (Gottstein et al. 1990) y por mimetismo molecular (Mitchell 1979).

Una de las ideas que más ha enriquecido el concepto de la relación hospedero-parásito, tanto en términos evolutivos así como de infección individual, es la proposición de la posible existencia de estructuras comunes entre hospedero y parásito. Esta convergencia molecular propuesta desde 1960 (Damian 1979) y denominada "Tolerancia adaptativa"

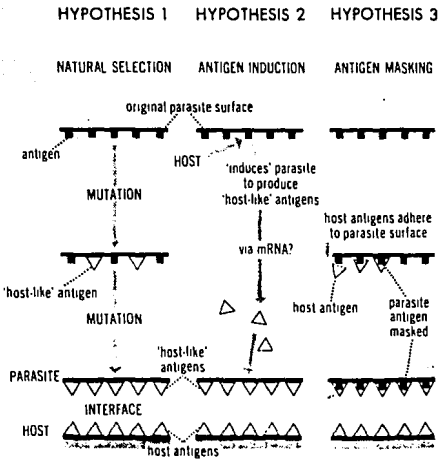


Fig. 1 Comparación diagramática entre el mimetismo molecular (selección natural), inducción antigénica y enmascaramiento antigénico por el Prof. J. D. Smith (Tomado de "Molecular Mimicry: antigen sharing by parasite and host and its consequence. Damian 1979).

(Sprent 1962), "Reacción antigénica cruzada" (Rowley and Jenkin 1962) y "Mimetismo molecular" (Damian 1964) puede ser vista como una explicación de la persistencia de los parásitos en un hospedero inmunológicamente competente pero no ha podido probar su importancia como mecanismo de evasión de la respuesta inmune. Otras posibles consecuencias que surgieron alrededor de esta idea fueron el polimorfismo antigénico del hospedero, la susceptibilidad a padecer una infección y la enfermedad inmunopatológica (autoinmune).

Del concepto de mimetismo molecular se han derivado dos modificaciones: la alternativa propuesta por Capron et al. (1968) y denominada hipótesis de la inducción antigénica y la hipótesis del enmascaramiento antigénico propuesta por Smithers, Terry and Hockley (1968). Como puede apreciarse en la figura 1, éstas tres alternativas difieren en el mecanismo y origen de las moléculas compartidas entre el hospedero y el parásito. La hipótesis de la selección natural (o del mimetismo molecular) y la hipótesis de la inducción antigénica son las que proponen una síntesis directa de antigenos similares a los del hospedero por parte del parásito, si bien en la primera son mecanismos naturales los que operan en el DNA del parásito, mientras que en la segunda es el propio hospedero el que induce la mutación en el genoma del parásito. La tercera hipótesis propone una simple adsorción de moléculas del hospedero al parásito.

La relevancia biológica del mimetismo molecular como se mencionó anteriormente puede tener consecuencias importantes en la estimulación de procesos autoinmunes en el hospedero (Rowley and Jenkin's 1962).

TABLA 3
ANTIGENOS DE REACTIVIDAD CRUZADA ENTRE PARASITOS Y HOSPEDEROS

Schistosoma mansoni-ratón	α-2 macroglobulina productos del MHC β-2 microglobulina antígenos de grupo sanguineo (ABO, Lewis) antígenos de Forsmann receptores para IgG
Plasmodium sp - hombre	α-2 macroglobulina antígenos del sistema ABO antígenos de Forsmann colágena fibrinógeno
Trypanosoma cruzi-hombre	antígenos del sistema ABO antígenos de Forsmann colágena tejido nervioso músculo cardíaco.
Taenia solium - cerdo	Inmunogloulinas IgG
Taenia crassiceps-ratón	albúmina transferrina Inmunoglobulinas IgG
Taenia saginata-bovinos	albúmina Inmunoglobulinas IgG
Echinococcus granulosus-hombre	Inmunoglobulinas
Ascaris lumbricoides - hombre	colágenas

Así, por ejemplo, los antigenos comunes encontrados entre Trypanosoma cruzi y sus hospederos se han relacionado con el daño que se detecta en el músculo cardiaco de los pacientes con tripanosomiasis (Sadigursky et al. 1982, McCormik & Rowland 1989). También la probabilidad de padecer enfermedades autoinmunes después de múltiples infecciones estreptocóccicas se ha relacionado con la reactividad cruzada que existe entre las proteínas M de los estreptococos y los antigenos de transplante humanos (Hirata & Terasaki 1970). Otros procesos autoimunes como el síndrome de Reiter y la artritis reactiva (Inman et al. 1986) se han asociado a antigenos HLA-B27 que cruzan antigénicamente con estructuras de Yersinia enterocolitica y Chlamydia trachomatis.

La convergencia antigénica entre hospedero y el agente infeccioso ha sido además descrita en Klebsiella sp., Shigella sp, Yersinia sp (Van Bohemen et al. 1984, Geczy et al. 1980) asi como en Streptococcus sp. (Pellegrino et al. 1972, Zabriskie et al. 1970, Hirata and Terasaki 1970, Rapaport et al. 1973). En el caso de parásitos, como la mayor parte de ellos requieren para completar su ciclo de vida de uno o más hospederos intermediarios y un hospedero definitivo, es lógico asumir que la reactividad cruzada entre parásito y hospedero pueda darse en cualquiera de estas fases del ciclo. Así en Schistosoma mansoni dos glicoproteínas (de 43 y 39 Kd) características de las etapas larvarias tempranas (miracidias) también se han encontrado en los caracoles hospederos (Biomphalaria glabrata) (Dissous et al. 1986). En este mismo sistema hospedero-parásito se ha encontrado homología entre las tropomiosinas (Dissous et al. 1990). Respecto a homología entre hospederos definitivos y parásitos, encontramos a

Schistosoma mansoni (Damian et al 1973, Smithers et al. 1969, Torpier et al. 1979, Sher et al. 1978), a Plasmodium sp. (McLaughlin et al. 1987) a Trypanosoma cruzi (Hudson & Hindmarsh 1985, Szarfman et al. 1982, Ouaissi et al. 1988, McCormikc & Rowland 1989, Petry et al. 1990) a Echinoccocus granulosus (Varela-Diaz and Coltorti 1973) a Ascaris lumbricoides (Michaeli et al. 1972) a Taenia crassiceps y Taenia saginata (Kalinna et al. 1989) y a Taenia solium (Willms and Arcos 1977). La naturaleza quimica de los antigenos compartidos va desde moléculas muy sencillas como antigenos de grupos sanguineos (sistema ABO y Lewis) o antigenos de Forsmann, hasta moléculas más complejas como las glicoproteinas del complejo mayor de histocompatibilidad (MHC), macroglobulinas, etc. La correspondencia de antigenos de reactividad cruzada entre parásito y hospederos definitivos se ilustra en la tabla 3. De singular importancia es la adquisición de productos del complejo mayor de histocompatibilidad, clase I y clase II, por S. mansoni (Sher et al. 1978), no sólo porque representa uno de los pocos casos de mimetismo molecular complejo, sino por el papel que desempeñan estas moléculas en el hospedero. Los antigenos clase I se han reconocido como marcadores de lo propio dado que se asocian funcionalmente al rechazo rápido de transplantes de tejidos por células T citotóxicas (Klein 1986). También de ellos depende la adquisición de la tolerancia inmunológica contra los antigenos propios (Marrack and Kappler 1988). Las moléculas clase II están limitadas a células del sistema inmunológico y su papel más importante, hasta la fecha, es la presentación del antigeno a células T cooperadoras (Klein 1986). Cabe señalar que la presencia de estos antigenos en los parásitos no parece desempeñar las mismas funciones que en las células del

competente. Gitter & Damian (1982) han sugerido que estas diferencias pueden deberse a que estos determinantes antigénicos se expresan de manera diferente en las células del hospedero. También podría deberse a eventos inherentes a las circunstancias de las interacciones intercelulares (proteínas de membrana, actividad celular, etc.) abriendo la posibilidad de que éstos antígenos de histocompatibilidad compartidos medien críticamente otras relaciones hospedero-parásito. Es muy probable que otros parásitos también expresen proteínas que estén relacionadas con los productos del MHC. Al respecto Harrison et al. (1989) han encontrado una glicoproteína en cisticercos de Taenia saginata que tiene la facultad de adherirse a la lectina de lenteja y que en ensayos de inmunoprecipitación bandea en la región de 45 Kd, de manera similar a los productos del MHC clase I.

Debido a que, en México, una de las parasitosis de mayor importancia por su elevada frecuencia y gravedad es la cisticercosis humana causada por Taenia solium, varios grupos de investigación han abordado éste problema a distintos niveles entre los que resaltan, por una lado, la posibilidad de desarrollar una vacuna y el empleo de drogas citocidas que controlen la parasitosis, y por otro lado estudiar algunos de los factores biológicos que la determinan, etc. Este último punto ha sido uno de los temas de interés de nuestro grupo. Sin embargo, ante la limitación de poder estudiar experimentalemente al cisticerco de Taenia solium, se comenzó a estudiar otro céstodo (el cisticerco de Taenia crassiceps) que antigénicamente está muy relacionado con el primero (Larralde et al. 1989). El principal atractivo de éste modelo radica en que el ratón es una de sus especies hospederas

naturales. La fisiología de éste animal así como su genética son es de las más conocidas entre los mamíferos. Otra ventaja consiste en que el ratón ofrece la posibilidad de estudiar el comportamiento de la infección en cepas singénicas, congénicas y recombinantes, de modo que es posible conocer el papel del MHC y de algunos otros genes en la evolución de ésta parasitosis. Otro aspecto de especial interés de nuestro modelo es que se instala fácilmente en el peritoneo, el cual es un compartimento muy accesible de estudiar y en el que el parásito se reproduce por gemación asexual. Si bien éste modelo ofrece ventajas muy importantes, también presenta un inconveniente que consiste en que la parasitosis que se desarrolla en el peritoneo de éstos animales no es natural, aunque desde el punto de vista de éste estudio no tendría grandes inconvenientes.

En principio este trabajo se concentró en estudiar el crecimiento de los cisticercos en cepas de ratones congénicas y singénicas a fin de identificar posibles diferencias de susceptibilidad que estuvieran determinadas genéticamente.

Los resultados que se obtuvieron, y que constituyen las bases teóricas del trabajo de investigación seguido, mostraron claramente que los genes del complejo mayor de histocompatibilidad del ratón (H-2) tienen una gran influencia en la instalación y crecimiento del parásito siendo las cepas de haplotipo H-2d las más susceptibles, en tanto que las de haplotipo H-2k y H-2b resultaron las menos las susceptibles. Además, se encontró que otros genes, diferentes a los H-2, no afectan significativamente la susceptibilidad en las hembras en tanto que sí lo hacen en machos, aunque en éstos últimos el efecto es

muy débil (Sciutto et al 1990a). Tampoco se encontraron diferencias significativas en la respuesta inmunitaria humoral entre las cepas con diferente susceptibilidad a la infección, siendo la respuesta mayor cuanto más intensa sea la carga parasitaria (Sciutto et al. 1990b).

Estos hallazgos nos llevaron a proponer la hipótesis de que el parásito se instala mejor en el hospedero que más se le parece antigénicamente, como uno de los fenómenos biológicos que pueden estar mediando las diferencias de susceptibilidad. Dado que entre cepas congénicas en H-2 (BALB/c y BALB/B) la única diferencia radica en los productos del MHC, es de esperarse que la similtud hospedero-parásito sea a nivel de éstos antigenos. Si esto resulta así, la menor susceptibilidad podría explicarse en términos del rechazo de injerto, mientras que la susceptibilidad sería una aceptación del mismo. El trabajo de investigación seguido y que constituye el objetivo de ésta tesis consistió en buscar antígenos del MHC del ratón en la superficie del cisticerco que puedan justificar las diferencias de susceptibilidad en las cepas antes mencionadas.

La presentación del trabajo de está organizada en dos secciones. La primera está constituida por la exposición de tres trabajos:

1.- " An Immunoenzymatic assay to measure the expression of murine histocompatibility antigens in macrophages and lymphocytes".

Este trabajo, que constituye uno de los seguimientos metodológicos para la demostración de la presencia de moléculas antigénicamente similares al H-2 en el cisticerco, es una aportación metodológica para estudiar otros antígenos superficiales en células tanto en reposo como

en distintos grados de diferenciación sin modificar la conformación de las moléculas en la célula.

2.- " Murine Taenia crassiceps cysticercosis: H-2 and Sex influence on susceptibility"

3.- " Role of antibodies in experimental murine cysticercosis caused by Taenia crassiceps".

Estos dos últimos trabajos constituyen las bases teóricas para el diseño de la hipótesis de ésta tesis. En el primero se define claramente que la susceptibilidad a esta parasitosis está fuertemente influenciada por los genes de haplotipo H-2 del ratón. En el segundo se reducen las posibilidades de que las diferencias de susceptibilidad observadas entre las cepas congénicas estudiadas puedan deberse a una diferente capacidad de respuesta inmune humoral.

Es importante aclarar que si bien los tres trabajos mencionados anteriormente forman parte de la presentación de esta tesis, es con el objeto de presentar las bases teóricas del trabajo de investigación realizados y no constituyen el objetivo central planteado.

Con base en los resultados expuestos en estos dos trabajos, comenzamos a estudiar diferencias de susceptibilidad que pudieran estar determinadas por características propias del parásito, específicamente investigamos la existencia, en el cisticerco, de antígenos de reactividad cruzada con las moléculas del MHC del ratón.

La segunda sección de este reporte presenta los resultados de este proyecto central de investigación, en un artículo titulado:

" Sharing of MHC (H-2) antigens and related DNA sequences between host

and parasite are strongly related to growth of Taenia crassiceps cysticerci in mice"

En este trabajo se persigue la búsqueda e identificación de determinantes antigénicos en el cisticerco de T. crassiceps similares a los antígenos del H-2 del ratón. Para ello se siguieron tres rutas distintas: la primera consistió en evaluar el efecto de aloinmunizaciones en ratones congénicos en H-2 (BALB/c y BALB/B) sobre la susceptibilidad a esta parasitosis a fin de establecer si la instalación del parásito obedece a fenómenos de rechazo/aceptación del "injerto". Posteriormente se realizaron ensayos de inhibición de ELISA así como Inmunoelectrotransferencias para evaluar el presencia de antígenos "H-2 like" en extractos totales del cisticerco. Por último se buscaron homologías genómicas entre el DNA del cisticerco tratado con EcoRI y una sonda cDNA que codifica para antígenos de transplante del ratón ($H\text{-}2K^d$).

