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"INTERACCIONES INMUNO-ENDÓCRINAS DURANTE LA CISTICERCOSIS MURINA: EL PAPEL DE LA PROGESTERONA Y LA DEHIDROEPIANDROSTERONA"

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Mira, yo he puesto hoy delante de ti la vida y el bien, la muerte y el mal; pues te ordeno hoy amar al SEÑOR tu Dios, andar en sus caminos y guardar sus mandamientos, sus estatutos y sus juicios, para que vivas y te multipliques, a fin de que el SEÑOR tu Dios te bendiga en la tierra que vas a entrar para poseerla.

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Índice

INTRODUCCIÓN. 6
RESUMEN 21
HIPÓTESIS 24
OBJETIVO GENERAL 24
MATERIALES Y MÉTODOS 25
RESULTADOS 30
CONCLUSIONES 43
ANEXOS (ARTÍCULOS PUBLICADOS): 45
REFERENCIAS: 105

I.- Introducción.

1.1 Inmunología General

La primera línea de defensa en los mamíferos en particular, y en los vertebrados en general, se proporciona por barreras mecánicas como la piel, que cubren superficies corporales y evitan físicamente que los microorganismos y otros agentes nocivos potenciales penetren a los tejidos situados por debajo de dicha cubierta.

Cualquier respuesta inmune implica, en primer lugar, el reconocimiento de un patógeno o cualquier otro material extraño al propio cuerpo; posteriormente, se lleva a cabo una reacción contra el antígeno para eliminarlo. La primera línea de defensa principal contra la infección la constituyen un importante grupo de células fagocíticas que forman parte de la inmunidad innata: monocitos, macrófagos y neutrófilos. Estas células se encargan de unirse a los organismos patógenos, internalizarlos y lisarlos, además de otras funciones (Roitt, 1998). En contraste con la inmunidad innata existe otra forma de inmunidad capaz de responder a una infección y adaptarse a ella: la inmunidad adaptativa. Ésta es específica para distinguir macromoléculas y tiene la habilidad para “recordar” y responder de inmediato a exposiciones repetidas contra el mismo antígeno. Los componentes de la inmunidad adaptativa son los linfocitos y sus productos. Las respuestas inmunes adaptativa e innata son componentes de un sistema integrado de defensa en el cual numerosas células y moléculas funcionan cooperativamente. Existen dos importantes vínculos entre uno y otro tipo de inmunidad: primero, la respuesta inmune innata hacia los organismos invasores estimula e influye sobre la acción inmune adaptativa. Segundo, la actividad inmunológica adaptativa utiliza algunos de los mecanismos efectores de la inmunidad innata para eliminar a los invasores, y con frecuencia funcionan potenciando la actividad antimicrobial de los mecanismos de defensa de la inmunidad innata (Abbas et al., 2000).

En el caso de algún patógeno que pueda superar las barreas superficiales y entrar al cuerpo, se cuenta con factores adicionales que protegen a los tejidos interiores. Algunas son proteínas solubles y otras macromoléculas, o también células que

circulan en la sangre y en el líquido extracelular haciéndolos inhóspitos para seres extraños.

En los vertebrados, y particularmente en los mamíferos, existen dos tipos de respuesta inmune adaptativa mediada por células efectoras, en cuyas respuestas radica, a) el potenciar otras células como la inmunidad celular (respuesta tipo Th-1) o la producción de anticuerpos (inmunidad humoral) capaces de reconocer cuerpos extraños invasores como las bacterias y virus y mantener al organismo libre de ellos (respuesta tipo Th-2). El sistema inmune humoral elimina principalmente a patógenos extracelulares y evita la diseminación de los patógenos intracelulares aprovechando que estos últimos se transmiten de célula a célula a través de fluidos extracelulares (ver figura 1) (página http://www.montpellier.com.ar/paginaqm/AvancesEnEndócrinologia/Inmunoendo/imagenes/PWMBasesA.gif).

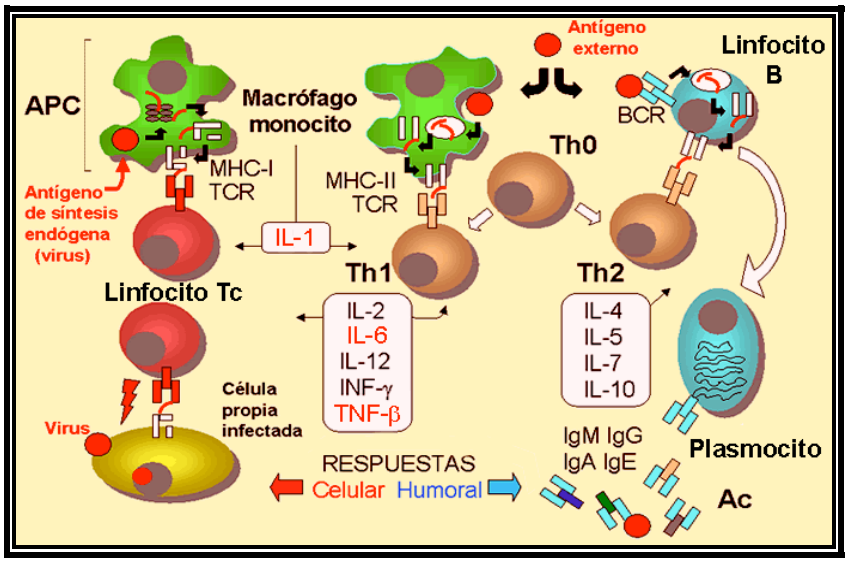


Figura 1. Representación esquemática de la respuesta inmune mediada por linfocitos cooperadores (Th de helper en inglés).

Esto se consigue mediante la producción de anticuerpos específicos. Los anticuerpos (Ac), por sí mismos, no suelen eliminar más que a ciertos virus o inactivar toxinas bacterianas. En la mayor parte de los casos, la eliminación efectiva del patógeno suele deberse a la inducción de las funciones efectoras de los anticuerpos, que dependen de la activación del complemento por la ruta clásica, misma que conlleva a la lisis del patógeno, quimiotaxis de fagocitos, opsonización de anticuerpos por inmunocomplejos (Ag-Ac), o citotoxicidad celular dependiente de inmunoglobulinas (ADCC). En esta última, los Ac se unen a

receptores (Fc) presentes en la superficie de células asesinas naturales (NK por sus siglas en ingles “Natural Killers”) y macrófagos.

La célula NK es un tipo linfocito citotóxico, es decir, tiene la capacidad de destruir células, principalmente aquellas que se encuentran infectadas por virus y otros microorganismos intracelulares. Sus funciones son llevadas a cabo por medio de un armamento granular intracitoplasmático. Este, se les conoce también como células LGL (Linfocito Granuloso Largo) debido al contenido que portan. Esta célula asesina tiene la capacidad de diferenciar las células infectadas por un virus, o las células tumorales que han tenido transformaciones malignas. Son capaces de identificar las células propias y extrañas.

Por lo tanto, en la respuesta humoral podemos distinguir dos grandes fases: la inducción de la producción de anticuerpos y la fase efectora, en la que estas inmunoglobulinas, directa o indirectamente, eliminan a los patógenos (Abbas, 2000).

Por otra parte, en la inmunidad celular, los linfocitos T participan principalmente en la eliminación de parásitos intracelulares (aunque no siempre es así). Estas células pueden activar a los fagocitos o a otras células, tales como las células NK. En respuesta a un estímulo antigénico, las células T secretan proteínas llamadas citocinas (ver figura 1), que tienen la función de estimular la proliferación y diferenciación celular. Dependiendo del tipo de citocina es el tipo de células inmunológicas que se activan y por consiguiente, el patrón de citocinas que se exprese puede determinar la eliminación de la infección. Esta respuesta origina una población de linfocitos citotóxicos, que es fundamental en la defensa de infecciones producidas por microorganismos intracelulares, como virus, bacterias y protozoarios (Abbas, 2000).

El éxito inmunitario depende de la notable propiedad de los linfocitos derivados del timo para reconocer y discriminar entre una amplia variedad de distintos antígenos extraños. Los linfocitos T no reconocen antígenos solubles, reconocen al antígeno en forma de fragmentos de péptidos unidos en las moléculas de las clases I y II del sitio del complejo principal de histocompatibilidad (MHC) (ver figura 2) (página:http://www.lesc.ic.ac.uk/ projects/appp_MHC.png).

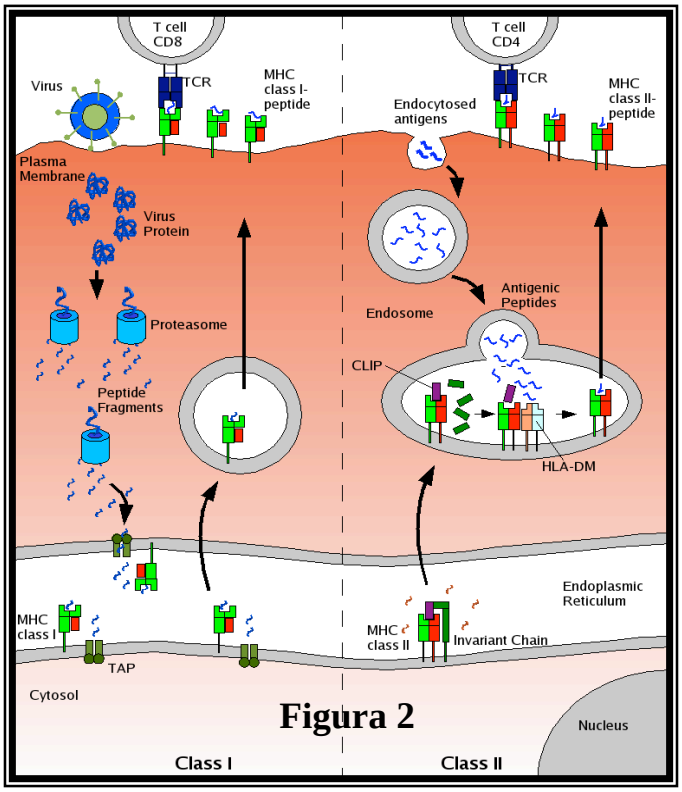


Figura 2. Figura representativa para el reconocimiento y procesamiento del antígeno; participación del complejo mayor de histocompatibilidad (MHC) y los linfocitos T.

Las células T cooperadoras (Th de helper en inglés) proporcionan las señales que sirven para aumentar las respuestas inmunitarias mediadas por células necesarias para que las células B se diferencien a células productoras de anticuerpos (Saalmüller A. 2006). Cuando se activan, las células Th producen interleucinas solubles que regulan la función de las células B, monocitos, macrófagos, y otras células del sistema inmunitario. La célula T colabora para producir la diferenciación de las células B a través del contacto directo entre los dos tipos de células que da como resultado, la estimulación directa de los receptores en las células B y también las exponen a grandes concentraciones locales de interleucinas derivada de Th (Saalmüller A. 2006). El repertorio de interleucinas (ILs) de las células Th vírgenes es muy limitado. En su encuentro inicial con el antígeno, las células Th producen principalmente IL- 2. Sin embargo, cuando las células Th se activan dan origen a células efectoras capaces de producir un conjunto considerable de diferentes interleucinas (Abbas, 2000). La mayor parte

de estas células efectoras pertenecen a 2 grupos distintos, nombrados como células Th-1 y Th-2, los cuales se diferencian por las interleucinas particulares que producen. Estos patrones divergentes de expresión de interleucinas, permiten que cada uno de estos grupos, promuevan los diferentes tipos de reacciones inmunitarias que sean más apropiadas para eliminar tipos particulares de microorganismos (Stites et al., 1997).

T-reguladoras

Actualmente existe una subpoblación de células T, llamada T-reguladoras (o inmunosupresoras). Estas células se caracterizan por tener marcadores de superficie específicos, tales cómo el fenotipo CD4+-CD25+. Cuando esta subpoblación de linfocitos T CD4+CD25+ es eliminada de ciertas cepas de ratones, los ratones desarrollan varias clases de enfermedades autoinmunes. Esto sugiere que la presencia de éstas células es esencial para efectuar los mecanismos de homeostasis inmunológica asociados a la protección contra la autoinmunidad. Aún no se saben exactamente la manera en que las células T reguladoras operan. Algunos piensan que estas células T reconocen y compiten por los mismos antígenos que aquéllos que activan a las células T citotóxicas, pero que las células T reguladoras se encuentran con epítopes diferentes. Otra posibilidad es que las células T citotóxicas solamente se multiplican cuando las células T reguladoras están ausentes. Sin embargo, al inhibir la participación de los linfocitos efectores, estas células pueden contribuir desarrollando la infección, el cáncer y otras enfermedades (Bluestone and Abbas 2003), por lo que todavía no se tiene pleno conocimiento de su función.