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APORTACIONES AL DISEÑO METODOLOGICO Y

TEORICO DEL TRABAJO DE INVESTIGACION

AN IMMUNOENZIMATIC ASSAY TO MEASURE THE EXPRESSION OF MURINE HISTOCOMPATIBILITY ANTIGENS IN MACROPHAGES AND LYMPHOCYTES.

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ABSTRACT

A simple, sensitive, rapid and reproducible enzyme immunoassay is described to quantify H-2 cellular antigens. Cellular antigens were measured through the subsequent binding of specific biotinylated antibodies and streptoavidin-peroxidase conjugate on cells in suspension. Endogenous peroxidase activity, in activated cells, was inhibited by addition of sodium azide and H_2O_2 in acid conditions. The assay specifically recognized between two congenic mice cells ($H\text{-}2^d$ and $H\text{-}2^b$) and two cell lines (EL-4 and P815) and was sensitive to as few as 2.5×10^4 cell/well. We also report here a version of this assay useful for the detection of soluble antigens by inhibition of the specific binding with antibodies after absortion of the antibody activity by problems antigens extracts or cell. This method can be useful for a number of potential applications.

INTRODUCTION

Cell surface antigens are detected through a variety of methods including immunofluorescence (Tumosa and Kahan 1989, Cikes and Friberg 1971), complement mediated cytotoxicity (Terasaki et al 1978, Stoker and Bernoco 1979), radioimmunoassay (RIA) (Takascs and Staehlin 1981, Pink and Ziegler 1979), ELISA (Hessian et al 1986, Baumgarten 1986) and fluorescence activating cell sorting (FACS) (Lanier and Warner 1981, Monroe and Cambier 1983, Sarkar et al 1980). All these methods are highly sensitive and relatively easy to perform, however, some are very costly or require of delicate standarization for give reproducible results or are hardly quantitative. We decided to develop an easy immunoenzymatic assay (ELISA), to study H-2 surface antigen expression considering it has the advantage of being potentially quantitative, stable and capable of processing a large number of samples in some hours very economically. Considering that activated cells increase their intrinsic peroxidase activity and that we plan to study antigen expression in this type of cells, we develop a procedure to inhibit this peroxidase activity in a non reversible way during the time of the assay without affecting the surface antigens presence.

We inform here of an ELISA procedure to quantify the expression of MHC products in cell suspensions of different types of murine cells, in different stages of differentation in a simple, sensitive, fast and reproducible way. The basic idea

behind this method is the detection of surface antigens by reaction with biotinylated specific polyclonal and monoclonal antibodies. The Ag-Ab reaction is developed using the peroxidase avidin system. The advantage of this method is that phagocytic cells could also be studied by previously inhibiting their endogenous peroxidase activity by the immunosassay proposed here by us. The assay did not modify cellular antigens and proved to be sensitive for studying H-2 expression. It also proved to be useful for the detection of antigens by inhibition of the specific binding with antibodies after absortion of the specific activity by problems antigens.

This ELISA may be advantageously applied to a variety of other immunological problems.

MATERIALS AND METHODS

Antibodies: purification and biotinylation

Polyclonal anti H-2^d and anti H-2^b alloantisera were obtained by eight serial intraperitoneal immunizations of H-2^d (BALB/c) and H-2^b (BALB/B) spleen cells in BALB/B and BALB/c mice respectively. One week after the last immunization the animals were bled. The resulting sera (obtained from 10 immunized mice) were combined into a single pool. Each alloantiserum was found to be active against erythrocytes of the strain used for immunization, at dilutions greater that 1:360 and neither serum was found to react with other congenic haplotype red cells (data not shown).

Monoclonal antibodies M.1.42 (rat-antimouse), M.5.114 (rat-antimouse IE^{dk}-IA^{bdq}, Bhattacharya et al 1981), 34.2.12 (mouse-anti H-$2D^d$, Ozato et al 1980, Ozato et al 1982) and 28.14.8S (mouse-anti H-$2D^b$, Ozato et al 1981) were provided by Dr. Carol Reiss (Dana Farber Cancer Institute). Sera from intact BALB/B and BALB/c mice were also used as controls of the specificity of the reaction. Antibodies from sera and ascites fluid were purified over an Affi-Gel Protein A MAPS II, (BIO-RAD).

For biotinylation, purified antibodies at concentrations of 100 ug/ml were dialized into 0.1M sodium bicarbonate pH 8. Biotin N-hydroxysuccinimide esther (Sigma), was dissolved in dimethyl

sulphoxide (Merck) at 100 ug/ml, 125 ul of this solution were added to each ml of antibody solution. The mixture was allowed to stand at room temperature for two hours and the reaction was stopped by addition of NH_4Cl, 1M pH 7.2 in 1/10 the volume of the total reaction volume. Then, the mixture was dialized against phosphate buffered saline (PBS: 0.15 M NaCl, 0.01 M sodium phosphate buffer, pH 7.2), sterilized by filtration (Milliphore No. 0.22) and stored at 4°C until used. Final dilutions of antibodies were performed freshly in each assay.

Cells

Peritoneal mice and tumoral lines cells were used in these immunoenzymatic assays to study the expression of H-2 surface antigens. Peritoneal cells from BALB/c and BALB/B mice were isolated from intact mice as well as from mice infected intraperitoneally with Taenia crassiceps cysticerci (Sciutto et al 1990). The EL-4 ($H-2^b$) cell line, a gift of Dr. Guillermo Alfaro (Instituto de Cancerologia, México D. F.) was maintained in C57Bl/6J mice (Gorer 1950), while P815 ($H-2^d$) cell line was maintained in (BALB/c x DBA/2)F1 mice (Ralph et al 1976).

Lymph node cells obtained from BALB/c mice by standard procedures were used in inhibition reactions for the detection of H-2 antigens.

Inhibition of endogenous cellular peroxidase activity

Endogenous peroxidase inhibition in activated peritoneal mice cells and in lines cells was carried out following the technique described here based in that published by Malorney et al (1988), as follows: one volume of the cells were treated with 50 volumes of a solution containing 0.1 M sodium azide (Sigma) in isotonic saline (0.15M NaCl), adding 0.04% of H_2O_2 (Merck) at room temperature for 20 min. Cells were washed twice with PBS and the pellet resuspended in a 0.1 M citrate/phosphate buffer pH 6.5 (one volume of cells per 50 volumes of solution) for 20 min at room temperature. After this, the cells in the pellet were washed three times with PBS and resuspended in 1% BSA in PBS to obtain the concentration used in the assay.

Cellular immunoenzimatic assay

To determine H-2 expression cells were diluted and washed with 1% BSA in PBS. All incubations were carried out at 4°C. The ELISA test was performed in V-shaped Immulon plates (Dynatech). Cells were added in 100 ul at various concentrations (2.5×10^4, 5×10^4, 10^5, 2.5×10^5, 5×10^5) per well, and spun for 7 min at 1,500 rpm. The supernatant was discarded and 100 ul per well of the diluted biotinylated antibodies (100 ug/ml) were added. Plates were incubated for 90 min and then washed three times. Ab-Ag reaction was developed by adding 100 ul of 1:2000 streptoavidin-peroxidase (Amersham) conjugate during 60 minutes. After washing three times, the enzyme was detected on the plate

by reaction with 100 ul of 0.4 mg/ml orthophenylendiamine (Sigma) and 0.03% H_2O_2 in citrate phosphate buffer (0.1M, pH 5) at room temperature for 20 minutes. The orthophenylendiamine reaction was stopped with 50 ul of 4M sulfuric acid. Optical density readings at 492 nm were done in a Behring automatic ELISA processor.

Inhibition reactions for the detection of H-2 antigens

H-2 antibodies (100 ug/ml) were absorbed with different amount of P815 ($H\text{-}2^d$) and EL-4 ($H\text{-}2^b$) cells (0.5×10^4, 1×10^4, 5×10^4, 10×10^4, 50×10^4 in 1% BSA in PBS) for 90 minutes at 4^oC. Then the suspensions were centrifuged and the supernatants were tested in the cellular immunoenzymatic assay against 1×10^5 BALB/c lymph node mice cells ($H\text{-}2^d$). Percentage of inhibition was calculated and graphed versus the amount of inhibitor cells used per well.

RESULTS

Quantification of histocompatibility antigens

Figure 1 shows that anti H-2 fixation (M.1.42) to H-2 molecules in resident peritoneal BALB/c macrophages depends on the amount of cells present per well. This method is sensitive enough to detect different levels of H-2 surface antigens as it is shown in Figure 1 where female BALB/c peritoneal cells express higher levels of H-2 antigens than male cells. The assay was sensitive to less than 2.5×10^4 cells per well. That this cell ELISA performed equally well with mononoclonal or polyclonal antibodies of different specificities in the determination of a variety of surface antigens, including naive proteins, is shown using peritoneal cells in Table I as well as in cell lines (Table II).

Identification of cellular haplotypes by Cell ELISA Inhibition

Figure 2 shows the identification of cellular haplotypes by cell ELISA inhibition of the specific reaction ($H\text{-}2^d$/anti $H\text{-}2^d$). Greater percentage of inhibition (70%) was observed when P815 ($H\text{-}2^d$) cells were used to inhibit the specific reaction, while a lower value (30%) was obtained when EL-4 ($H\text{-}2^b$) cells were used. Inhibition of endogenous cellular peroxidase activity.

Two of the cellular types used: macrophages and lymphocytes

have considerable intrinsic peroxidase activity, this activity is higher in macrophages than in the lymphocytes. Endogenous peroxidase activity in macrophages was completely inhibited by the treatment proposed with sodium azide in isotonic saline solution containing H_2O_2, and was only partially inhibited in lymphocytes (Table III). The acid substrate treatment that inhibit the peroxidase activity does not modify the surface antigenic properties as may be seen in Table I.

DISCUSSION

The present study reports the development of a simple, sensitive, rapid and economic ELISA method to measure the amount of the MHC products expression on different cell types. This assay does not involve cell fixation to ELISA plates, and uses cells in suspension, therefore decreasing possible modifications of cells and surface antigens (Drover and Marshall, 1986). This method also lessens the complications of unspecific reagent sticking, a common consequence of using poli-L-lysine to fix the cells to plastic (Epstein and Lunney, 1985).

Most other methods applying the cell-ELISA technique, require a large number of cells per well (1-5 x 10^5) (Epstein and Lunney 1985; Hessian et al 1986) but with the method described herein, different classes of histocompatibility antigens may be easily detected with approximately 10^4 cells per well.

This assay was sensitive enough to detect differences in the expression levels of histocompatibility antigens and its specificity may be judged, from the results obtained with the different antibodies (Table II) against H-2^d and H-2^b haplotypes.

The assay is carried out at 4°C and this decreases the possibilities of internalization and exchange of membrane proteins to a minimun. Unspecific sticking was evaluated using

normal immunoglobulins (purified from sera of intact mice) and the values obtained were very low. No interference by Fc receptors was detected, as in other assays (Nouri-Aria et al 1988; Baumgarten 1986). This could be due to a reduction in the fixation capacity of biotinylated immunoglobulins to the Fc receptor.

Our results also show that the high intrinsic peroxidase activity of macrophages is completely inhibited by the treatment with azide sodium in saline isotonic solution containing H_2O_2, without affecting their expression of MHC antigens. This is not so with the low intrinsic peroxidase activity of the lymphocytes which it could be inhibited only partially. These differences in enzymatic behaviour of cellular populations suggest that we may be dealing with peroxidase isoenzymes.

The consistency and reproducibility of our data allow us to propose this method as a reliable and accesible assay for measuring the expression of MHC antigens and other surface antigens in different types of cells and microenvironments.

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LEGENDES

Fig. 1 Optical densities obtained for different number of cells using cells plus mice IgG immunoglobulins (●) and cells plus MoAb anti H-2 (o), M.1.42. Each O. D. value is the average of three determinations per sample.

Fig. 2 Optical densities (A) and Percentage of Inhibition (B) obtained with P815 (■) and EL-4 (▲) as inhibitor cells of the specific reaction $H\text{-}2^d$ node lymphatic cells from BALB/c mice and anti $H\text{-}2^d$ polyclonal biotynilated antibody.

Table I

1.- Peritoneal cells (50% macrophages, 50% lymphocytes) used in this study were recovered from peritoneum of BALB/c and BALB/B mice, 30 days after infection with 10 cysts of Taenia crassiceps. Cells were previously treated to inhibit intrinsic peroxidase activity.

2.- For full analysis of specificities of MoAbs used, see references in material and methods. * Each value is the average of three determinations per sample $\pm$ S.D.

Table II

1.- For full analysis of specifities of MoAbs used, see references in material and methods. * Each value is the average of three determinations per sample ± S.D.

Table III

Peroxidase activity quantification in intact and in treated cells for the inhibition of the intrinsic peroxidase activity.

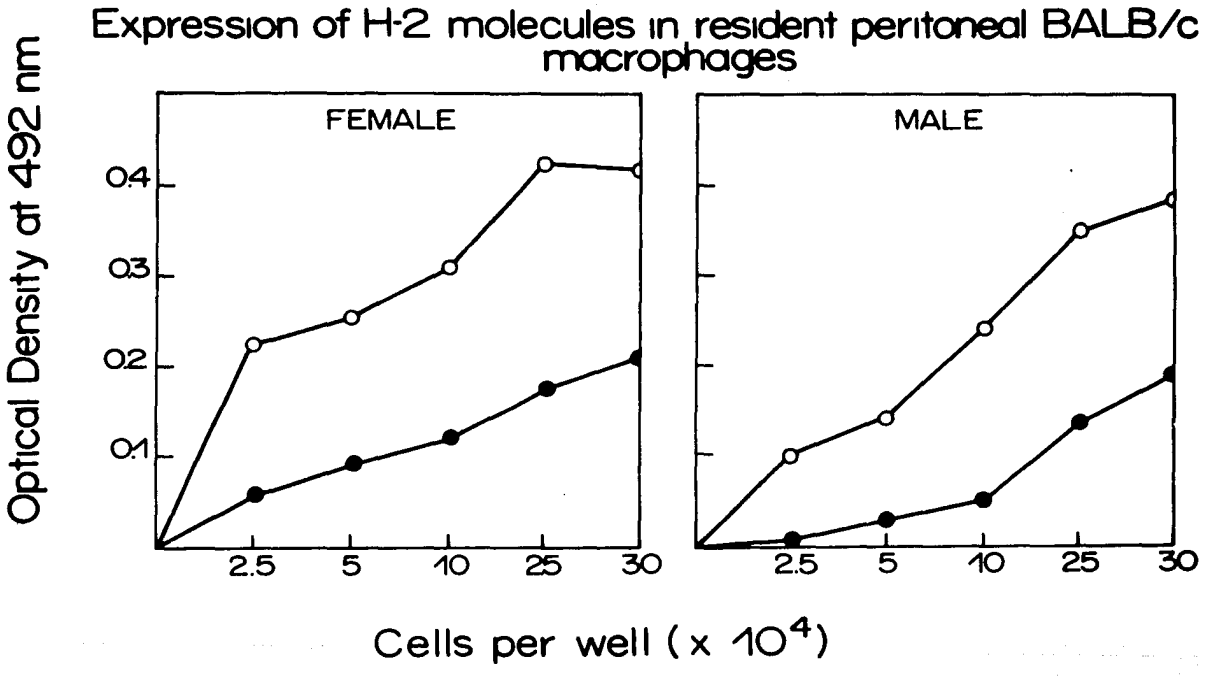
Expression of H-2 molecules in resident peritoneal BALB/c macrophages
FEMALE
MALE
Optical Density at 492 nm
0.4
0.3
0.2
0.1
2.5
5
10
25
30
Cells per well (x 10^4)

Figure 1

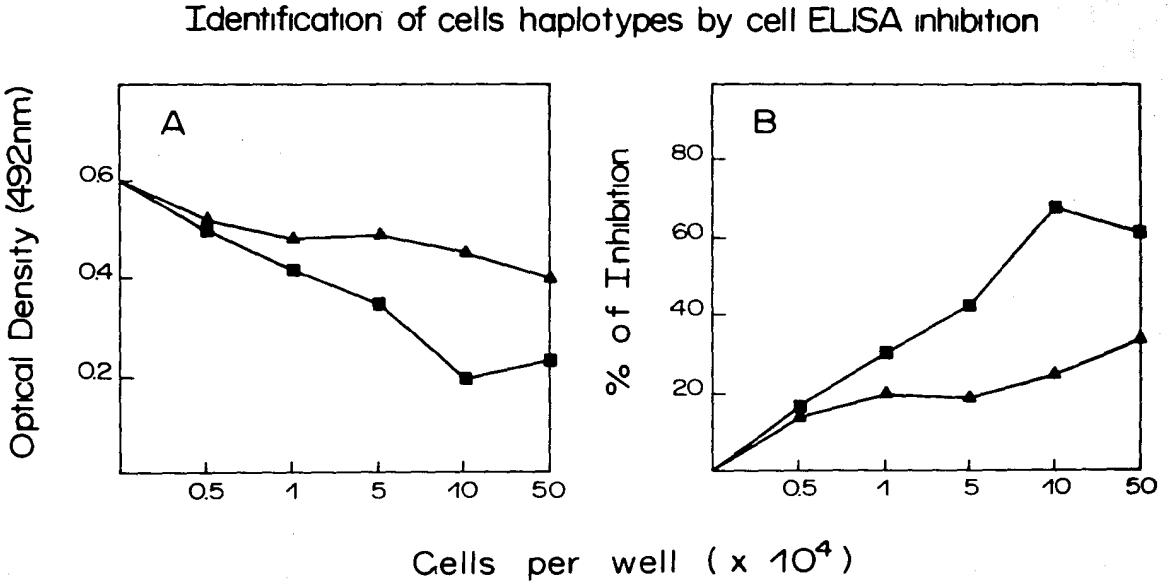
Identification of cells haplotypes by cell ELISA inhibition
A
B
Optical Density (492nm)
0.6
0.4
0.2
0.5
1
5
10
50
% of Inhibition
80
60
40
20
Cells per well (x 10⁴)

Figure 2

T A B L E I

IDENTIFICATION OF H-2 ANTIGENS BY CELL ELISA IN PERITONEAL CELLS

Antibody		OPTICAL DENSITY (492 nm)	
Name	Specificity[2]	Peritoneal cells[1]	
		H-2d	H-2b
Polyclonal	anti $H\text{-}2^{d}$	0.673±0.21*	0.348±0.03
34.2.12	anti $H\text{-}2D^{d}$	0.594±0.031	0.405±0.008
M.5.114	anti $IE^{dk}\text{-}IA^{bdq}$	0.509±0.015	0.507±0.015
Polyclonal	anti $H\text{-}2^{b}$	0.354±0.34	0.816±0.007
28.14.8S	anti $H\text{-}2D^{b}$	0.333±0.003	0.665±0.010
Pool of IgG mouse immunoglobulins from intact mice		0.169±0.006	0.207±0.009

T A B L E II

IDENTIFICATION OF H-2 ANTIGENS BY CELL ELISA IN CELL LINES

Antibody		OPTICAL DENSITY (492 nm)	
		Cell Lines	
Name	Specificity[1]	EL-4 (H-2^b)	P815 (H-2^d)
Polyclonal	anti H-2^d	0.195±0.001*	0.963±0.028
34.2.12	anti H-2D^d	0.182±0.013	0.563±0.30
M.5.114	anti IE^{dk} -IA^{bdq}	0.395±0.007	0.463±0.277
Pool of IgG mouse immunoglobulins from intact mice.		0.162±0.045	0.213±0.014

T A B L E III

INHIBITION OF INTRINSIC CELLULAR PEROXIDASE

BY ACID SUBSTRATE TREATMENT

Number of cells per well	OPTICAL DENSITY (492 nm)			
	MACROPHAGES (Resident peritoneal BALB/c cells)		LYMPHOCYTES EL-4 ($H\text{-}2^b$)	
	Non Inhibited	Inhibited	Non Inhibited	Inhibited
10^4	0.104*	0.013	0.099	0.066
10^5	0.480	0.014	0.156	0.096
10^6	0.745	0.013	0.405	0.228

* Average of three determinations

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

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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 and sex 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; Wasson et al., 1987) as well as genes outside the MHC (Wasson et al., 1983); to Trichuris muris (Else and Wakelin, 1988); to Schistosoma mansoni (Class and Deeleder, 1979) to Leishmania mexicana (Roberts et al., 1989) 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 et al., 1980). Finally, the immune response to secreted antigens of Ascaris suum is controlled at the level of H-2 in mice (Kennedy et al., 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. A 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 (Zeder, 1800) Rudolphi, 1810 isolated by Freeman in 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) (Sally et al., 1976) and were kept by serial passage in BALB/c female mice for five years by us.

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 X 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. Mice were sacrificed 30 days after infection 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 a 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 comparisons 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 ($4\sqrt{[\text{Number parasites} + 1]}$). This transformation does not imply significant numeric changes in parasite load. Transformation of 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 rigorous 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 further studied by 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 susceptible male BALB/c mice, in which parasites could be recovered as soon as one day after infection.

DISCUSSION

Several inbred strains of mice were experimentally 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 et al., 1987), the response of mice to

vaccination against schistosome infection (Sher et al., 1984) and the long-term response in systemic leishmaniasis caused by Leishmania donovani (Blackwell et al., 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 et al., 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 or host proteins in T. crassiceps adsorbed from the BALB/c stock mice in which the parasites have been kept for

several years now, as it has been recently documented (Kalinna et al., 1989). 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 detected in experimental oral egg infection of a number of different rodent species (Delvalle, 1989). If these sex associated differences vary because of differential parasite stage susceptibility or tissue 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 et al., 1986; Blalock et al., 1985).

As judged by parasite growth curves, the H-2 dependent control of parasite growth appears to occur very early in the infection or not at all, since no macroscopic parasites could be recovered from H-2b mice in the first 1-10 days of 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 in both sexes. 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 et al., 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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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]	30.0 ± 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]	0.0 ± 0.0 (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: Statistics labeled with the same literal are not significantly different from each other while those labeled with different literals are significantly different (P .01). Groups labeled with two literals are not significantly different from two groups differing significantly in parasite loads between themselves: one located at the high tail of the distribution and the other at the low tail.

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 PARASITE	
STRAIN OF MICE	H-2 HAPLOTYPE	S E X	ORF AVERAGE PARASITE LOAD ± * SE	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

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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 cysts (ORF) per mouse.

Table II. Mean of the number of parasites recovered from peritoneal cavity of ten male and female congenic BALB mice 30 days after infection with 10 cysts, (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.

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

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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 resistance to infection with parasite. The more susceptible conditions had higher antibody levels than the resistant conditions at equivalent parasite loads: $H\text{-}2^d$ (BALB/c) > $H\text{-}2^b$ (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. Qualitative Western Blot analysis of antigen of the frecuency of individual antigen recognition by circulating antibodies revealed extensive similarity between strains, sexes and immune status in responses to Taenia solium antigens. However, the frequencies of band recognition were higher for BALB/B resistant strain 24, 203 and 93 Kd antigens; for vaccinated mice in 78, 93 and 24 Kd. Thus, while the bulk of antibodies against cysticercal antigens appears, irrelevant if not favorable for against growth, several other less conspicuous antigens seem real ted to resistant states: a finding which suggest thatthey differ in their forms of influence upon the immune system.

INTRODUCTION

Taenia crassiceps is a larval cestode parasite of mice and rats that has proven useful as a laboratoy 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 in compelling that antiparasite antibodies are necessary for expression or resistance (Mitchell et al. 1982; Soulsby and Lloyd, 1982). Also, an association between the differing abilities of individual 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 has established the notion that fully

developed metacestodes are unharmed by antibodies, while early larvae are very susceptible (Rickard and Coman, 1977; Siebert and Godd, 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 assessed by use of quantitative enzyme linked immunosorbent assay (ELISA), analysis (Immunoplots) of 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

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 T. crassiceps was supplied by DR. B. Enders (Behringwerke, Germany) in 1984. The larvae were kept in our animal facilities by serial passaging (IP) in females 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 ug/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

Taenia crassiceps 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 ultracentrifugated 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).

Taenia solium vesicular fluid obtained as we described elsewhere (Larralde et al. 1986) was used as source of antigens in Western Blots.

Enzyme-Linked-Immunosorbent Assay (ELISA)

Sera were processed in ELISA as previously reported (Larralde et al. 1986). Three replicates of each serum were always analysed. Briefly, the plates were coated with 100 ul of a 10 ug/ml of Taenia crassiceps antigens, the mouse sera were diluted 1:200 in PBS-Tween (0.01%), and Ab-Ag reactions were developed with biotinylated sheep antimouse IgG (Amersham). A solution of

o-phenilendiamine (0.04% 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 ul of the serum to be tested, diluted 1:10 in PBS-Tween (0.01%) for 4 hrs at room temperature. Biotin conjugated polyclonal antimouse total Igs and Peroxidase-streptoavidin (Amersham), diluted 1:400 were used to develop the paper strips. The substrate used was 0-chloronaphtol (0.05 mg/ml, H_2O_2 0.03%).

Immunoplot

A simple plot of the frequency with which each individual antigen band in a Western Blot reacts with a set of sera collected from mice of a certain variable against the frequency of the same antigen band when reacted with sera of another variable is used to detect minor differences in the face of overwhelming similarities (Larralde et al. 1989a). The frequency of each band obtained with normal sera from that of the chosen variable was substracted to minimize background.

Passive Transfer of Humoral Immunity

Whole immune serum and purified immunoglobulins transfer experiments were designed to explore the role of the both 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 BALB/b mice. Male and female of both strains did no show significant differences in antibody levels at the same level of parasite load.

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 T. solium 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. Visual analysis of blots indicate to great similarities in antibody discrimiantion by the two strains of mice, by both sexes and by both vaccinated and naive infected mice. Identification of finer differences requires of a recently developed method of plotting Western Blot results, which follows (Larralde et al. 1989).

Immunoplots

Figure 3 shows all Immunoplots contrasting factors related with resistance and susceptibility to infection: resistant and susceptible strains A; male and female B; and vaccinated and naive mice C.

Linear correlation of the frequency differences Immunoplots revealed highly significant positive correlation in all 3 plots, meaning that most of the bands have similar frequency of recognition by infected mice. Only a small number of antigens are differentially recognized. Table 1 summarizes the discriminating antigens, which are defined as those antigens recognized by one category with a overall frequency higher than 50% but of only 30% by the other category.

BALB/B, ($H\text{-}2^b$) the resistant strain, recognized preferentially several antigens 24, 20, 32 and 93 Kd. Females (susceptible sex) esentially the same response than males while vaccination was associated to higher responses to three antigens 78, 93 and 24 Kd.

Passive Transfer of Humoral Immunity

The data shown in Table I 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 parasite in the transfered host.

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-2^b) [(resistant); BALB/c, H-2^d (susceptible)] 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 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 (Suquet, Green-Edwards and Mitchell, 1989) and early larvae being the susceptible stage of the parasite (Mitchell, 1989; Pond, Wassom and Hayes, 1988; Gansmuller et al. 1987). Because the form of experimental cysticercosis studied herein was produced by the injection of young metacestodes in the peritoneal cavity of recipient mice our results further strengthen the notion that antibody response, as a whole, is inconsecuental for the parasite. 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).

However, with the aid of Western Blots and immunoplotting, a set of individual antigens, otherwise inconspicuous in the multitude of irrelevant ones was found to be associated with resistance (78, 93 and 24 Kd). Whether immune responses to these antigens are involved in same way in expression of resistance to T. crassiceps metacestode infection remains to be determined but the identification of these antigens may have considerable relevance in the development of effective vaccines via natural or synthetic procedures as well as prived means of probing deeper in the molecular events regulating host parasite transactions.