1.2 Endocrinología general

En 1905 Ernest Starling fue el primero en utilizar el término de “hormona” para describir una sustancia producida en el intestino que estimulaba la secreción de jugos digestivos del páncreas, sustancia a la que bautizaron como secretina (Kraegen et al., 1970). Starling en colaboración con William Bayliss, fueron los

pioneros de la endocrinología actual. Su noción de hormona, rediseño el campo de la comunicación celular, incluyendo nuevos conceptos químicos y biológicos.

Las hormonas son moléculas secretadas hacia la circulación sanguínea que pueden actuar en tejidos distantes. La señalización de una célula a otra adyacente es llamada comunicación parácrina, mientras que sí el mecanismo de señalización es a distancia es llamado comunicación endócrina. Si una célula es estimulada por su propia señalización ésta es llamada comunicación autócrina. (Williams 2003). Tanto la comunicación parácrina y autócrina, como la endócrina pueden compartir mecanismos de señalización. La testosterona, por ejemplo, se secreta a la corriente sanguínea pero también actúa localmente en los testículos controlando la espermatogénesis. Otro ejemplo es el IGF-I (por sus siglas en inglés Insulin-like growth factor I). Ésta es una hormona que es secretada a la sangre y sus órganos blanco se encuentran distantes; sin embargo, también tiene órganos blanco locales (incluyendo las mismas células que lo liberan) por lo que la comunicación es parácrina y autócrina. (Adams 2002).

Las hormonas actúan en las células blanco por su interacción con sus receptores proteínicos altamente específicos. Las proteínas del receptor se pueden localizar en la membrana, en el citoplasma, y en el núcleo de la célula (Beato et al., 1996; Hammes 2003). Los receptores a hormonas polipeptídicas se encuentran asociados a la membrana celular, mientras que los receptores a esteroides sexuales y a otros mensajeros químicos (ligandos) lipofílicos como la hormona tiroidea, glucocorticoides, mineralocorticoides, vitamina D, entre otros., se encuentran unidos a receptores citoplasmáticos.

De hecho, los receptores de las hormonas esteroideas, están situadas en el interior de la célula. Estas hormonas actúan directamente sobre la expresión génica. (Hammes 2003). Los receptores a esteroides sexuales incluyen: el receptor a cortisol, a aldosterona, a estrógenos (ER), a progesterona (RP), y a andrógenos (AR). En algunos casos, hay múltiples genes que codifican para un solo receptor, por ejemplo ER (α y β) y en otros casos, los distintos promotores por un mecanismo de procesamiento alternativo del mensaje pueden actuar generando diferentes tipos de receptores para una misma hormona, por ejemplo

PR (A y B). Finalmente, algunos receptores pueden tener afinidad a múltiples hormonas, por ejemplo, AR tiene afinidad a la dehidrotestosterona (DHT), testosterona (T) y dehidroepiadrosterona (DHEA) (Williams 2003).

Por otro lado, existen además efectos no genómicos de las hormonas esteroideas sobre las distintas funciones celulares. Esto implica la generación de cascadas de fosforilación a través de segundos mensajeros. Por ejemplo, la unión del estradiol a receptores membranales inespecíficos (GABAergicos acoplados a proteínas G), induce la activación de fosfatidil inositol trifosfato (IP3), lo que provoca a su vez una fosforilación entrecruzada de la familia Src. Src es un cinsa que modula la señalización intracelular, misma que lleva a las cinasas MEK y a la expresión de factores de trascripción diferenciación o expresión de citocinas Th1 o Th2 (Falkenstein 2000).

Receptores clásicos (genómicos)

Los receptores a hormonas esteroideas se encuentran en el citoplasma unidos en un conjunto denominado complejo aporreceptor, éstas contiene proteínas chaperonas también conocidas como proteínas de choque térmico (HSPs del inglés Heat Shock Proteins). Generalmente, las proteínas chaperonas sirven como inhibidores de la trascripción, compitiendo con el sitio de unión al DNA. Cuando la hormona se une al receptor nuclear (generalmente formando heterodímeros), éste crea un complejo denominado “hormona-receptor” (HR), el cual, condiciona a que el receptor tenga una serie de cambios conformacionales incluyendo la internalizando del HR al núcleo y la liberación de las proteínas de choque térmico, dejando el sitio de unión con el DNA libre, en el cual se une el complejo HR con el sitio de unión al DNA.

El complejo HR determina la activación del gen respectivo del DNA, la secuencia del proceso de trascripción del ARN mensajero y su procesamiento, culminando con la producción de proteínas en el ribosoma.

1.3 Interacciones inmuno-endócrinas

Actualmente la cantidad de información referente a las interacciones entre los diferentes sistemas como el nervioso, endócrino e inmune es abundante. Los descubrimientos reportados en este ámbito, dan la base para poder interpretar aquellos mecanismos involucrados en el mantenimiento de la homeostasis (Morales-Montor et al., 2004). Se sabe, por ejemplo, que un estado de ansiedad (psicológicamente hablando) puede deprimir las funciones inmunológicas y hormonales que a su vez tiene repercusiones sobre el organismo para la eliminación de algunas enfermedades (Bartolomucci 2007). La evidencia, a nivel celular, molecular y funcional, de que los sistemas inmune y neuroendócrino mantienen una interconexión directa y bidireccional son considerables: A) Las células de los sistemas inmune, el nervioso y el endócrino pueden expresar receptores para citocinas, hormonas, neurotransmisores y neuropéptidos; B) los productos de estas células coexisten en el tejido linfoide, el nervioso y el endócrino; C) algunos mediadores endócrinos y nerviosos pueden afectar al sistema inmune y D) los inmunomediadores pueden afectar la función de estructuras endócrinas y nerviosas (Besedovsky and Del Rey, 1996). De esta manera, se conocen diversas sustancias solubles que son producidas por los tres sistemas y que actúan al nivel de diversas células blanco por medio de receptores específicos y comunes para los diversos tipos celulares (Sandi et al., 1989). Entre estas sustancias se puede mencionar, por parte del sistema nervioso, a los neurotransmisores, que actúan al nivel de diversas células inmunológicas, estimulando o inhibiendo una función determinada. Por parte del sistema inmune existen las citocinas que son producidas por diversos inmunocitos ante un estímulo externo y que, a su vez, pueden modificar el funcionamiento del sistema nervioso y endócrino actuando sobre diversos tejidos blanco, de forma autócrina, parácrina y endócrina (Blalock, 1989). Por parte del sistema neuroendócrino existen dos diferentes tipos de hormonas que tienen diversos efectos sobre el sistema inmune: las hormonas peptídicas y los esteroides: (Kennedy and Jones, 1991).

En estudios hechos principalmente en mamíferos, se ha observado la existencia de dos ejes de regulación de suma importancia para la homeostasis: el eje Hipotálamo-Pituitaria-Suprarrenales (HPA) y el eje Hipotálamo-Pituitaria-Gónada (HPG). Ambos ejes se ven afectados en alguna de sus fases, tejidos o células, por aquellos factores involucrados en la red neuroinmunoendócrina: neurotransmisores, citocinas y hormonas (Sandi et al., 1989).

La activación de los linfocitos y fagocitos, por parte de un antígeno, estimula la producción de interleucinas, como el factor incrementador de glucocorticoides (GIF) y el factor de necrosis tumoral (TNF); y las monocinas como la interleucina (IL)-1. Estas citocinas ejercen posteriormente un control directo a nivel del hipotálamo provocando la liberación de la hormona liberadora de corticotropina (CRH). La CRH, a su vez, estimula a la hipófisis anterior para liberar la hormona adrenocorticotrópica (ACTH) la cual tiene como órgano blanco a las glándulas suprarrenales (Sandi et al., 1989). Es en la corteza de estos órganos donde la ACTH estimula la liberación de glucocorticoides. Además, la ACTH puede ser liberada por linfocitos activados para estimular, de igual manera, la liberación de glucocorticoides por parte de las glándulas suprarrenales. Los niveles altos de glucocorticoides circulantes provocan directamente la inhibición de linfocitos T y B efectores mediante receptores a glucocorticoides y disminuyen la liberación de hormonas producidas en el timo (Van Laethem et al., 2001). Los niveles reducidos de hormonas tímicas, como la timulina, también inhibirán a los linfocitos efectores. La disminución de hormonas tímicas actuando en el hipotálamo y la hipófisis puede reducir la liberación de la hormona luteinizante (LH) y, por ende, disminuir la cantidad de esteroides sexuales secretados por las gónadas. Esta reducción de esteroides sexuales (estrógenos y andrógenos) provoca la liberación de hormonas tímicas posiblemente para reestimular a los linfocitos efectores e incrementar la liberación, hipófisiaria de LH y, de esta manera, incrementar la cantidad de esteroides sexuales liberados, lo que provoca la inhibición de las hormonas tímicas (Grossman et al., 1991).

Existe evidencia de que si el eje HPA está deprimido (muy bajos niveles de corticosteroides, por ejemplo) puede provocar una hiperactividad del sistema

inmune e incrementar de esta manera el riesgo de desarrollar enfermedades autoinmunes (Pennisi, 1997). La regulación del eje HPA también se ve afectada por los mecanismos de retroalimentación negativa: después de que la ACTH estimula a la corteza de las glándulas suprarrenales para liberar glucocorticoides. Estos últimos regulan negativamente al hipotálamo y a la hipófisis, para bloquear la activación del eje (Chiappelli et al., 1996). Uno de los efectos del incremento de corticosteroides es inhibir la producción de IL-1 y de esta manera reducir la respuesta inflamatoria, efecto que es la base de su utilización como fármaco anti-inflamatorio (Pennisi, 1997). El cortisol (un glucocorticoide) tiene un efecto directo sobre las células T y B, ya que modula su maduración, y afecta el tráfico y activación de células pro-inflamatorias; además, suprime la producción de IL-1, IL-2, IL-6, IL-8, IL-10, IL-12 y TNF-α (Grossman et al., 1991; Van Laethem et al., 2001). Otro esteroide importante producido por la corteza suprarrenal que tiene efecto en los inmunocitos es la dehidroepiandrosterona (DHEA). Este andrógeno tiene como células blanco a las células T, los monocitos y los macrófagos. Es capaz, además, de suprimir la producción de IL-6 y TNF-α e incrementar la de IL-2 e IFN-α (Morales-Montor et al., 2002a).

Estas interacciones bidireccionales entre el eje HPA y el sistema inmune representan un mecanismo importante que previene las respuestas inmunes extremas. La hipófisis controla directa o indirectamente la actividad de casi todas las glándulas endócrinas y, a su vez, está controlada a nivel del sistema nervioso central por el hipotálamo. La hipófisis es influida directamente por la IL-1 y la IL-6, principalmente sobre la secreción de la ACTH, la cual suprime la respuesta de anticuerpos a antígenos dependientes (eritrocitos de carnero) e independientemente de células T (Besedovsky and Del Rey, 1996).

Además de ACTH, la hipófisis produce otras hormonas entre las cuales se encuentran la hormona luteinizante (LH) y la hormona folículo estimulante (FSH). Estas hormonas son producidas de acuerdo a las señales recibidas en la hipófisis por parte del hipotálamo mediante la hormona liberadora de gonadotropinas (GNRH). Ambas hormonas, se unen a receptores en el ovario o en los testículos y

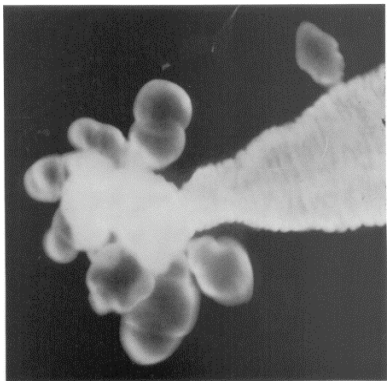
regulan la función gonadal promoviendo la gametogénesis y la producción de esteroides sexuales (Greenspan and Baxter, 1994).