Strains which differed genetically only at H-2 loci exhibed differences in the qualitative antibody response. These results support the conclusion that H-2 complex has a role in the control of the antibody response to T. crassiceps. Intra-H-2 genetic control of antibody production has been described for numerous T-dependent antigens (Mitchell, 1989) and for several parasites: Trichinella muris (Else and Wakelin, 1989), Trichinella spiralis (Fond, Wasson and Hayes, 1988) and Taenia taeniaeformis (Gibbens, Harrison and Parkhouse, 1986). Antigens associated with susceptibility or resistance could represent serological markers only as is the case of Sj26 (antigen associaed at resistance in mice to Schistosoma japonicum but that is infective in inducing high

level of resistance in vaccination, Davern et al. 1988) could inffect be or a critical antigen in the outcome of infection.

It would be reasonable to expect that a vaccine would induce resistance by ways similar to those of natural infection, but apparently T. crassiceps cysticercosis is not the case. Our results shown that antigens associated to sex of MHC related natural resistance differ from those related to immune protection by vaccination. This in turn suggest that different regulating networks are accesible and operated by MHC products gender related hormones and direct antigen stimulation of the immune system. Complex antigen mixtures such as those associated with parasites present many different epitopes to the immune system. some of these epitopes in association with major histocompatibility antigens are by different cell types using different receptors and epitopes recognition can activate a number of different effect or mechanisms.

In the other hand, parasites can modify the host immune response through altering the levels of sex hormones (Isseroff, Sylvester and Hels, 1986), hormones that play an influential role, in the regulation of the immune response (Alexander and Stinson, 1988) and so, by amplication on the control of parasitic infection. It is also known that parasites can modulate the host immune response inducing changes in the expression of MHC mole-

cules (Reiner, and Mcmaster, 1987).

Finally, since there is no question that vaccination effectively restrains parasite growth, but more so in the naturally resistant BALB/B ($H\text{-}2^b$) and in male gender (Sciutto et al. 1989), and the bulk on antibody being seeming irrelevant or propiciatiory of parasite growth, and considering the preceding information we can assume that the molecular mechanism, of the immune response to this parasite would be a complex and derive from and intrincate network of interactions that remains to be developed.

FIGURES AND LEGENDS

Figure 1.

Serum antibody response (as measured by ELISA OD 492 nm 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 gropus of ten for each sex and their antibody responses graphed against the logartim 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 T. solium vesicular fluid, overlaid with sera from BALB/B and BALB/c vaccinated and non vaccinated male and female mice and developed with anti-mouse-Ig. Each strip correspond to serum from individual mice bled 30 days after infection.

Figure 3.

.A Frequency differences Immunoplots of BALb/c (susceptible) and BALB/B (resistant) sera. B Frequency differences immunoplots of males and female sera. No antigens were found to be sex-associated. C. Frequency differences immunoplots of vaccinated and non vaccinated mice. Antibody response to antigens 78, 93, 24 Kds are preferentially associated to the resistant state of discrimination.

Squares are place at levels such that antigens falling therein are frequently recognized (frec. 0.5) by sera in the category and rarely so (freq. 0.3) by the other category. The antibody response to antigens 24, 203 and 93 Kds are preferentially associated to the resistant state of BALB/B.

Table 1.

[a]Mean ± standard error of the number of parasite recovered from 10 females BALB/c (susceptible) recipient mice transfered with 0.5 ml of immune sera from female or male BALB/B (resistant), or BALB/c (susceptible). [o]Serum from 30 days infected mice (ten parasites per mice). [•]Serum from 30 days vaccinated mice (100 ug 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.

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	[a]211 ± 106	N.D.
• vaccinated	*911 ± 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 ± 119	40 ± 30

Antigens from vesicular fluid developed with anti-Inmunoglobulins

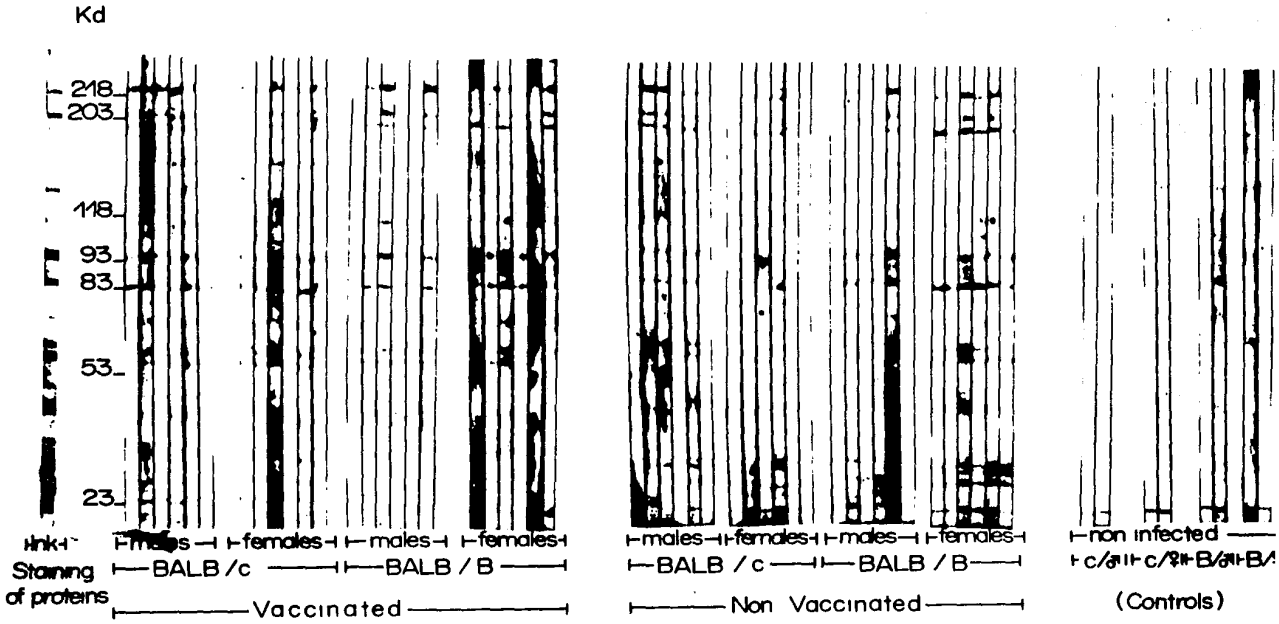

Figure 1

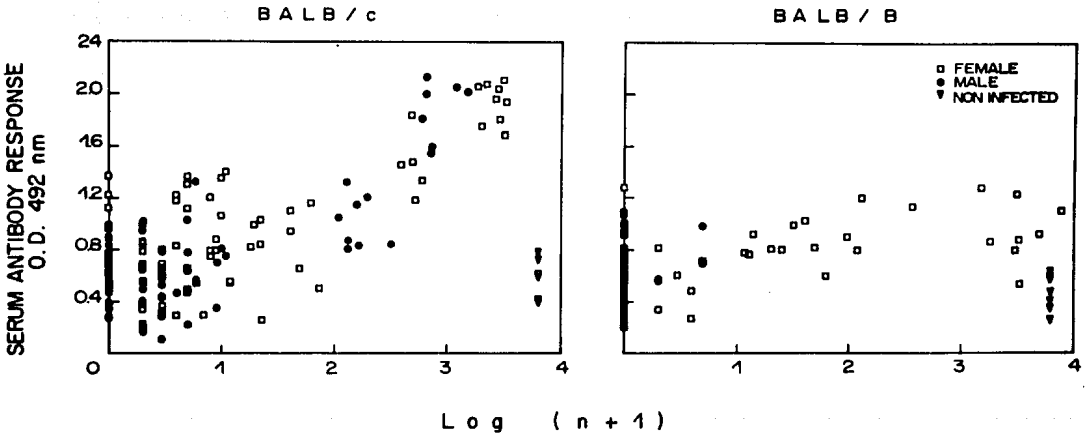
BALB/c
BALB/B
SERUM ANTIBODY RESPONSE
O.D. 492 nm
24
20
16
12
08
04
0
1
2
3
4
FEMALE
MALE
NON INFECTED
Log (n + 1)

Figure 2

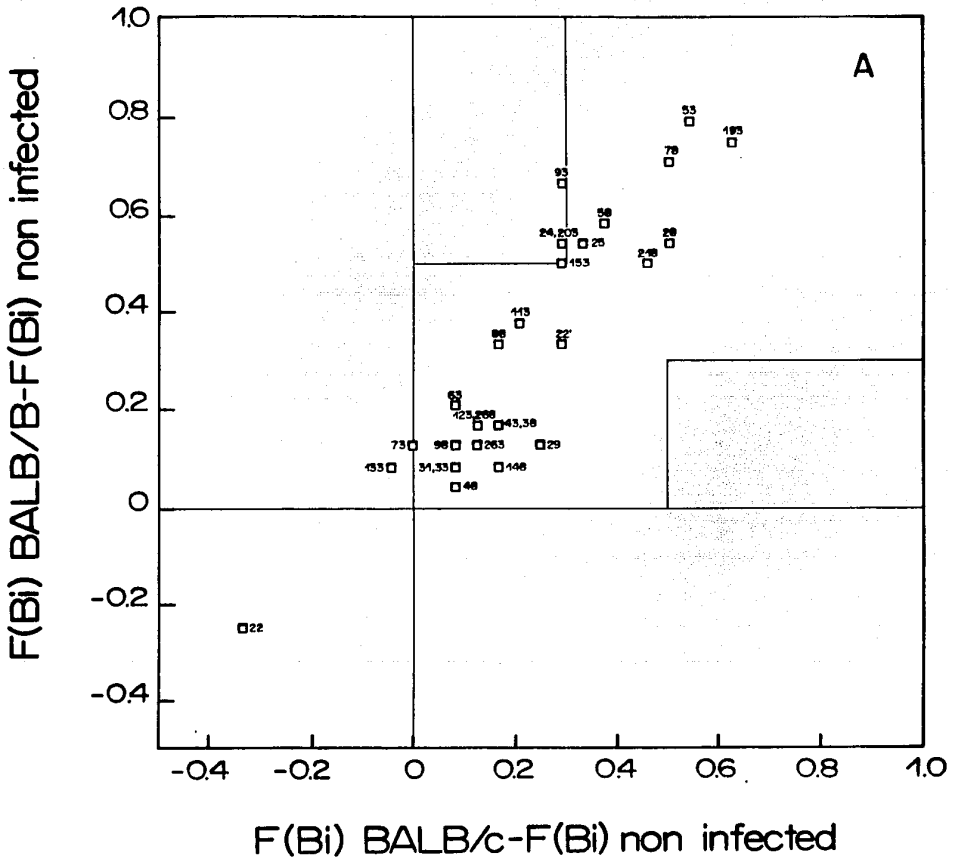
A
F(Bi) BALB/B-F(Bi) non infected
F(Bi) BALB/c-F(Bi) non infected
1.0
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1.0
53
195
79
93
58
24,205
25
153
28
248
113
86
22
63
123,268
43,38
73
98
263
29
155
31,33
146
46
22

Figure 3

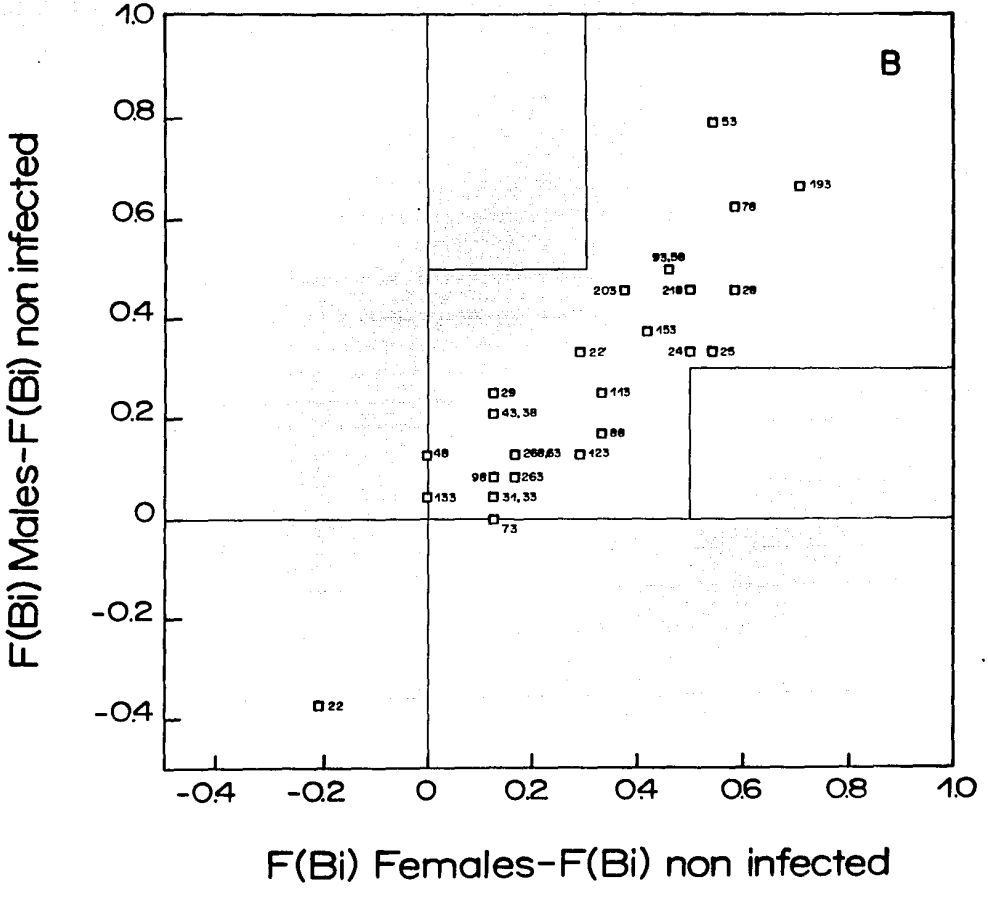
B
1.0
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1.0
F(Bi) Males-F(Bi) non infected
F(Bi) Females-F(Bi) non infected
53
193
78
93,56
205
218
28
153
22
24
25
29
113
43,38
86
48
268,63
123
98
263
133
34,33
73
22