Las hormonas sexuales parecen jugar un papel importante en las diferencias de susceptibilidad asociadas al sexo en ciertas enfermedades infecciosas y autoinmunes (Beagley y Gockel 2003). Se sabe que las hembras de diferentes especies tienen niveles más altos de inmunoglobulinas circulantes y presentan una respuesta inmune de tipo humoral más fuerte en contra de la infección. La producción de una variedad de anticuerpos autorreactivos también es más frecuente en las hembras (De León-Nava y Morales-Montor 2006). Se ha comprobado que los estrógenos incrementan la respuesta de células B tanto *in vivo* como *in vitro,* mientras los andrógenos y la progesterona deprimen la producción de anticuerpos. Las hormonas sexuales, además, modulan una gran cantidad de mecanismos implicados en la respuesta inmune, incluyendo la maduración y selección de timocitos, el tránsito celular, la producción de citocinas, la proliferación linfocitaria, la expresión y adhesión de moléculas y receptores del complejo mayor de histocompatibilidad clase II (Vargas-Villavicencio y Morales-Montor 2007). De acuerdo a estas observaciones, se sugiere que los estrógenos potencian la inmunidad mediada por células B y suprimen las condiciones dependientes de células T. La testosterona parece suprimir tanto la respuesta mediada por células T como la mediada por células B. Las hormonas sexuales pueden afectar al sistema inmune no sólo mediante efectos directos sobre células inmunocompetentes sino también indirectamente a través de cambios en el eje HPA: los estrógenos parecen incrementar, y los andrógenos disminuir la secreción de cortisol tanto en humanos como en animales de experimentación (Da Silva, 1999).

Uno de los factores de sistema inmune que influye sobre el eje HPG es la IL-6. Está proteína es una citocina multifuncional que regula varios aspectos de la respuesta inmune, reacciones de fase aguda y hematopoyesis. Normalmente está implicada en la regulación de la respuesta inmune humoral (Th 2), en infecciones provocadas por virus y bacterias y es una señal importante de “auxilio” que coordina las actividades de macrófagos, linfocitos y hepatocitos. Los efectos de

esta citocina en el sistema endócrino han sido extensamente demostrados: se ha comprobado que estimula la secreción de LH y FSH en células de hipófisis cultivadas; potencia la secreción de ACTH mediante la producción de CRH en el hipotálamo (Morales-Montor et al., 2001). Esta citocina también es producida por la hipófisis anterior y el hipotálamo. En algunos reportes, se ha demostrado que la IL-6 es un factor importante que afecta la actividad de la enzima encargada de transformar testosterona a estradiol: la P-450 aromatasa (Morales-Montor et al., 2001). También, existe evidencia de que en los humanos, la IL-6 incrementa los niveles plasmáticos de ACTH y cortisol sugiriendo que esta citocina tiene un papel regulador importante entre el sistema inmune y el eje HPA (Späth-Schwalbe et al., 1994).

1.4 Cisticercosis murina



La cisticercosis experimental murina inducida por inoculación intraperitoneal de los metacéstodos (cisticercos) de la *Taenia crassiceps* (ver foto superior izquierda) es un modelo experimental muy útil para estudiar diferentes aspectos de la relación hospedero-parásito, como por ejemplo, los factores de resistencia y susceptibilidad asociados al sexo; o bien los cambios endócrinos que ocurren en el hospedero durante la infección (Larralde et al., 1995). Es además, una alternativa excelente para estudiar otros factores biológicos que modulan el progreso de la infección, tales como el fondo genético asociado al complejo mayor de histocompatibilidad (MHC), la modulación de los mecanismos inmunes humoral y celular, así como la naturaleza de la inflamación y las células inmunocompetentes (Schiutto et al., 1991; Huerta et al., 1992; Bojail et al., 1993; Terrazas et al., 1998). Esta parasitosis, es semejante en muchos aspectos a la cisticercosis causada por los metacéstodos de la *Taenia solium*, el parásito que invade al hombre y al cerdo, y que representa un severo problema económico y de salud pública en países de América Latina, Asia y África. Como en otras parasitosis, el cisticerco de la *T.*

crassiceps causa una infección de naturaleza crónica, durante la cual, el parásito se multiplica constantemente sin afectar, aparentemente, la sobrevivencia del hospedero. Por lo tanto, la relación del cisticerco con su hospedero implica la interacción con diversos sistemas orgánicos, que da como resultado una relación equilibrada entre ambos organismos, dada la respuesta inmune reglamentaria del hospedero.

La cisticercosis murina está asociada a tres tipos de factores biológicos del hospedero: factores genéticos, gonadales e inmunitarios (Sciutto et al., 1991). Dentro de los factores genéticos, se ha demostrado que la susceptibilidad está asociada a la cepa de ratón, siendo la más susceptible a la cisticercosis la cepa BALB/cAnN mientras que la cepa BALB/cJ es altamente resistente. La susceptibilidad a la infección se asocia al locus del complejo mayor de histocompatibilidad (H-2), ya que cepas con el mismo fondo genético (BALB), pero con diferencias en el locus H-2, presentan diferencias en las cargas parasitarias, siendo la más susceptible la BALB/c H-2d (AnN). Otro factor que se relaciona con la resistencia es la expresión de un gen que codifica para una proteína del complejo mayor de histocompatibilidad (MHC): el antígeno Qa-2, ya que la cepa BALB/cAnN (susceptible) es Qa-2 negativa mientras que la cepa BALB/cJ (resistente) expresa la proteína Qa-2 (Fragoso et al., 1998). Sin importar la cepa de ratón utilizada, ni el locus del H-2 presente, las hembras siempre son más susceptibles que los machos a la infección aguda, lo que indica un fuerte control de la infección por factores gonadales (Sciutto et al., 1991).

Durante las primeras 4 semanas de infección (aguda) los machos y las hembras, de la cepa susceptible (BALB/cAnN), presentan una importante y bien marcada susceptibilidad asociada al sexo: las hembras presentan cargas parasitarias 4 veces mayores que los machos. Sin embargo, durante la infección crónica (más de 4 semanas) el parásito logra vencer la resistencia inicial del macho y termina por parasitarlo de la misma manera que a las hembras. Estos cambios coinciden con un efecto en los ratones macho de estrogenización y desandrogenización (Morales et al., 1996). Este efecto está mediado por las gónadas (en donde se lleva a cabo la producción mayor de testosterona) ya que al gonadectomizar y

posteriormente infectar a ratones macho, no se detectan los niveles de estradiol previamente reportados, y si los animales son tratados con testosterona o dehidrotestosterona, las cargas parasitarias disminuyen en un 50 % (Larralde et al., 1995). Además, este cambio en el microambiente hormonal del macho, afecta otras funciones del hospedero, ya que, conforme avanza el período de infección, los machos con cisticercosis pierden la respuesta de eyaculación, posteriormente, la conducta de intromisión y, finalmente, la conducta de monta. Esta conducta sexual queda totalmente restaurada al inyectar testosterona o dehidrotestosterona (Morales et al., 1996). El análisis histológico muestra que el tracto reproductivo de los ratones crónicamente infectados está completamente atrofiado mostrando una fuerte respuesta inflamatoria local (Morales-Montor et al., 1999a; Morales-Montor et al., 1999b). Al mismo tiempo, la expresión de la enzima P-450 aromatasa, encargada de metabolizar testosterona a estradiol, se incrementa en el testículo de los ratones macho crónicamente parasitados al mismo nivel de las hembras, y además existe una disminución de la expresión de la enzima 5α-reductasa de esteroides tipo II, sin que se afecte la expresión de la 20,22 desmolasa.

Por otro lado, recientemente se ha encontrado que en hembras preñadas (tanto en cerdas como en ratones), la infección se desarrolla de manera muy marcada (Morales et al., 2006). Esto sugiere que algunos factores biológicos producidos durante el embarazo (regulación de la respuesta inmunológica a través de la alta concentración de hormonas propias del embarazo, como la progesterona) pueden afectar el establecimiento o velocidad de reproducción del parásito (Morales et al., 2006), encontrando por lo menos dos mecanismos, ya sea afectando la respuesta inmune específica o sirviendo como factores positivos de crecimiento para el cisticerco. A pesar de ello, es necesario investigar si el efecto de la progesterona (por ser la hormona fundamental del embarazo) es directo, o si es transformada a sus metabolitos principales como son estradiol, androstendiona o estrona, o ambas (hormonas gonadales).

Además, previamente se estudió el efecto de los esteroides sexuales en la infección por *T. crassiceps* en ratones. Los estrógenos, como el 17β-estradiol, promueven la reproducción del parásito, mientras que los andrógenos como la

testosterona y la dehidrotestosterona tienen efectos contrarios, es decir, abaten la infección. Dado que a la fecha no han sido estudiados los andrógenos adrenales en este tipo de infección y que además, han tenido efectos principalmente en eliminación de parásitos, en este trabajo se estudió el papel biológico de la progesterona y la DHEA en la infección y sus posibles mecanismos de acción e interacción con el sistema inmune.

RESUMEN

Interacciones inmuno-endócrinas durante la cisticercosis murina: el papel de la progesterona y la DHEA.

Algunos estudios previos han demostrado que durante el embarazo en cerdos, la prevalencia de la cisticercosis por *Taenia solium* aumenta (número de cerdas infectadas) comparado con las hembras no embarazadas. En la cisticercosis murina por *Taenia crassiceps* ocurrió un efecto similar, pues se encontró que durante el embarazo el número de parásitos es el triple, comparado con hembras no embarazadas. Estos resultados sugirieron que las hormonas producidas durante el embarazo pueden afectar de manera específica el establecimiento y crecimiento del cisticerco, tanto de la *T. solium* en el cerdo, como de la *T. crassiceps* en el ratón. Con la finalidad de estudiar el efecto proliferativo del parásito asociado al embarazo, se investigó el papel que tuvo la progesterona (P_4) (por ser una de las hormonas principales en el embarazo) durante la infección experimental murina por *Taenia crassiceps.*

Se trataron ratones de la cepa BALB/c de ambos sexos con implantes subdermales de liberación prolongada de P_4. Posteriormente, fueron infectados con cisticercos de *T. crassiceps* en la cavidad peritoneal . Al cabo de 8 semanas, los animales fueron sacrificados, recuperando y cuantificando los parásitos encontrados. Además, se extrajo el bazo en donde se cuantificó el perfil de citocinas : tipo Th1, caracterizada por la secreción de IL-2 e IFN-γ (protectora contra la infección) y Th2, caracterizada por la secreción de IL-4 e IL-10 (inocua contra la infección) y también la expresión de los receptores para P_4 y estradiol (E_2). Aunado a esto, se extrajeron muestras de sangre en donde se cuantificaron los niveles séricos de progesterona y estradiol. Los resultados obtenidos demostraron un aumento de la carga parasitaria en animales tratados con P_4; así mismo, el perfil de citocinas encontrado fue Th2. También, se encontró incrementada la expresión del receptor para progesterona en las células de bazo de animales infectados y tratados con P_4, comparada con la de los controles. Por otro lado, al cuantificar los niveles hormonales de los animales infectados y

tratados con P_4, se demostró que en éstos se presentaban mayores concentraciones de estradiol, comparados con los controles. Estos resultados sugirieron la posibilidad de que la progesterona exógena pudiera metabolizarse a estradiol, y este último ser causante del efecto sobre proliferación del parásito. Se realizó un segundo experimento utilizando ratones de ambos sexos, gonadectomizados (eliminando la fuente principal de hormonas sexuales), estos fueron infectados y tratados con P_4 (se utilizó la misma estrategia experimental). Los resultados demostraron que los ratones de ambos sexos, tratados con P_4 y sin gónadas, hubo una protección contra la infección al 100%. El perfil inmunológico presentado en estos, fue Th1 (inmunidad que protege contra la cisticercosis). Por otro lado, este grupo infectado, tratado con P_4 y sin gónadas no presentó concentraciones cuantificables de estradiol. Sin embargo, al cuantificar los niveles de hormonas androgénicas (hormonas asociadas a la protección contra la infección), presentaron concentraciones mayores de DHEA en comparación con los ratones gonadectomizados no infectados. Estos nuevos resultados sugirieron la posibilidad de que la P_4 pudiera metabolizarse, a través de las glándulas suprarrenales, hacia el andrógeno DHEA permitiendo la inhibición de la reproducción parasitaria. Por lo tanto, se diseñaron los experimentos necesarios para determinar si la DHEA tendría un efecto inhibitorio en la carga parasitaria. Para este fin, se infectaron ratones de ambos sexos, a los cuales se les administraron periódicamente concentraciones farmacológicas de DHEA. Los resultados obtenidos demuestran que la DHEA *in vivo* tuvo un efecto cisticida, el efecto protector de la DHEA es similar al de los animales infectados, tratados con P_4 y gonadectomizados. Lo que nos sugiere que la P_4 en animales sin gónadas, se metabolizó a DHEA, siendo esta la hormona importante en la disminución de la carga parasitaria. Se realizaron otros experimentos para evaluar el efecto directo de las dos hormonas (P_4 y DHEA) sobre la cisticercosis. Los resultados demostraron que *in vitro,* la P_4 tuvo un efecto positivo en la reproducción del parásito, mientras que la DHEA tuvo el efecto contrario: disminuye en un 100% la reproducción del parásito. En conclusión, los resultados obtenidos sugieren un

efecto dicotómico de la P_4: por una parte, pudiera estar siendo bio-convertido a estradiol cuando el hospedero tiene gónadas, lo que provoca un efecto negativo para el hospedero (aumenta la carga parasitaria); y por el otro, en ausencia de gónadas, la P_4 es metabolizada en las glándulas suprarrenales, hacía DHEA, y así, esta hormona tiene un efecto protector para el hospedero. Este estudio apoya la existencia de mecanismos de comunicación bidireccional entre los sistemas neuroinmunoendócrino del hospedero y los propios del parásito, que regulan el establecimiento, crecimiento y reproducción del parásito. Estos mecanismos son de gran importancia, ya que integran una red compleja de interacciones neuro-inmuno-endócrinas hospedero-parásito, y tienen implicaciones importantes en los procesos co-evolutivos del hospedero y el parásito, así cómo en la preservación de las especies.