Figure 3

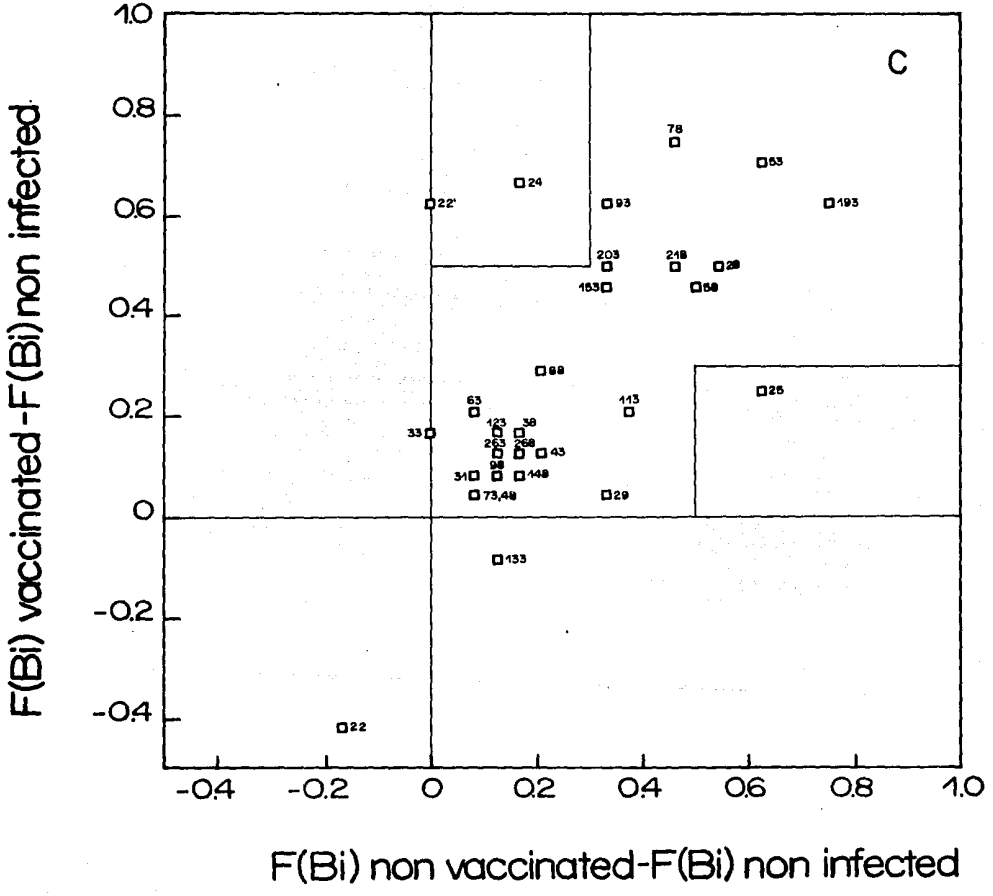
C
F(Bi) vaccinated-F(Bi) non infected
F(Bi) non vaccinated-F(Bi) non infected
1.0
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1.0
78
53
24
22'
93
193
203
218
28
163
58
89
25
63
113
123
38
33
263
268
43
34
148
73,48
29
133
22

Figure 3

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TRABAJO DE INVESTIGACION

SHARING OF MHC (H-2) ANTIGENS AND RELATED DNA SEQUENCES BETWEEN HOST AND PARASITE ARE STRONGLY RELATED TO GROWTH OF Taenia crassiceps CYSTICERCI IN MICE

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Running Title: H-2 and mice susceptibility to *Taenia crassiceps* cysticercosis.

Abbreviations used in this paper.

Ab: antibody; pAb: polyclonal antibody; mAb: monoclonal antibody; SSC: stock solution 0.15M NaCl/ 0.015 M sodium citrate; PCMB: sodium p-hidroximercuribenzoate.

ABSTRACT

Cysticerci of Taenia crassiceps recovered from the peritoneal cavity of mice express antigens that crossreact with mouse histocompatibility (MHC) antigens class I and are closely related to parasite survival and growth within the host. These parasite MHC-like antigens were detected by their capacity to specifically inhibit the reaction of H-2^d cells and/or H-2^b cells with a panel of polyclonal (pAb) and monoclonal (mAb) antibodies (anti H-2^d pAb, anti H-2^b pAb, anti H-$2D^d$ mAb, anti H-$2D^b$ mAb, anti IE^{dk}-IA^{bdq} mAb, anti H-2 mAb) and by their direct Western Blot reactivity with these same antibodies. That these MHC-like antigens are coded for in the cysticercus genome was demonstrated by probing parasite DNA with a ^{32}-cDNA clone (pH-2IIa) encoding for mouse class I MHC antigens (H-$2K^d$). Very strong hybridization indicated to the presence of nucleotide sequences in the parasite genome homologous to those coding for H-2^d antigens in the mouse. That the host's immune response to these MHC-like antigens -H-2^d specially- is involved in parasite survival and growth was shown by the increased resistance to cysticercosis of BALB/B mice immunized with H-2^d cells, while BALB/c mice immunized with H-2^b cells were not protected.

These findings support the notion that the basic principles of proper antigen matching that rule transplantation immunity may be involved in host-parasite relationships with complex parasites, such as metacestodes, and could be of relevance to understand human susceptibility to cestode disease.

INTRODUCTION

Previous studies in experimental murine Taenia crassiceps cysticercosis pointed to significant differences in susceptibility to peritoneal infection between H-2 congenic strains. BALB/c ($H\text{-}2^d$) mice were the most susceptible and the least protected by vaccination, while BALB/B ($H\text{-}2^b$) and BALB/K ($H\text{-}2^k$) mice were comparatively resistant and more effectively protected by the vaccines (1,2).

In other parasitic diseases there are records of susceptibility differences associated to H-2 that have been attributed to immunological phenomena of higher efficency in the resistant strains (3-6). However, differences in host susceptibility determined by the parasite are poorly explored, excepting perhaps in schistosomiasis, in which H-2 antigens found on the surface of the parasites differentially affect the growth of the parasite, but again, they are of host origin (7-9).

Here we report of a search for $H\text{-}2^d$ antigens coded in the parasite DNA (Taenia crassiceps cysticercus) conducted to examine the hypothesis that correct matching in histocompatibility antigens between host and parasite may be involved in the establishment and growth of these parasites in the host, in a manner similar to the acceptance on rejection of a tissue transplant.

MATERIAL AND METHODS

Mice: Infection and Immunizations

Female BALB/c and BALB/B mice (5-7 weeks old), bred in our animal facilities by the "single line breeding system" during 20 generations, starting with original stock from Jackson Labs in 1982, were infected intraperitoneally with 10 cisticerci of Taenia crassiceps ORF (10). Mice were sacrificed 30 days after infection and the parasite load was determined as previously reported (1).

The ORF strain was kept by serial passage in BALB/c female mice for five years (ORFd) and in BALB/B female mice for more than two years (ORFb).

In order to evaluate the effect of the presence of MHC like products on the susceptibility to cysticerci infection, allograft immunized BALB/B and BALB/c mice were obtained by eight serial immunizations with $H\text{-}2^d$ or $H\text{-}2^b$ spleen cells respectively (10^7 cells once a weeks per mouse). One week after the last immunization groups of ten mice each were infected with ten cysts of the ORFd or ORFb parasites. Groups of non immunized mice were also infected as controls.

Antibodies: purification and biotinylation

Polyclonal anti/H-2^d and anti/H-2^b alloantisera were obtained by eight serial intraperitoneal immunizations of H-2^d (BALB/c) and H-2^b (BALB/B) spleen cells in BALB/B and BALB/c mice respectively. One week after the last immunization the animals were bled and the resulting sera (obtained from 10 immunized mice) were combined into a single pool. Each alloantiserum was found to be active against erythrocytes of the strain used for immunization, at dilutions greater than 1:360 and neither serum was found to react with other congenic haplotype cells (data not shown).

Monoclonal antibodies M.1.42 (rat antimouse H-2), M.5.114 (rat antimouse IE^{dk}-IA^{bdq}) (11), 34.2.12 (mouse anti H-$2D^d$), (12,13) and 28.14.8S (mouse anti H-$2D^b$) (14), were provided by Dr. Carol Reiss (Dana Farber Cancer Institute, Boston). Mouse anti-DNP and rabbit anti-mouse immunoglobulins were obtained by conventional procedures (15,16). Immunoglobulins from intact mice were also used as control of the specificity of the reaction. Antibodies from sera and ascites fluid were purified over an Affi-Gel Protein A MAPS II, (BIORAD).

For biotinylation, purified antibodies at concentrations of 100 ug/ml were dialized into 0.1 M sodium bicarbonate pH 8. Biotin N-Hydroxysuccinimide esther (Sigma), was dissolved in dimethyl sulphoxide (Merck) at 100 ug/ml, 125ul of this solution

were added to each ml of antibody solution. The mixture was allowed to stand at room temperature for two hours and the reac-tion was stopped by addition of NH_4Cl, 1M pH 7.2 in 1/10 the volume of the total reaction volume. Then, the mixture was dialized against phosphate buffered saline (PBS: 0.15 M NaCl, 0.01M sodium phosphate buffer, pH 7.2), sterilized by filtration (Millipore No. 0.22) and stored at 4°C until used. Final dilutions of antibodies were performed at the time of the assay.

Cells

Peritoneal mice cells and tumoral line cells were used in the immunoenzymatic assays. Peritoneal cells from BALB/c and BALB/B mice were isolated from intact mice as well as from mice infected intraperitoneally with Taenia crassiceps cysticerci. The EL-4 ($H-2^b$) cell line, a gift from Dr. Guillermo Alfaro (Instituto de Cancerología, México, D. F.) was maintained in C57Bl/6J (17). P815 ($H-2^d$) cell line was passaged in (BALB/c x DBA/2)F1 mice (18).

Parasite Antigen Preparation

Metacestodes of Taenia crassiceps were collected from the peritoneal cavity of infected BALB/c and BALB/B mice, and extensively washed with ice-chilled phosphate buffered saline (PBS, pH 7.4, NaN_3 2%, PCMB 0.04%, PMSF 0.006%, EDTA 2.5 mM). Washed cysticerci were suspended in an equal volume of the same solution and gently mixed overnight at 4° C. The suspension was then centrifuged (15,000 rpm, 60 min., 4°C), and the supernant

was aliquoted and freezed until used. Protein content was determined by the method of Lowry (19).

Cellular immunoenzymatic assay

Crossreactivity between parasite and H-2 antigens was detected by inhibition of an ELISA assay for H-2 antigens. For this ELISA, endogenous peroxidase peritoneal cell activity inhibition was carried out following the technique described by Malorney (20) with some modifications. Briefly, one volume of the cells were treated with 50 volumes of a solution of 0.1M sodium azide (Sigma) in isotonic saline (0.15M NaCl), adding 0.04% of H_2O_2 (Merck) at room temperture for 20 minutes. Cells were washed twice with PBS and the pellet resuspended in a 0.1M citrate/phosphate buffer pH 6.5 (one volume of cells per 50 volumes of solution) for 20 minutes at room temperature. After this, the cells in the pellet were washed three times with PBS and resuspended in 1% BSA in PBS to obtain the concentration used in the assay. All incubations were carried out at 4°C., 100 ul of cells ($2x10^5$ cells/ml), was mixed with 100 ul of biotinylated antibody in V shaped Immulon plates (Dynatech) and incubated at 4°C for 90 min. Ab-Ag reaction was developed by adding 100 ul of 1:2000 avidin-peroxidase (Amersham) conjugate during 60 minutes. After washing three times, enzyme was detected on the plate by the reaction with 100 ul of 0.4 mg/ml orthophenylendiamine (Sigma) and 0.03% H_2O_2 in citrate phosphate buffer (0.1M, pH 5) at room temperature for 20 minutes. The orthophenylendiamine reaction was stopped with 50 ul of 4M sulfuric acid. Optical

density readings at 492 nm were done in a Behring automatic ELISA processor. The inhibition reaction used different amounts of parasite antigens (0 to 3 ug/ml) that were mixed with the specific biotinylated antibodies prior to their addition to the cells. The specificity of this inhibition reaction was evaluated by measuring the amount of inhibition obtained when incubating DNP labeled cells with biotinylated mouse anti DNP-IgG adsorbed with parasite antigens and with murine cells incubating with biotinylated rabbit IgG antimouse also adsorbed.

Western Blot

Western blottings were carried out according to Bittner, Kupferer and Morris (21). T. crassiceps antigen was boiled in the presence of SDS and 2- mercaptoethanol and separated on a 10% SDS polyacrylamide gels using the method of Laemmli (22). Proteins were transferred from gels to nitrocellulose membrane electrophoretically. After saturating any remaining protein-reactive sites on the nitrocellulose with 3% BSA in PBS- 0.3% Tween 20, the membrane was cut into strips and incubated with the anti-H-2 polyclonal anti-H-2^d, anti-H-2^b antibodies; monoclonal M.1.42, M.5.114, 34.2.12 and 28.14.8S antibodies; anti-mouse and normal biotinylated immunoglobulins (1:10 dilution in PBS- 0.3% Tween 20) for four hours at room temperature. After extensive washing, the strips were incubated with streptoavidin peroxidase (Amersham) diluted 1:400 in PBS- 0.3% Tween 20 for one hour at room temperature. Enzyme activity on the paper was developed by

immersing the strips in a solution containing 30mg of 4- ortho-chloronaphtol (Sigma) 10 ml of methanol, 50 ml of PBS and 50 ml of H_2O_2 added immediately prior to use.

Preparation of DNA

High molecular weight DNA was prepared from BALB/c and BALB/B mice liver by the method of Britten et al (23). Parasite DNA was isolated from two populations of Taenia crassiceps cysticerci: ORFd and ORFb. DNA was extracted as follows. Cysticerci was washed and homogenated in SSC 1X (0.015M sodium citrate, 0.15M NaCl, pH=7.4) with 10 up-down strokes of a motor-driven polytron (Brink-mann Instruments) in ice-chilled bath and centrifuged at 4500 rpm for 20 minutes. A pellet of 100 ul was resuspended in one mililiter of lysis buffer containing 50mM EDTA, pH=8, 0.5% Sarcosyl and 100 ug of proteinase K. The solution was incubated at 50°C for four hours. The lysate was then extracted three times with an equal volume of phenol, once with phenol:chloroform:isoamilic (25:24:1) and twice with chloroform:isoamilic (24:1). After dialysis with 10mM tris pH=8, 0.1mM EDTA, RNA was discarded by ribonuclease treatment (24), followed by the extraction and dialyses described before. After adjusting to 0.3% with sodium acetate, DNA was precipitated with two volumes of ethanol. DNA was obtained by cesium chloride gradient centrifugation in the presence of ethidium bromide as described previously (24).

Probe cDNA Radiolabelling

A probe, inserted in the pH-2IIa clone and containing 442pb from H-2K^d transplantation antigens cloned in pBR322, were used in this assay. The pH-2IIa clone its the 3'end probe for transplantation antigens and codes for amino acids 167 to 352 (25-27). ^{32}P labebeling of the probe to an estimated specific activity of 4.4×10^4 cpm/ug was accomplished by nick translation before use (28).

Southern blot analysis

Parasite and mice DNA digested with EcoRI were separeted by electrophoresis in 1.0% (w/v) agarose gels, stained with ethidium bromide and transferred to nitrocellulose by the method of Southern (29). The nick translated DNA probe were denatured by heating at 62^oC. Hybridization was carried out in 20 ml 5X Denhardt's solution (30), 2M EDTA, 2X SSC, 120 mM sodium phosphate pH=7, 50% formamide and 0.2% sodium dodecyl sulphate (SDS) at 37^oC overnight with 450 of pH-2IIa ^{32}P-probe. Filters were washed under conditions of high stringency two times with 0.1% SDS, 1X SSC, twice with 0.01% SDS, 0.1X SSC and twice with 0.1X SSC at 37^oC for 10 minutes each wash.

RESULTS

Parasite Antigens inhibited the H-2 anti H-2 reaction in cell ELISA

In Figure 1 it is shown how Taenia crassiceps antigens of cysticerci maintained in BALB/c as well as in BALB/B mice specifically inhibited the reaction between class I histocompatibility antigens (Figure 1 A, B, D and E) and class II (Figure 1 A, B, C and F) present in the $H\text{-}2^d$ (Figure 1 A-C) and $H\text{-}2^b$ (Figure 1 D-F) peritoneal cells, with their respective antibodies (monoclonal and polyclonal). Inhibition was specific considering that the reaction between dinitrophenylated cells and mice anti-DNP antibodies (closed circules), as well as the reaction between mouse cells and rabbit anti-mouse cells (open squares), were not inhibited at all, even with the maximum concentration of parasite antigens used. Although all H-2 anti-H-2 reactions were inhibited by the parasite antigens, the higher inhibitions were obtained in the $H\text{-}2^d$ anti $H\text{-}2^d$ system (Figure 1 A-C), suggesting that in the parasite antigenic extract the $H\text{-}2^d$-like determinants are the most prominently expressed. Similar results were obtained using tumoral line cells of $H\text{-}2^b$ and $H\text{-}2^d$ haplotypes (data not shown).

Effect of previous allograft immunizations on susceptibility

Table 1 shows the effect of previous allograft immunizations of BALB/c mice with $H\text{-}2^b$ congenic mouse cells, and of BALB/B mice with $H\text{-}2^d$ congenic mouse cells, upon resistance to ulterior parasite challenge. BALB/c susceptible mice did not show significant modifications in

the expected parasite load due to immunization with H-2^b cells. In contrast, BALB/B resistant mice were made significantly more resistant by inducing transplantation immunity against H-2^d cells. These effects were independent of whether the parasites had been grown in H-2^b or H-2^d hosts.

Histocompatibility epitopes in parasite antigens detected by Western Blot.

Figure 2 shows the extensive crossreactivity, specially in the 24-67 Kd range between parasite and H-2^d histocompatibility antigens, as revealed with a panel of polyclonal and monoclonal anti H-2^d antibodies (Figure 2A-D). Little or no reaction was found with rabbit anti-mouse sera and non-immune mouse immunoglobulins (Figure 2 E and F). H-2^b hyperimmune polyclonal serum against H-2^d cellular (splenocytes) antigens (Figure 2B) recognized many more bands in the parasite extracts than did monoclonal immunoglobulins against H-2^d (Figure 2A), specially several close to Kd 46 and above Kd 60. Anti-class-I monoclonal antibodies (Figure 2C) recognized the same parasite antigens than did anti-class II (Figure 2D), with minor intensity differences. That Western Blots show little non-specific reactions is indicated by the negative controls: polyclonal antimouse serum (Figure 2E) and non-immune mouse IgG (Figure 2F). Western Blots did not significantly differ with source of parasite antigens, both preparations of cysticerci cultivated in BALB/c or BALB/B produced similar reaction patterns.

H-2-like genes in the cysticercus genome.

High molecular weight genomic DNA was obtained from Taenia crassiceps cysticerci grown in BALB/c mice, as well as DNA from BALB/c and BALB/B mice. The DNA preparations were digested with restriction endonucleases, electrophoresed and transferred to nitrocellulose. The probe ^{32}-P labeled cDNA encoding H-2K^{d} antigen (pH-2IIa) strongly hybridized with high molecular weight DNA from Taenia crassiceps (Figure 3E) and with low molecular weight DNA from BALB/c mice (Figure 3C) and almost insignificantly with BALB/B DNA (Figure 3D). A low reaction was obtained with the DNA of pBR322 (Figure 3A) and a clear one with unlabeled probe (Figure 3B).

DISCUSSION

Our results show that the cysticercus of Taenia crassiceps not only shares H-2 epitopes with it's murine host, as determined by inhibition ELISA and Western Blot, but also that the parasite genome shows extensive genetic homology with mouse H-2^d genome, as indicated by the strong binding of H-2^d class I cDNA probe by the cysticercus DNA. This cDNA probe, contains one or more repeated elements (25), which could also be present in the DNA parasite and partly responsible of the strong hibridization observed. Also, this cDNA probe shows homology with the constant domain of the mouse immunoglobulins u gene (25), thus our results can not exclud cross reactions with these other genes, specially considering that Taenia solium cysticerci was found to be metabolically active to synthetize a protein in vitro which cross react with porcine immunoglobulins (49). In the other hand, the induction of transplantation immunity in BALB/B (H-2^b) mice against antigens from H-2^d splenic cells resulted in cross-protective immunity against challenging T. crassiceps cysticerci raised in either H-2^d or H-2^b hosts. In contrast immunization of H-2^d against H-2^b antigens was ineffective for protection. Thus, taken everything considered, our experiments indicate that correct matching in -at least- H-2^d antigens (or epitopes) is deeply involved in the establishment and growth of T. crassiceps cysticerci in the peritoneal cavity of mice. Thus, BALB/B (H-2^b) mice capacity to immunologically react against H-2^d antigens renders them capable of better resisting the establishment and growth of T. crassiceps cysticerci, while BALB/c (H-2^d) being tolerant to

self antigens, is less capable of resisting challenge.

In general terms, our findings refer to the biological basis of murine differences in susceptibility to cysticercosis and point to the principles of transplantation immunity ruling also the relationships between host and complex parasites. A few years back we found a strong association between genetic background and susceptibility of mice to experimental T. crassiceps cysticercosis (31). More careful work esta blished that susceptibility was related with the H-2 genes (2), while genes in the rest of the genome seemed comparatively irrelevant, excepting those envolved in sex determination (unpublished observations). In congenic strains of BALB background $H\text{-}2^d$ was identified as the susceptible haplotype, while $H\text{-}2^b$ and $H\text{-}2^k$ were resistant (2). Because the challenging parasites had been serially cultured in BALB/c ($H\text{-}2^d$) female mice, the possibility that they had adsorbed MHC antigens from their hosts, and thus were mistakenly taken as "self" structures by $H\text{-}2^d$ mice and correctly as "foreing" by $H\text{-}2^b$, revived the notion of antigen similarities between host and parasite playing a significant role in their relationship (32,33). Most recently many molecules have been found are shared between host and parasite (34-37). However, the sharing of many of these molecules -immunoglobulins, collagen, -2 macroglobulin, Forsmann antigens etc- is not really surprising as they perform general biological functions that one would expect would be conserved throughout evolution. In contrast, there is little in the literature about sharing of histocompatibility antigens, which through evolution produced extremely unique individuals (7,8,38). Should these MHC antigens be shared between host and

parasite it was assumed would greatly influence the outcome of their relationship. More so perhaps for cestode than for any other parasites, as cestodes are extremely complex biological structures that include nerves, muscles, gonads, digestive system and more (39). Perhaps cestodes, metacestodes specially, so complex themselves and so intimately located inside their hosts, -where they may reside for years without major complications for both (40-43)- more resemble a veritable tissue trans plant than other comparatively simpler parasitic organisms. If so, these host-parasite interactions should roughly follow the basic laws of transplantation immunity, where a certain degree of matching in histocompatibility antigens between donor and receptor is imperative for the graft to take (for the parasite to survive). Of course, literature abunds in instances where MHC and susceptibility to infection associate (3-6) but it rarely invokes a correct matching of histocompatibility antigens, in a transplantation immunity context, as a key factor in parasite establishment and survival (9). As research proved the role of MHC in immunity to be of paramount importance for antigen presentation and regulation of the immune response to a number of antigens (44), including those of parasites (45), it strengthened the notion, that it would be suicidal for a parasite to share MHC molecules with it's host, as it would probably hasten it's immune recognition and destruction.

However, our results definitively show that mice and Taenia crassiceps cysticerci share H-2^d-like epitopes and DNA sequences and that the ability to respond to these, is directly related to the

host's ability to resist a challenging infection, very much like the factors involved in the rejection of a transplant.

This finding could be of practical significance for T. solium cysticercosis of human and porcine hosts, as it could contribute to explain the selective distribution of neurocysticercosis within families or litters and, in fact, within populations exposed to essentially the same risk (46-48).

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Legends

Fig. 1 Taenia crassiceps antigens from parasites grown in BALB/B (■) or BALB/c (○) mice inhibit H-2^d (A,B,C) and H-2^b (D,E,F) specific reactions in a dose dependent manner. No inhibition was detected in two controls systems used (● , DNP anti-DNP; □ , mouse-rabbit anti mouse).

Fig.2 Western blot T. crassiceps antigens reacted with anti H-2 monoclonal antibody, A; anti H-2^d polyclonal antibody, B; anti H-$2D^d$ monoclonal antibody, C; anti IE^{dk}-IA^{bdq} monoclonal antibody, D; rabbit anti mouse antibodies, E; non immune mouse immunoglobulins, F. Extensive crossreactivity of parasite antigens with polyclonal anti H-2^d antibodies regardless of the strain of mice where parasite were grown was detected.

Fig. 3 Hybridization of ^{32}P-cDNA (coding for H-$2K^d$ class I transplantation antigens) with genomic DNA derived from BALB/c mice (C), BALB/B mice (D) and from Taenia crassiceps cysticerci grown in BALB/c mice (E). Lanes A and B represent two control systems with pBR322 and unlabeled probe respectively. Lanes C, D and E were restricted with EcoRI. All lanes contained 7 ug of DNA.

Table 1. Effect of previous immunizations with histocompatibility antigens in host susceptibility to T. crassiceps infections. Parasite resistant BALB/B (H-2^b) mice immunized against BALB/c

splenic cells developed protective immunity against T. crassiceps cysticerci grown in either BALB/B or BALB/c hosts. In contrast, susceptible BALB/c mice remained equally susceptible after immunization with H-2^b cells. The ability to respond to H-2^d antigens is, thus, critically involved in the host's resistance to T. crassiceps cysticercosis. The sexual differences in susceptibility, in both strains, immunized or not, is manifest.

TABLE I

PARASITE LOAD IN IMMUNIZED AND NON IMMUNIZED RESISTANT (BALB/B) AND SUSCEPTIBLE (BALB/c) MICE WITH HISTOCOMPATIBILITY ANTIGENS

		HOSTS			
		BALB/B (H-2b)		BALB/c (H-2d)	
		Non Immunized	Immunized (anti H-2d)	Non Immunized	Immunized (anti H-2b)
Parasites	+ pH-2b	48.33 ± 23.3*a	2.2 ± 2.5b	98.25 ± 29.4d	78.1 ± 19.56d
	++ pH-2d	20.4 ± 6.8 c	2.8 ± 1.93b	120.8 ± 46.88de	143.68± 48.78e

\+ parasites grown in BALB/B mice for four generations.
++ parasites grown in BALB/c mice for more than four generations.
* Mean ± s.d. of the parasite load recovered from ten mice per group 30 days after infection with 10 cyts.
a,b,c,d and e: Statistics labeled with the same literal are not significantly different from each other while those labeled with different literals are significantly different (P 0.1). Groups labeled with two literals are not significantly different from two groups differing significantly in parasite loads between themselves.