II. Hipótesis

La progesterona afecta positivamente el crecimiento, reproducción y establecimiento del cisticerco de la *Taenia crassiceps* en su hospedero murino, mientras que la DHEA afecta, en los mismos parámetros, negativamente.

III. Objetivo General

Determinar cómo la progesterona y la DHEA afectan el establecimiento, crecimiento y reproducción del cisticerco de la *Taenia crassiceps* en su hospedero murino.

IV. Objetivos Particulares

1) Determinar el efecto de la progesterona y la DHEA sobre el crecimiento, reproducción y establecimiento del parásito en ambos sexos del hospedero murino.
2) Determinar sí el efecto de la progesterona y la DHEA, están dado por la modulación de la respuesta inmunológica específica contra el parásito.
3) Esclarecer si el efecto de la progesterona y la DHEA se producen por una modulación diferencial de los receptores a hormonas esteroides en los linfocitos del hospedero.
4) Establecer si el efecto de la progesterona y DHEA, se produce de manera directa sobre el parásito, evaluando su efecto *in vitro* en cultivos del cisticerco.

V. Materiales y Métodos

5.1 Material Biológico

Todos ratones de ambos sexos de 4-6 semanas de edad de la cepa BALB/cAnN fueron alimentados con Purina Diet 5015 y agua *ad libitum.* El cuidado de los animales y las prácticas experimentales se realizó en el Instituto de Investigaciones Biomédicas bajo las normas internacionales del cuidado y manejo de animales de experimentación.

5.2 Parásitos e infección experimental

La cepa ORF de *Taenia crassiceps* (que es de rápido crecimiento), aislada por Freeman en 1962 es la que se utilizó para infectar a los ratones. Los parásitos fueron extraídos de hembras BALB/cAnN donadoras, y se inocularon de manera intraperitoneal en el ratón. Se utilizaron 10 cisticercos (de alrededor de ≈2mm de diámetro), sin gemas, suspendidos en 0.3 ml de PBS estéril (0.15M NaCl, 0.01M buffer de fosfato de sodio, pH 7.2) utilizando una jeringa de 0.25 de diámetro (PLASTIPAK).

5.3 Tratamiento hormonal

Se llevaron a cabo tres experimentos en los cuales se describen a continuación:

A) con progesterona

A cada ratón seleccionado se le administró de manera intradermal y sobre los omóplatos (utilizando un Trocar de alta precisión) un implante de liberación prolongada de 5 mg de progesterona con duración de 8 semanas (Innovative Research of America). Otro grupo de animales fue tratado de la misma manera pero con implante placebo (implante vehículo).

B) con DHEA

A cada ratón seleccionado, se le administró una concentración farmacológica (0.5 mg/ masa/kg) de DHEA, ésta fue inyectada de manera subdermal 3 veces a la semana. Otro grupo de ratones se le administró una solución placebo (aceite mineral) de la misma manera que los animales tratados con DHEA.

5.4 Gonadectomías

A cada grupo seleccionado les fueron extraídas las gónadas (testículos y ovarios) por cirugía. Previamente los ratones fueron anestesiados con una dosis de 1.26 mg de pentobarbital sódico (Pfizer) y suturados con hilo reabsorbible (aguja circular del número 4-0) (Atramat). Al final de la cirugía, los animales se mantuvieron aislados por 7 días de reposo para su recuperación.

5.6 Cuantificación parasitaria

Se inspeccionó la cavidad peritoneal y los cisticercos encontrados fueron recuperados y contados en cada ratón sacrificado. Previamente fue lavada vigorosamente (con PBS 1X) la cavidad peritoneal del ratón extrayendo el total de los cisticercos alojados.

5.7 Cuantificación de hormonas esteroides

El suero de los animales fue utilizado para la determinación de los esteroides. Éstos fueron concentrados empleando la técnica éter-acetona. A continuación las muestras fueron solubilizadas en solución amortiguadora de RIA.

Se cuantificaron los niveles de progesterona, estradiol, testosterona, DHEA utilizando KITs de ELISA (D.S. Labs) siguiendo las instrucciones del fabricante para cada kit; posteriormente, las muestras fueron analizadas en un lector de ELISA a 450 nm. Los datos obtenidos se ajustaron a una regresión lineal (para obtener la concentración de cada hormona) utilizando el paquete estadístico Prisma.

5.8 Análisis estadístico

Todos los datos fueron analizados empleando un análisis de varianza comparando las diferencias de las medias de cada grupo tratado y control. El software utilizado para comparar el valor estadístico fue Prisma (GraphPad Software Incorporated).

5.8 RT-PCR de Bazo

5.8.1 Extracción de bazo y gónadas

En condiciones estériles fueron extraídos (por cirugía) los tejidos de cada ratón correspondiente (bazo y gónadas). Posteriormente, cada muestra fue colocada de manera individual en tubos con tapa eppendorf de 2ml con 1 ml de TRIZOL. Al final fueron colocadas a una temperatura de -70°C.

5.8.2 Obtención y cuantificación del RNA

A cada muestra se le extrajo el RNA (utilizando la técnica fenol-cloroformo) siguiendo la instrucción del fabricante (Invitrogen).

Para determinar la cantidad de RNA obtenida en cada muestra, se obtuvo una alícuota y ésta fue leída en un espectroscopio (GeneQuant) utilizando a una densidad óptica de 260 y 280 nm.

Posteriormente, con la formula general (RNA = ABS_{260}X 1 D. O. X dilución =ng/μl) utilizado en el software del espectroscópico, se obtuvo la concentración de cada muestra.

5.8.3 Obtención de cDNA

El RNA obtenido (de todas las muestras) fue transformado a cDNA utilizando la técnica RT o retro-trascripción (Invitrogen) utilizando un KIt y siguiendo el instructivo del fabricante.

5.8.4 Determinación de la expresión de las interleucinas y los receptores hormonales

Los distintos genes (ver el apartado 5.8.5) fueron amplificados por PCR de cDNA utilizando el Kit (biotecnologías universitarias) y siguiendo el instructivo del fabricante.

Los oligos (cebadores) empleados fueron diseñados a partir de las secuencias evolutivas más conservadas entre los mamíferos (rata, ratón, humano). (Ver el apartado 5.8.5)

5.8.5 Estandarización de la temp. y ciclos de los cebadores utilizados:

Abreviatura	Nombre	Secuencia	TM_c, ciclos y pares de bases
RP-A	Receptor a progesterona A	**SENSE 5`** CAG TGG TGG ATT TCA TCC ATG **3`** **ANTSENSE 5`** CTT CCA GAG GGT AGG TGC AG **3`**	60°C 25 ciclos 198 pares de b.
RP-B	Receptor a progesterona B	**SENSE 5** GGA GGC AGA AAT TCC AGA CC **3`** **ANTSENSE 5** GAC AAC AAC CCT TTG GTA GC **3`**	60°C 25 ciclos 197 pares de b.
ER-α	Receptor a estrógenos alfa	**SENSE 5`** AGA CTG TCC AGC AGT AAC GAG **3`** **ANTISENSE 5`** TCG TAA CAC TTG CGC AGC CG **3`**	58.8°C 35 ciclos 251 pares de b.
ER-β	Receptor a estrógenos beta	**SENSE 5`** CAT CTG GGT ATC ATT ACG GTG **3`** **ANTSENSE 5`** GGC ACT TCT CTG TCT TCG TAC **3`**	60°C 30 ciclos 239 pares de b.
β–actin	β–actina	**SENSE 5`** GGG TCA GAA GGA CTC CTA TG **3`** **ANTSENSE 5`** GGT CTC AAA CAT GAT CTG GG**3`**	<62^{o}C 20-35 ciclos 238 pares de b.
IL-2	Interleucina 2	**SENSE 5`** TGA TGG ACC TAC AGG AGC TCC TGA T **3`** **ANTSENSE 5`** GAG TCA AAT CCA GAA CAT GCC GCA G **3`**	38^{o}C 63 ciclos 168 pares de b.
IL-4	Interleucina 4	**SENSE 5`** CGA AGA ACA CCA CAG AGA GTG AGC T **3`** **ANTISENSE 5`** GACTCATTCATGGTG CAGCTTATCG **3`**	68°C 35 ciclos 181 pares de b.
IL-6	Interleucina 6	**SENSE 5`** ATG AAG TTC CTC TCT GCA AGA G **3`** **ANTISENSE 5`** CAC TAG GTT TGC CGA GTA GAT **3`**	62°C 30 ciclos 638 pares de b.
IL-10	Interleucina 10	**SENSE 5`** ACC TGG TAG AAG TGA TGC CCC AGG CA **3`** **ANTISENSE 5`** CTA TGC AGT TGA TGA AGA TGT CAA A **3`**	35^{o}C 63 ciclos 237 pares de b.
TNF-α	Factor de necrosis tumoral alfa	**SENSE 5`** GGC AGG TCT ACT TTG GAG TCA TTG C **3`** **ANTISENSE 5`** ACA TTC GAG GCT CCA GTG AAT TCG G **3`**	63°C 30 ciclos 300 pares de b.
IFN γ	Interferón gamma	**SENSE 5`** AGC GGC TGA CTG AAC TCA GAT TGT AG **3`** **ANTISENSE 5`** GTC ACA GTT TTC AGC TGT ATA GGG **3`**	30°C 60 ciclos 247 pares de b

5.9 Estudios *in Vitro*

5.9.1 Preparación del medio de cultivo

El medio de cultivo utilizado fue: AIM-V libre de hormona (Invitrogen) suplementado de la siguiente forma y sin suero bovino fetal:

Porcentaje	Reactivos
5	HEPES.
2	aa no esenciales.
2	Penicilina / estrepto.
2	L-glutamina.

5.9.2 Cultivo *in Vitro*

Con el objeto de observar el efecto de la progesterona y la DHEA *in Vitro* sobre el crecimiento y reproducción de los parásitos, éstos fueron cultivados en destinas concentraciones hormonales (el estradiol fue utilizado como control positivo para comparar con las demás hormonas):

DHEA	Progesterona y estradiol
0 μg/ml	0 μg/ml
1 μg/ml	1 μg/ml
2 μg/ml	2 μg/ml
4 μg/ml	4 μg/ml

Como dato adicional, en este experimento se utilizaron hormonas solubles en agua (Sigma) y se registraron observaciones diarias de motilidad, número de gemas, número de parásitos y tamaño de los mismos (utilizando un microscopio invertido a 40x y 100x de aumento).

VI. RESULTADOS

Cargas parasitaria

El primer objetivo fue determinar sí la progesterona y la DHEA pueden afectar el establecimiento, y reproducción *in vivo*, del cisticerco de la *Taenia crassiceps* en el hospedero murino (interacción hospedero-parasito); para lo cuál se realizaron los experimentos expuestos en el anexo A1, descrito a continuación:

En la figura 1 del anexo A1 se muestran los datos individuales de las cargas parasitarias obtenidas en los ratones infectados de ambos sexos y tratados con la progesterona. A pesar de que hubo una variación individual en el número de parásitos, el análisis estadístico mostró diferencias entre cada uno de los tratamientos. En los ratones controles (C), las hembras (♀) fueron más susceptibles a la infección (**<P; 0.01) que los machos (♂). El tratamiento con progesterona, en los ♂, triplicó el número de parásitos (a partir el 35.8 del ± 16.3 a 122.1 el ± 17.3), mientras que en las ♀ fue menor, puesto que la carga parasitaria sólo aumentó en aproximadamente dos veces (de 247.1 del ± 97.6 a 394.9 el ± 84.7) (** < P; 0.01), comparados con el grupo control (el tratamiento con el placebo no tuvo efecto), ver la figura 1 del anexo A1.