H-2 LIKE DETERMINANTS IN *Taenia crassiceps* CYSTICERCI DETECTED BY CELL ELISA INHIBITION

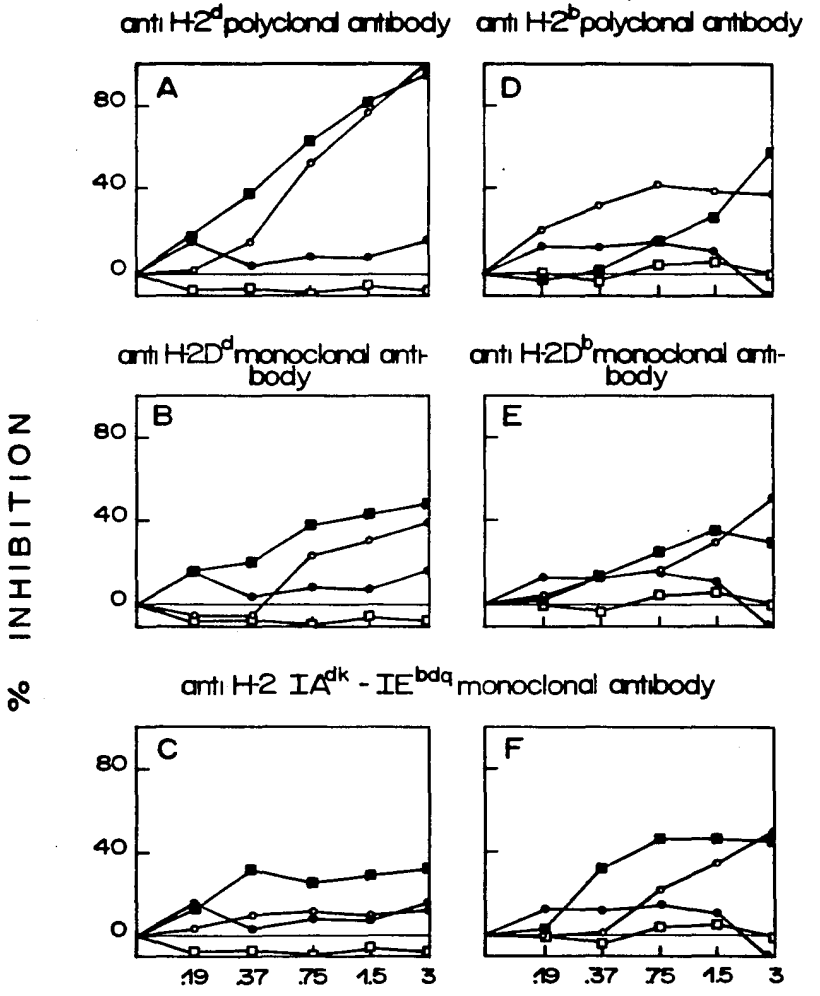


Fig. 1

CROSS REACTIVITY BETWEEN *T. crassiceps* AND H-2^{d} ANTIGENS

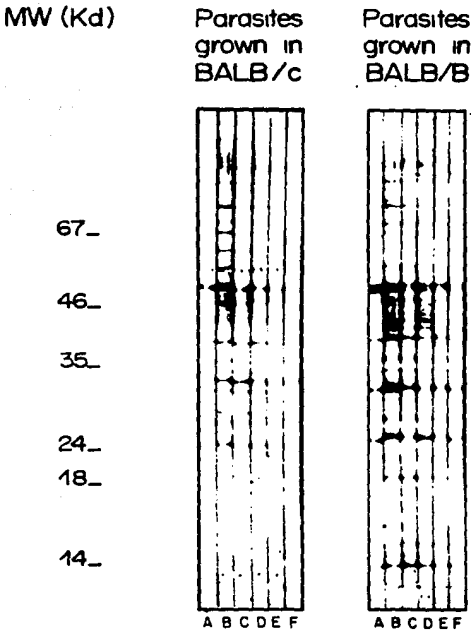
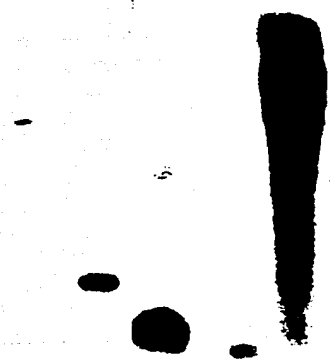


Fig. 2

Southern Blot Analysis of DNA from parasites and host



A B C D E

Fig. 3

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DISCUSION Y PERSPECTIVAS

Los resultados presentados en este trabajo de investigación muestran que la susceptibilidad de los ratones de la cepa BALB/c en la infección causada por el cisticerco de Taenia crassiceps es influenciada por los genes del complejo mayor de histocompatibilidad. De especial interés son los hallazgos de que las cepas de haplotipo $H\text{-}2^d$ sean las más susceptibles en comparación con las cepas que tienen los haplotipos $H\text{-}2^k$ y $H\text{-}2^b$. El resto del fondo genómico del hospedero no parece tener un papel tan importante ni inmediato en la tasa de multiplicación del parásito en el peritoneo. Estos resultados permiten excluir como eventos críticos aquellos que se desarrollan independientemente del MHC y que podrían influir en la mayor o menor susceptibilidad del hospedero. El efecto aparentamente exclusivo del H-2 en la susceptibilidad puede restringir notablemente el estudio de los mecanismos moleculares a aquellos involucrados en la respuesta inmunológica dirigida contra los antígenos del parásito en un determinado hospedero, dado el conocimiento existente sobre las funciones asociadas a estos genes. En otras parasitosis se han identificado algunos genes a los que se les ha asociado la resistencia del hospedero, como los genes Lsh para Leishmania donovani (De Tolla, Scott and Farell, 1981) o los genes Ity para Salmonella typhimurium (Plant and Glynn 1979), sin embargo se desconocen los mecanismos a través de los cuales se ejerce tal efecto. Así pues, si bien las asociaciones entre susceptibilidad y MHC no constituyen una regla general en todas las parasitosis, en ésta cisticercosis murina los fenómenos inmunológicos que dependen de los productos del H-2 son los que fundamentalmente

están determinando las diferencias de susceptibilidad.

Habiendo pues identificado a los genes del H-2 como los factores fundamentales en determinar la susceptibilidad del hospedero, se abren dos grandes líneas de investigación a seguir. La primera contempla aquellos mecanismos inmunológicos del hospedero que dependen de las actividades biológicas de y los productos del MHC, la segunda corresponde a propiedades propias del parásito que puedan favorecer de un modo diferencial su capacidad de adaptación en distintos hospederos. Considerando que la cepa A/J, (una recombinante natural k/d, [K^k, I^k, S^d, D^d], Klein 1986) fue susceptible, es muy probable que puedan ser excluidos como eventos críticos en la instalación de ésta parasitosis, aquellos que se determinan en el contexto de las moléculas clase II y que sean los genes asociados a un rechazo de transplantes (clase I) los más probablemente involucrados y por lo tanto los más inmediatos a estudiar. Sin embargo los resultados obtenidos con esta cepa recombinante no excluyen que factores como el complemento (región S) puedan tener un papel importante en la susceptibilidad. Al respecto, una consideración de nuestros resultados sugeriría que el sistema del complemento no está críticamente involucrado en las diferencias de carga parasitaria observadas, ya que la cepa C3H/HeJ, que naturalmente es deficiente en C4 y por ello susceptible a algunas infecciones como la teniasis por Taenia taeniaeformis (Mitchell, Goding and Rickard, 1977), mostró ser resistente a la cisticercosis por Taenia crassiceps. Los datos presentados anteriormente aunados a las observaciones de que el complemento no parece tener un efecto importante, sugieren que los principales mecanismos

dependientes del MHC que afectan la carga parasitaria estarían localizados en la región D.

En el intento de explorar mecanismos inmunológicos dependientes del hospedero que pudiesen asociarse a las diferencias de susceptibilidad y considerando el papel tan importante que tienen los anticuerpos en muchas parasitosis por céstodos, se estudió la respuesta inmune humoral frente a esta parasitosis. Las siguientes consideraciones de nuestros resultados nos sugieren que ésta respuesta inmune no está directamente asociada, al menos de una manera sencilla, con las diferencias en la carga parasitaria encontradas: a) Se pudo constatar que existe una correlación positiva entre el título de anticuerpos y el número de parásitos. Así la cepa BALB/B que sostuvo una menor carga parasitaria también presentó los valores más bajos de anticuerpos, en tanto que los animales susceptibles BALB/c con mayor carga parasitaria, presentaron los mayores niveles de anticuerpos. b) Las cepas más susceptibles no modificaron su mayor carga parasitaria al recibir suero de animales menos susceptibles, c) No se encontraron diferencias cualitativas de respuesta inmune humoral según el análisis de espectros antigénicos obtenidos en las inmunoelectrotransferencias, los cuales resultaron muy similares entre cepas que presentaban diferentes grados de susceptibilidad. Estos resultados sugieren que los factores del hospedero que determinan su grado de susceptibilidad no están asociados de una manera sencilla con la producción de anticuerpos, sino que mas bien involucran mecanismos relacionados con la respuesta inmune celular. Al menos así lo sugieren las observaciones de Terrazas et al. (1990), quienes han encon-

trado que la timectomia neonatal además de aumentar significativo en la carga parasitaria de la cepa susceptible no modifica los niveles de anticuerpos.

Otro nivel de estudio fue la búsqueda de características propias del parásito que pudiesen correlacionarse con las diferencias de susceptibilidad. Específicamente se estudió la propuesta de que la homología entre hospedero y parásito a nivel de moléculas del H-2 pudiese constituir un medio a través del cual se comprendieran las diferencias en la carga parasitaria entre las cepas estudiadas. Este nivel de estudio se enriqueció notablemente al encontrar que en otras parasitosis como en la esquistosomiasis causada por Schistosoma mansoni los parasitos se recubren con moléculas H-2 del hospedero (Sher, Hall y Vadas 1978) que si bien no se han logrado correlacionar con protección para el parásito, tampoco parece existir que puedan favorecer su reconocimiento (McLaren, Clegg and Smithers, 1975). Asimismo los estudios conducidos por Damian (1979), sugieren que la homología antigénica entre Nematospiroides dubius y el ratón, determina la susceptibilidad de esta especie hospedera a ésta parasitosis.

Los datos presentados en éste trabajo indican que los cisticercos de Taenia crassiceps recuperados tanto de animales susceptibles (BALB/c) como resistentes (BALB/B) sintetizan moléculas que comparten epitopes con los antigenos de histocompatibilidad del hospedero. Estas moléculas presentes en el extracto antigénico obtenido de parásitos fueron detectadas por su capacidad de inhibir sistemas especi-

ficos de reacción entre H-2^d /anti H-2^d y H-2^b /anti H-2^b, aunque los mayores porcentajes de inhibición obtenidos fueron con el sistema H-2^d, con lo cual cabe la posibilidad de considerar que sean estos determinantes los más prominentemente involucrados en el extracto antigénico parasitario y que la inhibición del sistema H-2^b/anti H-2^b se presente como consecuencia de la gran homología antigénica que existe entre los productos del MHC (Klein 1986).

La extensa reactividad cruzada observada al confrontar éstos antígenos parasitarios por Immunoelectrotransferencias, con un panel de anticuerpos anti H-2 de diferentes especificidades, sugiere que éstos antígenos "H-2 like" pueden estar asociados a otras moléculas del parásito o bien que pueden estar expresados de manera distinta de como se encuentran en las células del hospedero.

Si bien los resultados obtenidos demostraban la presencia de antígenos "H-2 like" en el parásito, no explicaban las diferencias en la carga parasitaria entre la cepa más susceptible (BALB/c, H-2^d) y la menos susceptible (BALB/B, H-2^b) ya que aún cuando el extracto antigénico se obtuvo de parásitos mantenidos por más de 2 años en cada una de las cepas de ratones (para eliminar de los parásitos productos de absorción del hospedero), siempre se observó reactividad entre los antígenos parasitarios y anticuerpos anti H-2 de una especificidad de haplotipo distinta de la cepa en la que se mantuvieron a los parásitos.

Los resultados de las aloinmunizaciones revelaron que la induc-

ción de una inmunidad de transplante contra antígenos H-2^d, previa al desafío, reduce notablemente la carga parasitaria, no modificándose la carga parasitaria esperada cuando la inmunidad es contra antígenos H-2^b, estableciéndose que la habilidad del hospedero de poder responder inmunológicamente contra los antígenos H-2^d es, al menos, uno de los eventos más importantes que controlan la tasa de multiplicación del parásito dentro del hospedero. Así, con este estudio, el conocimiento de la relación hospedero-parásito se enriquece notablemente al revelar que la generación de una inmunidad de transplante inducida por antígenos "MHC like" propios del parásito es uno de los mecanismos que están críticamente involucrados en el control de la cisticercosis murina. Cabe considerar además que es muy probable que algunos otros factores dependientes directa o indirectamente del MHC puedan estar involucrados en la interacción hospedero-parásito y que sean críticos en determinar el destino final de la relación.

Finalmente un último aspecto a discutir, es el origen de estas moléculas. Muchos reportes de homología entre hospedero-parásito, situan a la adsorción como el evento más importante (Sher et al. 1978, Varela Díaz and Coltorti 1973, Cleg, Smithers and Terry 1971, Dean and Sell 1972, Goldring et al. 1976). También el parásito puede sintetizar moléculas similares a las del hospedero, como por ejemplo la producción de una molécula similar a la 2 -macroglobulina murina por Schistosoma mansoni (Damian, Green and Hubbard 1973). El evento de síntesis no necesariamente tiene que darse como consecuencia de la estancia del parásito en el hospedero a fin de evadir la respuesta inmune del mismo, sino que dado que los primeros son organismos más evoluciona-

dos y que presentan estructuras complejas y organizadas, bien cabria pensar en la posibilidad de que esta similtud sea tan sólo una coincidencia. Con base en los antecedentes anteriormente expuestos y en los resultados presentados a continuación, proponemos que estos antigenos "H-2 like" pueden ser productos del propio cisticerco:

a) La manifestación de moléculas de elevada reactividad cruzada con anticuerpos anti H-2^d en el extracto de parásitos mantenidos por más de 5 generaciones en cepas de haplotipo H-2^b, de manera que dado estos se reproducen por gemación asexual (Freeman 1963) y de que la infección se comienza con tan solo 10 cisticercos peque|os (Sciutto et al 1990) es de esperarse que las nuevas generaciones de no conserven nada de un hospedero con el que nunca tuvieron contacto.

b) La hibridización positiva obtenida entre el genoma del parásito y la sonda ^{32}P-cDNA (que codifica para antigenos MHC clase I del ratón: H-$2K^d$). La hibridización tan intensa observada podria incluir la reacción cruzada con genes de las inmunoglobulinas, dado que esta sonda tiene secuencias homólogas con los genes u de IgM. Sin embargo la baja reactividad de la sonda (H-$2K^d$) con el DNA de ratones BALB/B (H-2^b) intensifica la especificidad de la sonda.