Un segundo experimento similar al anterior se realizó. En este, se eliminó la fuente natural de hormonas sexuales, las gónadas (GX); posteriormente, se realizó la misma estrategia experimental. (Ver figura 2 del anexo A2). Los resultados mostraron una disminución en la carga parasitaria en animales de ambos sexos GX. El efecto de la gonadectomía igualó el número de parásitos entre las ♀ y los ♂ infectados con gónadas. Además, todos los ratones de ambos sexos GX, infectados y tratados con progesterona tuvieron cero parásitos (figura 2 del anexo A2).

Un tercer experimento fue realizado. En este, fueron infectados un grupo de ratones completos (con gónadas). Posteriormente, se les administró una concentración fisiológica de DHEA. En el anexo A3 se observó que el tratamiento con la DHEA en la infección, la carga parasitaria disminuyó un 50% en ♀

(***P<0.001) y un 40% en los ♂ (***p<0.001) con respecto a los ratones controles (animales infectados sin tratamiento con la DHEA). El placebo no tuvo efecto.

Perfil inmunológico

El segundo objetivo fue determinar sí el efecto de la progesterona y la DHEA en la interacción hospedero-parasito es modulado por una respuesta inmunológica específica en contra o a favor del parásito. Puesto que la infección por *T. crassiceps* está bajo una regulación inmunológica, en donde la inmunidad de tipo Th1 (IL-2, TNF-α e IFN-γ) le confiere protección y la de tipo Th-2 (IL-4, IL-6 e IL-10) le confiere susceptibilidad; se decidió cuantificar el perfil inmunológico Th1 y Th-2 (dejando aun lado la respuesta inmune innata por no ser crucial para la infección) en los ratones de ambos sexos, infectados y tratados con progesterona.

De las muestras del primer experimento en donde reportamos un número mayor de parásitos en los animales infectados y tratados con progesterona (figura 1 de anexo A1), se cuantificó la respuesta inmune protectora contra la cisticercosis murina (Th-1). La figura 4 del anexo A1 se muestra que la expresión de IL-2 fue mínima en todos los tratamientos (ND), por lo que no hubo diferencia significativa en ningún tratamiento ni en ambos sexos. Por otra parte, se observó una sobre-expresión de IFN-γ y TNF-α en los ratones infectados y tratados con progesterona, comparados con los ratones no infectados. Para el caso de la inmunidad Th-2 (ver la figura 3 del anexo A1), la expresión de las interleucinas 4, 6, 10 (perfil Th-2), fueron incrementadas tanto en las hembras como en los machos en respuesta a la infección, comparada con las hembras no infectadas. El tratamiento con la progesterona no modificó el perfil inmunológico de Th2, la expresión de estas citocinas fueron similares a los niveles encontrados en los animales no infectados. El placebo (o vehículo) no tuvo efecto.

De las muestras del segundo experimento que se describió en: “la carga parasitaria”, se realizó un perfil de citocinas, tanto para Th1 como para Th2. En la figura 4 del anexo A2 se muestran los datos obtenidos para el tipo de respuesta inmune celular Th1. La expresión de IL-2 fue detectada en los animales GX, mientras que en los animales completos no fue así (figura 4 del anexo A2). Tanto

la infección como el tratamiento placebo no afectaron la expresión de citocinas Th1 en ambos sexos. Para el caso de la inmunidad humoral Th2, la gonadectomía disminuyó marcadamente la expresión tanto IL-4 como de IL-10 en ambos sexos, comparada con los animales controles (con gónadas). El tratamiento con la progesterona en los ratones infectados de ambos sexos no afectó el patrón de expresión de estas citocinas Th2. Los grupos controles (vehículo) se comportaron de manera semejante al grupo infectado.

Debido a que en el tercer experimento (tratamiento con la DHEA) obtuvimos un menor número de parásitos, se decidió ver sí el efecto protector de la DHEA estuvo asociado a un aumento de la respuesta inmune celular específica (Th-1) o, a una disminución de la respuesta inmune humoral e inócua para esta infección (Th2). La figura 3 del anexo A3 se muestra los datos obtenidos del perfil de citocinas características para el tipo Th1 y Th2. Para el caso del perfil de citocinas Th1, los ratones no infectados y tratados con la DHEA expresaron niveles más altos de IL-2 (las ♀ 3 veces y los ♂ 2) comparados con los ratones no infectados e infectados no tratados (figura 3 de anexo A3). Los datos muestran la existencia de un dimorfismo sexual en la expresión IL-2, ya que las ♀ no infectados expresaron más IL-2 que los ♂. Los ratones infectados y tratados con la DHEA presentaron una expresión de IL-2 similares a los grupos tratados con la DHEA no infectados. Con respecto a la expresión de IFN-γ, los datos no mostraron diferencias estadísticas entre los grupos analizados. El tratamiento con el vehículo no tuvo ningún efecto. Para el caso del perfil de citocinas Th2, no hubo diferencia en los animales infectados y tratados con la DHEA tanto en la expresión de IL-4 como en la IL-10. El tratamiento con el vehículo no tuvo efecto comparado con el grupo infectado.

Perfil endocrinológico

El tercer objetivo fue el esclarecer sí el efecto de la progesterona y la DHEA se producen por una modulación diferencial de los receptores a hormonas esteroideas en los linfocitos del hospedero. Debido a que los esteroides sexuales pudieran afectar las funciones del sistema inmune y por consecuencia la

respuesta hacia la eliminación o no de la infección; decidimos determinar la expresión, por RT-PCR, de los receptores a hormonas sexuales en bazo.

Se utilizaron las muestras del primer experimento descrito tanto en la carga parasitaria como el perfil inmunológico, se determinó la expresión del mensajero de los receptores de estrógenos en sus dos tipos (α y β) y del receptor de progesterona (A, B). En la figura 6 del anexo A1, se muestra la expresión relativa de ambos tipos del receptor (ER-α y ER-β), el cual, se incrementó en los esplenocitos de ratones infectados en ambos sexos; por el contrario, el tratamiento con progesterona no modificó este patrón de expresión. Para el caso del receptor de progesterona (PR) tipo A, (figura 5 del anexo A1) la expresión mostró diferencias entre los distintos sexos, ya que en los ratones infectados y tratados con progesterona, las ♀ tuvieron mayor expresión del receptor que los ♂. Este patrón fue similar en los animales no infectados. Para el caso del PR-B la expresión se incrementó en animales infectados de ambos sexos ($P < 0.01$), comparado con sus controles; los animales infectados y tratados con placebo no tuvieron diferencias, comparado con los animales infectados. Por otra parte, la expresión del RP-B disminuyó en animales infectados y tratados con progesterona, mientras que en las hembras este efecto fue opuesto, mostrando un incremento de la expresión del receptor en los animales infectados y tratados con progesterona. En cuanto a la cuantificación hormonal (ver figura 2 del anexo A1), los niveles de progesterona disminuyeron un 50% en las ♀ infectadas (2.39 ± 1.07, comparado con animales no infectados (5.20 ± 1.37), En los ♂ controles e infectados (0.18 ± 0.02 ng/ml y 0.16 ± 0.05 ng/ml respectivamente) no hubo cambios en los niveles de progesterona, en ambos casos tuvieron bajas concentraciones circulantes de ésta. Los ratones infectados y tratados (en ambos sexos) con progesterona, se incrementaron los niveles séricos hasta alcanzar valores cercanos a las ♀ no infectadas, en ambos sexos (4.32 ± 1.33 en ♀ y 4.78 ± 1.11 en ♂) (figura 2 del anexo A1). Con respecto a la concentración de estradiol, los ♂ infectados presentaron niveles tan altos (26.85 ± 3.18) como las ♀ no infectadas (25.16 ± 5.46), mientras que las ♀ infectadas no mostraron cambios en los niveles de estradiol, comparadas con sus controles no infectados. Los

niveles de estradiol fueron incrementados (2 veces) en los animales infectados y tratados con progesterona tanto en las ♀ (56.15 ± 4.21) como en los ♂ (53.42 ± 6.14) comparados con los ratones infectados y tratados con el placebo. La ♀ no mostró cambios en los niveles séricos de testosterona. El tratamiento con progesterona no afectaron los niveles de testosterona en animales infectado de ambos sexos. Los grupos infectados y tratados con el placebo no tuvieron efectos significativos (figura 2 de anexo A1).

Para el caso del segundo experimento, (en donde se evaluó la GX) se amplificó, también, la expresión del receptor a hormonas esteroideas: progesterona y andrógenos. La figura 5 del anexo A2 muestra la expresión del mensajero del PR-A y del PR-B en los ratones ♂ y ♀ expuestos a diversos tratamientos. Para el caso del receptor a progesterona A, la gonadectomía aumentó (del doble) la expresión del receptor en los ratones ♀ y ♂ GX, comparados con los animales infectados y tratados con progesterona pero sin GX. Por otra parte, en los ratones ♂ GX infectados y tratados con progesterona, la expresión de PR-B fue sobreexpresada (figura 5 del anexo A2). La expresión del receptor de andrógenos (AR) fue mayor en los esplenocitos de los ♂ completos no infectados (***$P<0.01$), sin embargo, el mRNA del AR también fue expresado en ♀ aunque en menor grado. La GX y la infección en ambos sexos disminuyeron la expresión de AR en los ratones ♂ un 50%, mientras que el patrón de expresión de AR en ♀ no tuvo efecto. Los ratones GX e infectados de ambos sexos, así como los ratones infectados GX y tratados con progesterona, mostraron un patrón similar al grupo GX no infectado (figura 6 del anexo A2). Los niveles de DHEA en los ratones GX infectados y tratados con progesterona aumentaron tres veces con respecto a los ratones GX no infectados y a los ratones GX infectados (figura 2 del anexo A2). La concentración de progesterona en los animales GX fue semejante a los grupos intactos (con gónadas).

Para correlacionar el tercer experimento hecho anteriormente, en donde se evaluó el papel de DHEA, se decidió realizar una curva de concentración sérica de DHEA en ratones infectados y controles durante las primeras 16 semanas de infección. La figura 2 del anexo A3 se muestra que conforme avanza el tiempo de infección,

la concentración de DHEA se ve disminuida; siendo en la semana 16, cuando la concentración sérica de DHEA, presenta una disminución del 50% comparado con el grupo control sin infección. Las ♀ controles tuvieron mayores concentraciones séricas de DHEA que los ♂ del mismo grupo. Por otra parte, la expresión del mensajero del AR (figura 4 del anexo A3) se incrementó 2 veces en los ratones de ambos sexos infectados y tratados con dicha hormona, comparados con los controles no infectados, infectados y vehículo (en ambos sexos). Además, el nivel de expresión del AR fue semejante a los obtenidos en tejidos endócrinos como los testículos o glándulas seminales, los cualas fueron utilizados como control positivo, (datos no mostrados).

Cultivo in vitro

La siguiente figura (a) corresponde al número de gemas (una medida indirecta para cuantificar la reproducción) promedio durante 10 días de cultivo.

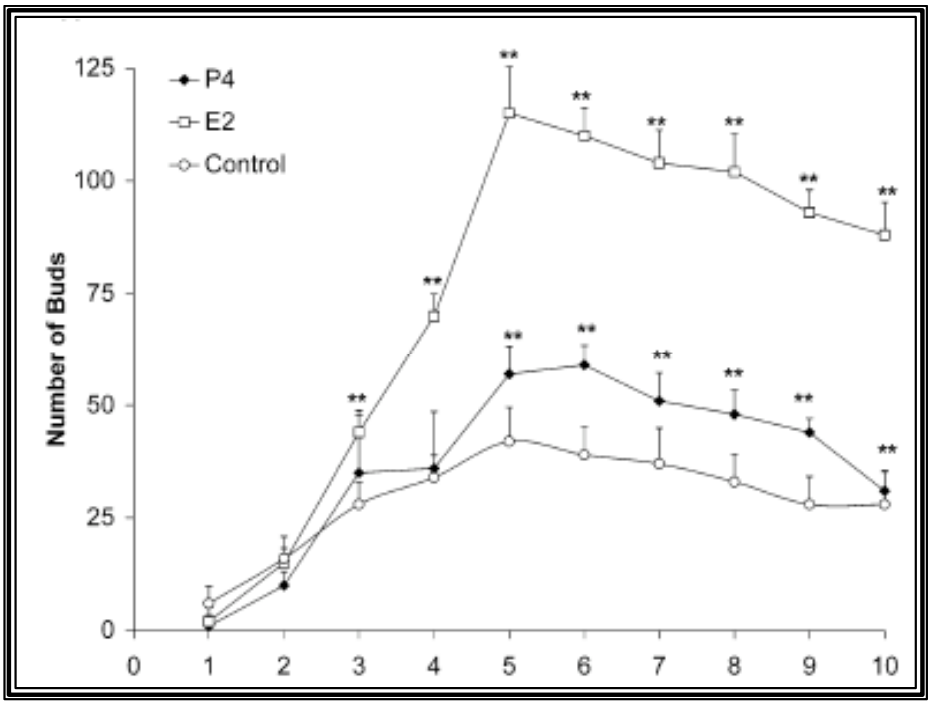
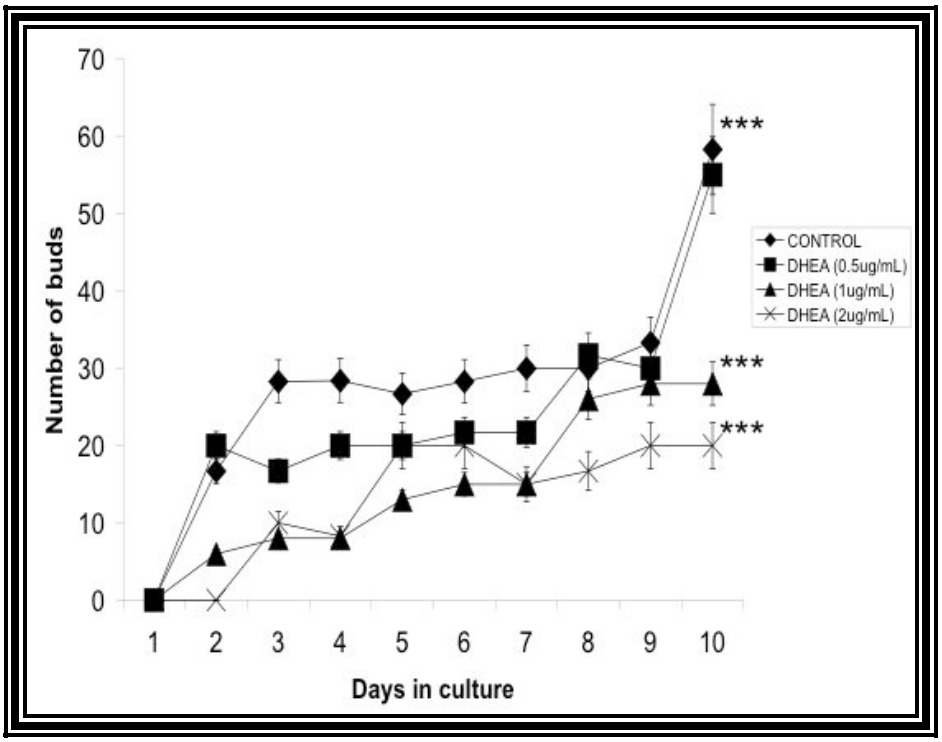


Figura a. Curva temporal en donde se observa el número de gemas los cisticercos de la *T. crassiceps* después de la exposición al estradiol y a la progesterona. Cada punto representa el promedio ± desviación estándar de dos experimentos hechos en distintos tiempos (n = 5). $P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. La concentración de hormonas es como sigue: E2, 40ug/ml, P4, 20ug/ml.

Los resultados anteriores muestran que la progesterona aumentó el número de gemas del parásito, hasta alcanzar un aumento de 65% en 5 días después de

cultivo. Posteriormente, el tratamiento con P4 no modificó la viabilidad de los cisticercos durante los 10 días del estudio (gráfica a). Otro experimento *in vitro* se realizaron para demostrar sí la DHEA puede afectar el parásito directamente. El tratamiento *in vitro* con DHEA en la cisticercosis tuvo efecto inhibitorio en el parásito, tanto en las dosis fisiológicas como farmacológicas. El tratamiento con DHEA afectó la reproducción de los cisticercos (cuantificación del número de gemas). El efecto resultó ser también dependiente del tiempo, ya que alcanzó su máximo a los 10 días de cultivo utilizando la dosis subletal (gráfica b).



GRÁFICA b. Curva de dosis respuesta y temporal de la reproducción de los cisticercos de la *T. crassiceps* después de la exposición a DHEA. Cada punto representa el promedio ± desviación estándar de dos experimentos diferentes (n = 5). P <0.05, **P < 0.01, ***P < 0.001.

VII DISCUSIÓN

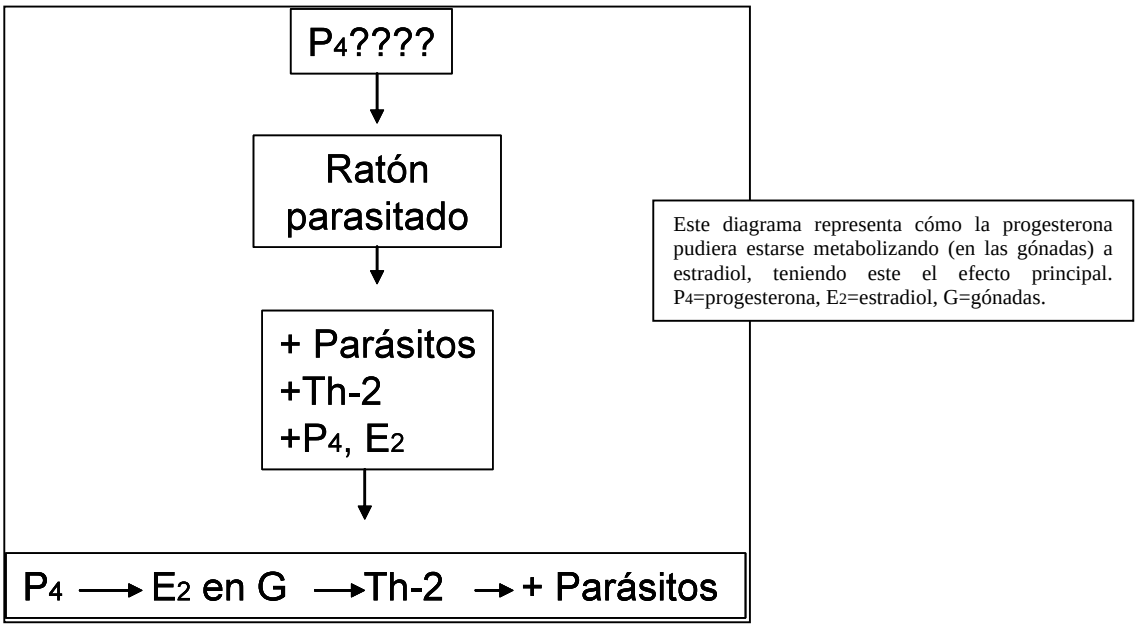
Previo los estudios del las hormonas sexuales en la infección por *T. crassiceps* en el hospedero murino, nosotros evaluamos el efecto de la progesterona en la infección.

1) En los experimentos iniciales, el tratamiento con la progesterona en animales completos (con gónadas) e infectados, tuvieron un aumento en la carga parasitaria. Por otro lado, un dato interesante en este experimento fueron los niveles séricos de la progesterona, al ser cuantificados, mostraron que la concentración de ésta no era tan alta como se esperaría (puesto que nosotros suministramos concentraciones farmacológicas). Este hecho nos resultó interesante, por lo que se planteó la posibilidad de la bio-conversión de la progesterona en otra hormona. Esta interrogante fue contestada al cuantificar otras hormonas como el estradiol y testosterona. Para el caso del estradiol, este fue mayor de lo esperado en los animales infectados y tratados con progesterona. La testosterona no tuvo efecto. Lo anterior sugirió la posibilidad de una conversión (en las gónadas) a estradiol. Sí suponemos que la progesterona se metabolise a estradiol, éste pudiera ser la causa de tener el efecto proliferativo (mayor número de parásitos en el hospedero) encontrado, y además un efecto negativo sobre el sistema inmune. Para probar sí este efecto (mayor concentración estradiol) podría cambiar el perfil del sistema inmune, se cuantificaron los niveles de citocinas, y se demostró que había una disminución importante de la respuesta inmune celular Th1 (IFN-γ e IL-2) en los ratones que presentaban concentraciones altas de estradiol (ratones infectados y tratados con progesterona de ambos sexos). Este estudio es complementario al reportado previamente para el tratamiento con estradiol y la testosterona en la cisticercosis murina. (Morales-Montor et al., 2001; Morales-Montor et al., 2002a; Morales-Montor et al 2002b).

Puesto que muchos mecanismos están presentes la regulación de las funciones del sistema inmune por los esteroides sexuales, decidíamos amplificar los receptores nucleares de progesterona y estradiol en el bazo de todos los animales tratados y observar sí la expresión es modulada por el tratamiento y/o por la infección. Los datos encontrados muestran que ambas isoformas del receptor a

progesterona (A y B), así como del receptor a estradiol (α y β) están presentes en los distintos tratamientos. Además, son regulados de manera semejante a los encontrados en el sistema endócrino. Con estos resultados podemos sugerir que las células inmunológicas pueden estar afectadas directamente por la regulación de la unión de la hormona con el receptor a la progesterona y al estradiol, esto implica la existencia de receptores nucleares específicos en tejido inmune.

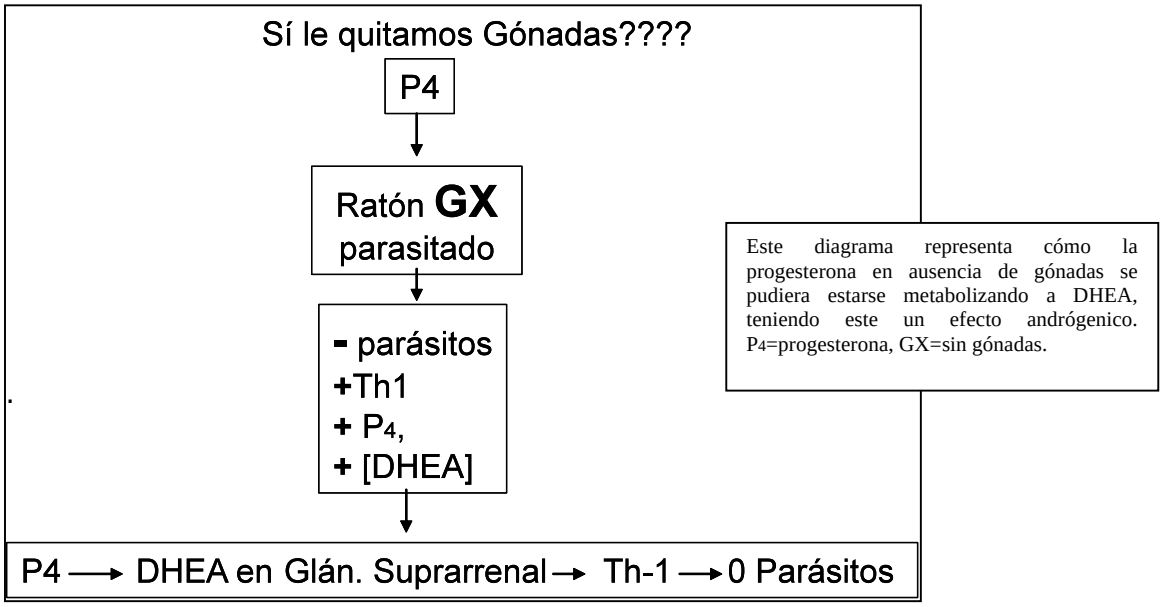
En resumen podemos observar el diagrama a continuación:



2) Previo al experimento anterior, se realizó otro experimento muy semejante pero en un ambiente endocrinológico más controlado, es decir, libre de esteroides sexuales (eliminación de gónada o GX). Nuestros resultados mostraron que los ratones ♂ y ♀ Gx tratados con progesterona estuvieron protegidos totalmente contra la infección, comparados con los ratones tratados con el vehículo. Estos resultados mostraron niveles de protección superiores a los que se reportaron previamente en la literatura, incluyendo la vacunación (Suitto et al., 1990). El efecto fue notable y consistente, ya que no se observó ninguna variación en el número de parásitos, aún cuando las muestras fueron procesadas de manera individual en cada experimento. Al igual que el experimento anterior, los niveles séricos de progesterona no eran tan altos como los esperados; sin embargo, a diferencia del experimento anterior (en donde la concentración de

estradiol presentaba un aumento), los niveles séricos de DHEA presentaron un aumento significativo. Este hecho apuntó a que la progesterona pudiera metabolizarse a DHEA (en glándulas suprarrenales) explicando (parcialmente) así, los resultados en la disminución de la carga parasitaria. El conocimiento de que los esteroides sexuales son uno de los factores biológicos más importantes en el hospedero y que pueden alterar el curso de las infecciones parasitarias ha sido previamente demostrado tanto en este modelo de infección como en la infección natural (Morales et al., 2006). Aunado a este experimento, recientemente se demostró que la GX en cerdos ♂ aumentaba la prevalencia a la infección por cisticercosis de *T solium* desde un 25% hasta el 50% (Morales et al., 2006). Esta observación, sugiere que los andrógenos juegan papel importante en la susceptibilidad a la infección tanto en la *T. solium* en cerdos como en la *T. crassiceps* en ratones. Por otra parte, un hallazgo muy importante fue el encontrar un expresión de IL-2 en las células de bazo de ♂ y ♀ GX y tratados con progesterona, a diferencia del experimento anterior (animales con gónadas). Estos resultados apoyan la idea de que la regulación hormonal actúa significativamente en el sistema inmune; debido a esto, se decidió amplificar los receptores clásicos de las hormonas esteroideas como la progesterona y los andrógenos en tejido inmunológico, bazo, de todos los animales tratados. Los resultados mostraron una expresión diferencial en ambos isoformas del receptor de la progesterona (A y B), así como también en el receptor de andrógeno. Interesantemente, se demostró que el tratamiento con la progesterona en los ratones infectados de ambos sexos; solamente una isoforma del receptor clásico de la progesterona (RP-B) fue regulada en el tejido inmunológico. Sin embargo, el receptor de andrógeno mostró una inhibición en los ratones ♂ GX e infectados, no así en las ♀ GX, sugiriendo una vez más, que la DHEA, producto del metabolismo de la progesterona, pudiera estar actuando en la infección, teniendo como resultado la diferencia en la carga parasitaria tanto en los tratados como en los no tratados con progesterona.

En resumen podemos observar el diagrama a continuación:



3) Debido al experimento anterior en donde sugerimos la conversión de progesterona a DHEA a través de las glándulas suprarrenales y, además, los hallazgos en la literatura, que han demostrado que ésta, puede afectar otras infecciones, como la eschistosomiasis humana, promoviendo la resistencia a ésta infección (Fulford et al., 1998), decidimos ver el efecto de esta hormona en la infección por *T crassiceps.* Nuestros experimentos reportaron una protección de los ratones infectados y tratados con DHEA. Además, el efecto protector de la DHEA ha sido demostrado en otras infecciones parasitarias. La DHEA administrada de manera exógena, incrementa los niveles de anticuerpos reduciendo la parasitemia de *T. cruzi* en ratas (dos Santos et al., 2005). En otro estudio, se encontró que la DHEA incrementó la actividad lítica de los macrófagos en ratones ♂ protegiendo contra *T. cruzi* (dos Santos et al., 2005). En otro parásito protozoario humano, *Criptosporidium parvuum* (criptosporidiosis); el tratamiento con DHEA en los ratones redujo significativamente el número de parásitos. Los ratones tratados con DHEA tuvieron un número mayor de células CD4+ y CD8+ en células de bazo, a diferencia de los ratones que no fueron tratados. Estos resultados sugirieron que la DHEA administrada de manera exógena induce una sobreregulación del sistema inmune que pudo ser clave en el tratamiento contra la criptosporidiosis (Rasmussen et al., 1993; Rasmussen et al., 1995). Debido a la

idea de que el mecanismo molecular por el cual los esteroides adrenales pudieran estar afectando la función de sistema inmune, y a que pudiera ser el mismo mecanismo por el que la DHEA actúa en tejido endócrino, es decir, mediado por receptor nuclear; se decidió amplificar el receptor a andrógenos (AR) en el bazo de todos los ratones tratados. En nuestros resultados se encontraron que la expresión de AR estaba inhibida en ratones infectados de ambos sexos. Sin embargo, el tratamiento con DHEA en ambos sexos mostró una sobre-regulación de la expresión, sugiriendo que la DHEA probablemente esta actuando a través de mecanismos que involucren un receptor nuclear en el sistema inmune. La ventaja para el hospedero del tener un receptor hormonal en el bazo es que tal vez pueda estimular al sistema inmune evitando así el establecimiento, y la reproducción del parásito. En resumen podemos observar el diagrama a continuación:

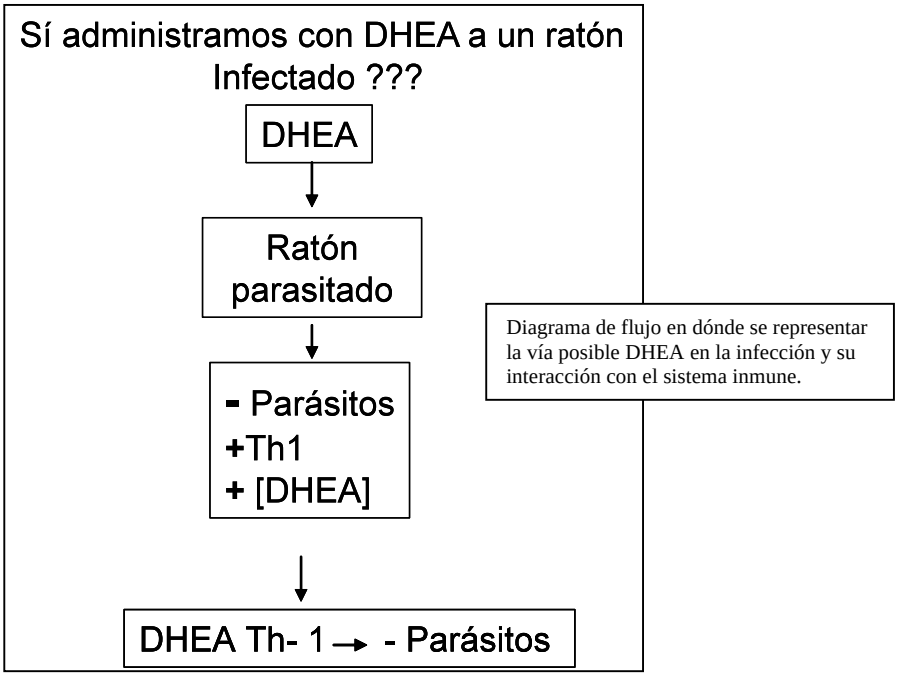


Diagrama de flujo en dónde se representar la vía posible DHEA en la infección y su interacción con el sistema inmune.

4) Con respecto a los efectos opuestos encontrados en los experimentos anteriores y a que se ha demostrado en otras infecciones, que el tratamiento con DHEA *in vitro* ejerce efectos inhibitorios de viabilidad, crecimiento y reproducción en contra del parásito (Morales-Montor et al., 2001); se decidió observar el efecto directo de las hormonas: progesterona y DHEA, en cultivos *in vitro* de cisticercos de la *T. crassiceps*. Nuestros resultados apuntan que la DHEA, *in Vitro,* inhibe importante la reproducción y la viabilidad de cisticerco de *T. crassiceps* (menor

numero de gemas). Esto nos sugiere la posibilidad de tener, en el parasito, receptores hormonales (probablemente nucleares) semejantes al mamífero hospedero. Lo anterior apoya la idea de una relación hospedero-parasito y su interacción directa y bidireccional compartiendo el entorno inmuno-endócrino en que se encuentran. A continuación se muestra, en resumen, lo antes mencionado.

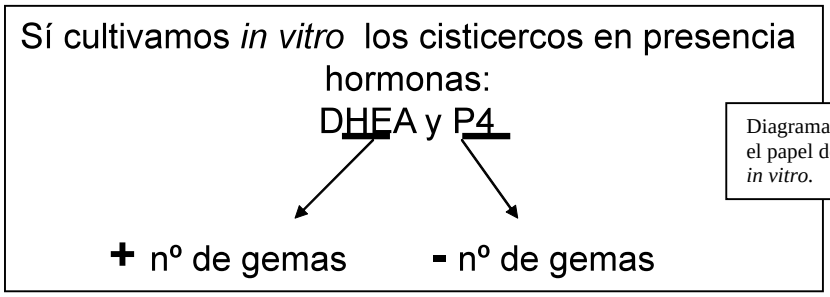


Diagrama de flujo en dónde se representar el papel de la Progesterona y la DHEA *in vitro.*

VIII. Conclusiones

Efecto de la progesterona en la infección

1) Los resultados anteriores muestran la importancia de considerar al sexo de cada hospedero para su estudio y representarlos de manera separada, ya que existen diferencias intrínsecas que los hace diferente entre ellos.
2) Los resultados demuestran que la progesterona sólo es un intermediario en la infección.
   A) Por un lado, la progesterona incrementa las cargas parasitarias, probablemente a que se metaboliza a estradiol y este tener el efecto principal en la infección.
   B) Por otro lado, en ausencia de las gónadas, la progesterona protege al 100% contra la infección, lo que sugiere que la progesterona podría metabolizarse a un andrógeno (DHEA) y este ser el causante del efecto observado en la infección.
3) El efecto de la progesterona involucra una polarización del sistema inmune dependiente de las hormonas sexuales o gónadas:
   - Para el caso de los animales completos, los datos indicaron un aumento en la inmunidad Th-2 (inmunidad inocua).
   - Para el caso de los animales GX, los datos revelaron un aumento en la inmunidad Th-1 (inmunidad protectora).
4) El efecto de la progesterona involucra una sistema endocrinos dual:
   - Para el caso de los animales completos, los datos indicaron un aumento en la expresión de receptores a estradiol y a la progesterona.
   - Para el caso de los animales GX, los datos revelaron un aumento en la expresión del receptor a andrógenos.
5) La progesterona de manera directa aumentó el crecimiento y reproducción del cisticerco de *T. crassiceps in vitro.*

Efecto de la DHEA en la infección

1. El tratamiento con DHEA disminuyó la carga parasitara.

2. El efecto de la DHEA pudiera estar involucrado un mecanismo endocrino.
3. El tratamiento con la DHEA, de manera directa, inhibe el crecimiento y reproducción del cisticerco de *T. crassiceps in Vitro.*

IX. Anexos (artículos publicados):

A1 Vargas Villavicencio JA., Larralde C, De Leon Nava M., Morales-Montor J. Regulation of the immune response to cestode infection by progesterone is due to its metabolism to estradiol. Microbes Infect. 2005;7(3):485-493.

A2 J.A. Vargas-Villavicencio, Carlos Larralde and Jorge Morales-Montor. Gonadectomy and progesterone treatment induce protection in murine cysticercosis. Parasite Immunol. 2006;28(12):667-74.

A3 J.A. Vargas-Villavicencio, and J. Morales-Montor. Treatment with dehydroepiandrosterone *in vivo* and *in vitro* inhibits reproduction, growth and viability of *Taenia crassiceps* metacestodes. Int J Parasitol. 2008;38:775–781.

A4 J.A. Vargas-Villavicencio, G. Escobedo, J. Morales-Montor: Tamoxifen treatment induces protection in murine cysticercosis. J Parasitol. 2007;93(6):1512–1517.

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A6 Peña N., Morales J., Morales-Montor J., Vargas-Villavicencio A., Fleury A., Zarco L., Aluja A. S de, Larralde C., Fragoso G., and Sciutto E. Impact of naturally acquired *Taenia solium* cysticercosis on the hormonal levels of free ranging rural boars. Vet Parasitol. 2007;149(1-2):134-137.

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Original article

Regulation of the immune response to cestode infection by progesterone is due to its metabolism to estradiol

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Abstract

The aim of this work was to investigate the role of progesterone during *Taenia crassiceps* cysticercosis, and the immunological mechanisms involved in its effects, by relating progesterone treatment to whole parasite counts, to host humoral and cellular immune response, to the presence or absence of nuclear receptors to sex steroids in splenocytes, and to serum sex steroid levels in infected mice of both genders. Progesterone treatment increased parasite loads two-fold in females and three-fold in males compared with control mice. The expression of the Th2 cytokine profile (IL-4, IL-6 and IL-10) was markedly increased in infected mice of both genders, while progesterone treatment returned this expression to basal levels. However, the Th1 cytokine profile (IFN-γ and TNF-α) was not affected by infection, whilst progesterone treatment increased the expression of both cytokines two-fold compared to uninfected, infected and placebo-treated mice. Testosterone serum levels decreased in infected male mice by 95%, and treatment with progesterone did not affect them. In females, no change in testosterone levels was observed. Progesterone levels increased three-fold only in progesterone-treated infected mice of both sexes, while estradiol levels in female and male progesterone-treated infected mice increased two-fold compared to infected control mice. The infection markedly induced the expression of progesterone receptor (PR) isoforms A and B in splenocytes of infected mice of both genders (five-fold). Metabolism of progesterone to estradiol was demonstrated by the use of the anti-estrogen tamoxifen, which reduced parasite loads 100% in infected mice of both sexes treated with progesterone. These results suggest that progesterone, possibly through its metabolism to estradiol, affects establishment, growth and reproduction of the helminth parasite *T. crassiceps*.