Quedan aun por conocer los mecanismos por los que el cisticerco ha adquirido la capacidad de sintesis de estas moléculas, los cuales pueden ser consecuencia de una adquisición directa del gene del hospedero mediada por mecanismos de intercambio genético a través de particulas virales, o ser productos de una mutación del genoma del parásito que se originó como consecuencia de su estancia en el hospedero o ser

simplemente elementos constitutivos de los parásitos que casualmente resultaron ser similares a los antígenos del H-2 del ratón.

Desde un punto de vista aplicativo, este trabajo establece metodologías que permiten la búsqueda de antígenos similares entre hospedero y parásitos en otros sistemas, así como interpretaciones del mimetismo molecular a nivel de moléculas del MHC que puedan explicar las diferencias de susceptibilidad-resistencia en el fenómeno del parasitismo.

Finalmente, el presente trabajo desarrollado con el modelo experimental de cisticercosis murina por Taenia crassiceps, ha orientado líneas de investigación dirigidas a la búsqueda de los factores biológicos que median la susceptibilidad a esta parasitosis, en especial la búsqueda de epítopes compartidos entre el cisticerco de Taenia solium y los antígenos del complejo mayor de histocompatibilidad del hombre (HLA).

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APENDICE

REVISTA LATINOAMERICANA DE MICROBIOLOGIA
APARTADO POSTAL 4-862 06400 MEXICO, D. F.

México, D.F. a 22 de octubre de 1990.

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Su trabajo "SUSCEPTIBILIDAD GENETICA A INFECCIONES PARASITARIAS MURINAS", número 90819, - ha sido evaluado por el comité editorial de la Revista Latinoamericana de Microbiología. La opinión emitida es favorable en general, sin embargo, es - necesario hacer algunas correcciones, señaladas en el texto, y aclaraciones en otros puntos igualmente indicados.

- La tabla 1, si bien se publicará en una página, conviene que se presente mas amplia con las aclaraciones o correcciones que en ellas se indican.

- Finalmente, ya que se trata de infecciones de ratones se sugiere que se indique así, con la aclaración de que se trata de Mus musculus variedad - albina, puesto que el término "murino" es menos preciso.

En espera de su versión corregida, quedo de Ud.

ATENTAMENTE

DR. JORGE ORTIGOZA FERADO.
COEDITOR.

*clm

SUSCEPTIBILIDAD GENETICA A INFECCIONES PARASITARIAS EN EL RATON

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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 arbitrarios 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 huésped al parásito.

Las 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 fenotípicos participantes de la respuesta inmune del huésped contra el parásito, a través de los cuales controla su crecimiento (ie. anticuerpos), mientras que los productos fenotípicos 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 proteínas y factores solubles así como para proteínas de superficies celulares.

Dentro de los genes que codifican para proteínas 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 proteínas de superficies celulares se encuentra un grupo de glicoproteínas que pertenecen al Complejo Mayor de Histocompatibilidad (MHC), a las que se les ha asociado, entre otras funciones, las de presentación de antígenos a células immunocompetentes (35,74), así 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 antígenos (9) y, los receptores de moléculas liberadas por las propias células linfoides (interleucinas) presentes en linfocitos T, B y en macrófagos (79).

Susceptibilidad asociada a proteínas solubles codificadas por GRI.

Anticuerpos:

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

La susceptibilidad a diversas enfermedades parasitarias está asociada a la especificidad y al isotipo de algunas inmunoglobulinas. Así, por ejemplo, en la triquinosis experimental murina, un factor crítico 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 asociación antígeno-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). De este modo, las cepas resistentes presentan altos títulos de IgE e IgG específicas, inmunoglobulinas para las 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 tricuriasis experimental murina se ha encontrado que las cepas más susceptibles presentan mayores títulos de anticuerpos de clase IgM contra antígenos de secreción - excreción. (16). Otro ejemplo lo constituye Taenia taeniaeformis, para la que se ha reportado mayores títulos de anticuerpos en la cepa resistente que en la cepa susceptible, y su fase crítica de acción parece residir en los primeros días de infección, ya que son las larvas tempranas las susceptibles a anticuerpos. (26,47,54).

Respecto a la asociación entre susceptibilidad y repertorio de antígenos reconocidos, en el modelo de infección murina con Schistosoma japonicum, la cepa resistente 129/J reconoce un antígeno 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 señalar 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 proteínas 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 la 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 huésped 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 antígenos del huésped).

Factores solubles reguladores de la Respuesta Inmune:

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 huésped. 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 antígenos de huevecillos de Schistosoma japonicum disminuyen la actividad de la IL-2, a través de un mecanismo mediado por células T supresoras (73).

Susceptibilidad asociada a proteínas celulares codificadas por GRI.

Entre las proteínas de superficies celulares codificadas por GRI se encuentran las moléculas receptoras para antígenos, 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 proteínas sólo se han descrito asociaciones entre la susceptibilidad a infecciones y los genes que codifican para los antígenos 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 *Nematospiroides dubius*. En ambas parasitosis las cepas resistentes (H-2^s, H-2^b, H-2^q, H-2^f) presentan como característica común ser I-E^- y las más susceptibles (H-2^k) ser I-E^+. Se ha propuesto que la respuesta inmune en el contexto de antígenos I-E es fundamentalmente supresora (56), en tanto que en el contexto de I-A se induce una respuesta proliferativa, lo que podría 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 antígeno (87). Sólo en estos casos se ha podido proponer asociación entre el genoma y sus productos fenotípicos; 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 características que se ha intentado asociar a diferencias en la susceptibilidad a infecciones.

GNRI relacionados con el cromosoma sexual

En la Tabla 4 se señalan 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 huésped 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 típico de esta clase de susceptibilidad es el paludismo humano, para el cual la resistencia del huésped está asociada a la clase de hemoglobina de sus eritrocitos. Así, los individuos con anemia hemolítica congénica, (cuya hemoglobina tiene una mutuación puntual en la cadena β de la hemoglobina) tienen menor capacidad para acarrear oxígeno, 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 sanguíneo se encuentra asociado con la incapacidad del parásito para penetrar la célula del huésped (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 está 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 dependerá 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 huésped.

Si bien el estudio de la genética de vertebrados ha permitido identificar regiones genómicas que determinan la susceptibilidad del huésped 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), en 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 huésped.

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 huésped.

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 huésped, lo que implicaría diferencias intrínsecas de susceptibilidad del parásito al huésped.

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

Modificación del genoma del huésped por el parásito

Es posible que el genoma del parásito y del huésped 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 huésped. Esta homología proteica podría ser el resultado de la interacción entre el genoma del parásito y del huésped. (71).

Un ejemplo de que los parásitos pueden modificar la expresión genómica del huésped se presenta en la esquistosomiasis (producida por *Schistosoma mansoni*) en la cual el parásito disminuye la traducción de proteínas 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 huèsped y otro como su parásito, el huèsped ofrece diferentes ambientes heterogéneos, en lo que se refiere a lo propicio para que el parásito crezca. Por otro lado, el parásito como especie, es potencialmente heterogéneo, respecto a su patogenicidad o a su capacidad de reproducción en el huèsped. Una vez en contacto, el convivio huèsped-parásito puede generar en cada una de ellas, modificaciones que cambien el destino de esta relación, modificándose la susceptibilidad inicial. Según estos criterios, las diferencias de susceptibilidad del huésped hacia el 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 huésped-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 huèsped-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 parasitosis.

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.

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PARASITARIAS MURINAS:
PROPIEDADES

PARASITO	RESISTENTE Cepa	Haplotipo	SUSCEPTIBLE Cepa	Haplotipo	Heredabilidad	Criterio de medición de la infección	Ref
Trypanosoma cruzi (Protozoario hemático)	BIO	b	A	a	Resistencia dominante (Poligénico)	Variaciones en los niveles de parasitemia en sangre.	
	BALB/c	d	A, AW, CBA, SW	b			
	CBA/BIO.BR	k	DBA/2	d			
	DBA/1, SJL	q	AKR, CBA	k			
	B6	b					
Trypanosoma brucei (Protozoario hemático)	B6	b	CBA/He	k	Resistencia dominante o codominante (Poligénico)	Variaciones en los niveles de parasitemia en sangre.	12
Trypanosoma congolense (Protozoario hemático)	B6	b	AJ			Variaciones en los niveles de parasitemia en sangre y muerte.	52,53
							58
Leishmania trópica (Protozoario intracromofágico)	CBA, AKR	k	BALB/c	d	Susceptibilidad dominante	Desarrollo de lesión cutánea persistente.	
	A	a	B6	b			31
			DBA/2, BIO.D2	d	Un solo gene autosómico.		
L. donovani (Protozoario intracromofágico)	A	a	BALB/c, BIO.D2	d		No. de parásitos presentes en núcleos de células hepáticas.	5,6,7
	DBA/2	d	B6	b			
	CBA/He	k					
L. brazilliensis (Protozoario intracromofágico)			A	a		Desarrollo de lesión cutánea persistente.	37
			B6	b			
			BALB/c	d			
			CBA	k			
			SWR	q			
L. mexicana (Protozoario intracromofágico)	CBA, AKR	k	CBA	k	Resistencia dominante o codominante.	Desarrollo de lesión cutánea persistente.	60
	B6	b	BALB/c	d			
Giardia muris (Protozoario intestinal)	BALB/c	d	CBA/He	k		Desarrollo de infección crónica.	67
Toxoplasma gondii Multigénico (Protozoario de células endoteliales y leucocitos mononucleares)	B6	b	BALB/c	d		Indice de mortalidad	3,86
	BIO.D2	d					
	CBA	k					
	DBA/1	q					
Plasmodium berghei (Protozoario intraeritrocítico)	B6	b	A	a	Resistencia dominante multigénico	Variaciones en niveles de parasitemia y muerte.	23,65

ELMINTOS

PARASITO	RESISTENTE Cepa	Haplotipo	SUSCEPTIBLE Cepa	Haplotipo	Heredabilidad	Criterio de medición de la infección	Ref
Echinococcus multilocularis Fase larvaria (Equinococo) hepática del hámster	A	a	AKR	k		Cambios hematológicos y alteraciones graves en el sistema linforeticular.	67
	BIO	b					
	BALB/c, DBA/2	d					
	CBA/CBA	k					
	DBA/1	q					
Nematospiroides dubius (Nemátodo intestinal)	B6	b	CBA	k	Resistencia dominante (poligénica)	NO. de larvas eliminadas antes del día "n". Resistencia a reinfección.	42,64
	BALB/c	d	A	a			
Ascaris suum (Larvas migrantes en tránsito en pulmón. El adulto es intestinal)	CBA	k	B6	b	Resistencia dominante	No. de larvas en pulmón.	37
	CBA	k	CBA/N, BIO.R	k			
Schistosoma mansoni (Trematodo hemático)	A, BIO.A	a	CBA, BIO	b		No. de gusanos recuperados.	75
	B6, BIO	b	BALB/R	b			
	DBA/2, BIO.D2	d	BALB/c	d			
	CBA	k					
	SJL	s					
S. japonicum (Trematodo hemático)	129/J		B6	b		No. de gusanos recuperados.	49
Taenia taeniaeformis (Fase larvaria hepática del cisticelo)	B6	b	CBA, AKR	k		No. de cisticercos en hígado.	57,60
	BALB/c, DBA/2	d	CBA/N				
Trichinella spiralis Fase adulta intestinal (larvas musculares)	A	a	BIO.M(17R)	aq1	Resistencia dominante	No. de larvas eliminadas antes del día "n" o No. de larvas en tejido.	76,80
	BALB/c	d	B6	b			81,82
	CBA, AKR	k	BIO.A(1R)	h1			
	DBA/1, BIO.Q	q	BIO.AM	h3			
	BIO.S	s	BIO.A(4R)	h4			
			BIO.A(5R)	i5			
			AKR, BIO.K	k			
			BIO.ABM	m			
			BIO.P	p			
			BIO.RIII	r			
			S.SL	s			
			BIO.NTT	t3			
Trichuris muris (Nemátodo intestinal)	BALB/c	d	BIO	b	Resistencia dominante	No. de larvas eliminadas antes del día "n"	78
	BALB/B	b	BIO.D2/n	d			
	BALB/K, CBA	k	BIO.BR	k			
	RIII		BIO.G	Q			
			CBA/2				
Taenia crassiceps (Fase larvaria del cisticelo)	BALB/K	k	BALB/c	d		No. de cisticercos recuperados en cavidad peritoneal	66
	BALB/B	b					
	B6	b					

TABLA 3

PROTEINAS DE SUPERFICIE CELULAR CODIFICADAS POR GRI

		Sitio de codificación en Murinos	Funciones principales	Ref
Receptores para Antígenos	LB (Ig's)	CH Ch 12 Clk Ch 16 Cll Ch 6	Reconocimiento de antígeno soluble o libre y en superficies celulares de células presentadoras de antígeno	55
	LT	RCT γ Ch 13 RCT β Ch 6 RCT α 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 específica 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 linfocitolisis y rechazo de transplantes.	35
	I-A/I-E	Ch 17	Moléculas presentadoras de antígeno, mediadoras de funciones supresoras y cooperadoras.	36

TABLA 2

PROTEINAS REGULADORAS SECRETADAS POR EL SISTEMA LINFOHEMATICO

	Funciones principales	Sitio de codificación en ratón	
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.	Ch 11	69,29
IL-4	Factor estimulatorio de linfocitos B. Inducción de MHC Clase II.	Ch 11	34
IL-5	Factor de crecimiento de linfocitos B.	Ch 11	34
IL-6	Factor de diferenciación de linfocitos B. Inducción del receptor de IL-2 en células T.	Ch 5	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	10.39,62
IFN-γ	Induce moléculas del MHC clase I y clase II. Potente activador de la función de macrófagos.	Ch 10	39,62

TABLA 4

PATRONES DE SUSCEPTIBILIDAD A ENFERMEDADES PARASITARIAS MURINAS ASOCIADAS A SEXO

Parasito	Susceptible	Resistente	Cepa	Ref.
PROTOZOARIOS				
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	H	M		2
Brugia pahangi	M	H	C57BL/6	88
HELMINTOS				
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 taeniaeformis	M	H	C3H/He	47

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