Keywords: Progesterone; Immunoendocrine; Helminth infection; Cysticercosis; Metabolism; Estradiol

1. Introduction

Intraperitoneal infection in mice with the metacestode of the helminth parasite *Taenia crassiceps* causes murine cysticercosis [1–3]. This infection is well known as a source of cross-reacting antigens useful in immunodiagnosis of human cestode disease [4–6], as well as a practical model for testing candidate vaccines against porcine *Taenia solium* cysticercosis [7–9]. It is also a manageable experimental system designed to explore the role of biological factors involved in host susceptibility [10–12].

To date, reciprocal endocrine interactions between host and parasite are receiving increased attention regarding their role in parasite success [13]. Particularly, in *T. crassiceps* cysticercosis, females of all studied murine strains sustain larger intensities of infection than males [14]. However, during chronic infection (more than 4 weeks), the difference disappears and the males show a feminization process characterized by high serum estradiol levels (200 times their normal values), while testosterone levels are 90% decreased [15,16]. Concomitantly, there is a specific shift from a Th1 (protective) to Th2 (inocuos) immune response in the infected host, characterized by a marked decrease of IL-2 and IFN-γ in both sexes, while the secretion of cytokines involved in the specific humoral response is enhanced (IL-6, IL-10 and IL-4) [17]. On the other hand, immunological experiments have suggested that E_2 positively regulates parasite reproduction in hosts of both genders, presumably by interfering with the thymus-dependent cellular immune mechanisms that obstruct parasite growth (Th1) and enhancing those that facilitate it

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(Th2) [11,17]. Thus, susceptibility to cysticercosis in mice may involve the joint action of the immune system and the gonads, both driven by a parasite, which is able to change the restrictive normal male hormonal milieu during chronic infection to a more permissive female environment [15,16,18].

Several reports suggest that patterns of resistance and susceptibility to infections are closely linked to changes in progesterone concentrations during the estrous cycle [19]. Most studies, including those in *T. crassiceps*, have examined the effect of sex hormones (estrogens and androgens) on the establishment of helminths in the host, and little information is available regarding other hormonal influences [20]. Typically, increased concentrations of progesterone down-regulate immune cell functions, whereas reduced progesterone concentrations up-regulate them [21]. Previous studies have clearly shown that both susceptibility and immune responses in the female genital tract are regulated by sex hormones, particularly progesterone [19,22–24]. In a rat model, it was previously shown that the stage of the estrous cycle and treatment with sex hormones, specifically estradiol and progesterone, influence both the inductive and effector arm of the immune system in the genital tract [23]. Thus, it has been postulated that antigen presentation, antibody levels and presence of immune cells are all under hormonal regulation [25,26]. It has been shown that hormonally treated rats exhibit remarkable changes in susceptibility and immune responses, depending on the received hormone treatment. Progesterone-treated rats became heavily infected following genital exposure to *Chlamydia* and showed severe inflammation, while estradiol-treated rats remained uninfected with no signs of inflammation [22]. In *T. solium* pig cysticercosis, pregnancy increased prevalence in wild-living hosts [27]. Thus, the hormonal environment at the time of infection may play a significant role in determining both susceptibility and immune responses [28].

Progesterone and estrogens exert a number of effects on the immune system of mammals possibly via intracellular progesterone receptors (PR) and estrogen receptors (ER). The PR are expressed as two isoforms: the full-length form (PR-B) and the N-terminally truncated form (PR-A) [29]. It has been shown that PR isoforms are functionally distinct in their biological actions, depending on the studied tissue (PR). The ER is also expressed as two isoforms: ER-α and ER-β [28], both of which are also functionally distinct, depending on the tissue in which it is expressed.

Because the parasite load increased during pregnancy in both natural [27] and experimental cysticercosis (E. Sciutto, personal commun.), we supposed that progesterone plays an important role during parasite growth. The aim of this work was to investigate the role of progesterone treatment during *T. crassiceps* cysticercosis, and the immunological mechanisms involved in its effects. This was investigated by relating progesterone treatment to whole parasite counts, the host humoral and cellular immune response, the expression of PR and ER in splenocytes and the host's hormonal status in infected mice of both sexes. Our results point to a possible control of the infection by progesterone metabolism to estradiol, and the inhibition of the cellular immune response to *T. crassiceps* cysticerci in murine cysticercosis.

2. Materials and methods

2.1. Mice and experimental infections

Six-week-old BALB/c AnN mice of both sexes were used in this study. Female mice were all synchronized to be at the same oestrous cycle phase before infection. They were fed with Purina Diet 5015 and water ad libitum, and light/dark cycle (14 h light:10 h dark). The fast-growing ORF strain of *T. crassiceps* isolated by Freeman in 1962 [1] was used for infection of mice in all experiments. Larvae for experimental infection were obtained from female donor mice infected 3–6 months earlier. Ten small (approximately 2-mm diameter) non-budding *T. crassiceps* larvae were suspended in 0.3 ml of PBS (0.15 M NaCl, 0.01 M sodium phosphate buffer, pH 7.2) and injected intraperitoneally into 42-day-old mice using a 0.25-gauge needle. After an 8-week period of infection, mice were sacrificed by cervical dislocation after deep pentobarbital anesthesia, and all the cysticerci found inside the peritoneal cavity were counted. A complete parasite count was performed visually in each mouse after sacrifice, by collecting parasites after thoroughly rinsing with PBS. Parasites were never found outside the peritoneal cavity.

Animal care and experimentation practices at the Instituto de Investigaciones Biomédicas are frequently evaluated by the University Animal Care and Use Committee and by governmental agencies to ensure compliance with established international regulations and guidelines.

2.2. Hormonal treatments

Progesterone and tamoxifen were introduced in the form of subcutaneous 21-day-release pellets (0.05 mg) or placebo (5.0 mg), using a precision trochar (10-gauge needle; Innovative Research of America, Toledo, OH). After another week of steroid treatment, mice were infected as described above. The effects of progesterone upon parasite loads and immunological parameters were measured 8 weeks after infection.

2.3. Castration and hormonal treatments

Four-week-old mice of both sexes were castrated under pentobarbital anesthesia (100 μl of pentobarbital plus 900 μl of PBS), as previously reported [28]. Mice were then allowed a 1-week recovery period before progesterone treatment. After another week of steroid treatment, mice were infected as described above. The effects of progesterone upon parasite load were then recorded as described.

2.4. Serum steroid levels

Blood for steroid determinations was collected by cardiac puncture, performed in mice under deep anesthesia. After

incubation for 5 h at room temperature, and 18 h at 4°C, the blood clot was centrifuged and serum was obtained. Steroids were ether-extracted and solubilized in buffer and then used for immunoassay. The concentrations of progesterone, estradiol and testosterone were determined by liquid-phase kinetics enzyme immunoassay kits (Diagnostics Lab Inc., Webster, TX), according to the manufacturer's instructions. After reactions were developed, the samples were read at 450 nm in an ELISA reader.

2.5. RNA extraction

Total RNA was isolated from testes, uterus and ovary (positive expression control tissues for sex steroid receptors) and splenocytes of control, infected, placebo control, placebo-infected, progesterone non-infected and progesterone-treated *T. crassiceps*-infected mice by the extraction method using TRIzol reagent (Gibco-BRL, NY, USA). Briefly, each tissue was removed and immediately disrupted in TRIzol reagent (1 ml/0.1 g tissue), and 0.2 ml of chloroform was added per ml of TRIzol. The aqueous phase was recovered after 10-min centrifugation at 14,000 × rpm. RNA was precipitated with isopropyl alcohol, washed with 75% ethanol, and re-dissolved in RNase-free water. RNA concentration was determined by absorbance at 260 nm and its purity was verified after electrophoresis in 1.0% denaturing agarose gel in the presence of 2.2 M formaldehyde. Total RNA from all extracted tissues was reverse-transcribed, followed by specific PCR amplification of the IL-2, IFN-γ, IL-4, IL-6, IL-10, TNF-α, INF-γ, PR-A, PR-B, ER-α, ER-β and β-actin gene sequences.

2.6. IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, PR (A and B) and ER (α and β) expression in splenocytes

Nucleotide sequences of the primers used for amplification are shown in Table 1. Briefly, 1 μg of total RNA from each tissue was incubated at 37 °C for 1 h with 400 units of M-MLV reverse transcriptase (Applied Biosystems, Boston, MA) in 20 μl of reaction volume containing 50 mM of each dNTP and 0.05 μg oligo (dt) primer (Gibco, NY). Ten microliters of the cDNA reaction were subjected to PCR in order to amplify specific sequences of the specified genes. The 50-μl PCR reaction included 10 μl of previously synthesized cDNA, 25 μl of 10× PCR buffer (Biotecnologías Universitarias, Mexico), 1 mM MgCl, 0.2 mM of each dNTP, 0.05 μM of each primer, and 2.5 units of *Taq* DNA polymerase (Biotecnologías Universitarias). Twenty microliters of the total PCR reaction products of each sample were electrophoresed on 2% agarose gel. PCR products were visualized by staining with ethydium bromide. A single band was detected in all cases, as expected. In order to determine if all amplified genes as well as the constitutively expressed control gene (β-actin) were in the exponential phase of amplification, and to make sure that changes in expression were not artifactual (such as β-actin being in the stationary phase), we performed RNA, cycling and temperature curves for each analyzed gene.

2.7. Densitometric analysis

Hybridization signals were quantified by densitometric scanning of multiple autoradiograms of various exposures, and were represented as the ratio of the signal from the problem gene relative to the expression of β-actin, a constitutively expressed gene used as internal control (relative expression).

2.8. Experimental design and statistical analysis

The experimental design is a three factorial experiment. The independent variables were (1) treatment (two levels: progesterone or placebo); (2) gender (two levels: male, female); (3) infection (two levels: yes, no). The dependent variables were the number of parasites, serum sex steroids and the expression of PR-A, PR-B, ER-α, ER-β and IL-4, IL-6, IL-10 IFN-γ and TNF-α in the tissue sample, as measured by the optical density (OD) of the corresponding gel divided by the OD of β-actin in the same tissue sample in the same gel, used as a control gene for amplification technology. The complete design was repeated twice, and the tissues used in each experiment at each time of infection were those pooled from five normal or infected mice. Statistical analysis of variance components was performed with Prism 2.01 soft-

Table 1
Sequences of the primers used for PCR amplification of reverse-transcribed total spleen RNA

Gene	Sense primer	Antisense primer
IL-2	5′-tgatggacctacaggagctcctgag	5′-gagtcaaatccagaacatgccgcag
IL-4	5′-cgaagaacaccacagagagtgagct	5′-gactcattcatggtgcagcttatcg
IL-6	5′-atgaagttcctctctgcaagag	5′-cactaggtttgccgagtagat
IL-10	5′-acctggtagaagtgatgccccaggca	5′-ctatgcagttgatgaagatgtcaaa
IFN-γ	5′-agcggctgactgaactcagattgtag	5′-gtcacagttttcagctgtataggg
TNF-α	5′-ggcaggtctactttggagtcattgc	5′-acattcgaggctccagtgaattcgg
PR-A	5′-cagtggtggatttcatccatg	5′-cttccagagggtaggtcgag
PR-B	5′-ggaggcagaaattccagacc	5′-gacaacaacccttggtagc
ER-α	5′-agactgtccagcagtaacgag	5′-tcgtaacacttgcgcagccg
ER-β	5′-catctgggtatcattacggtg	5′-ggcacttctctgtcttcgtac
β-Actin	5′-gggtcagaaggattcctatg	5′-ggtctcaaacatgatctggg

Primers were designed based on these sequenced mouse genes (Gene databank, NCBI, NIH).

ware (graphpad Software incorporated). When applied, *post hoc* individual contrasts of group means by Tukey test used the sum of residual and three factor interactions variance to test for significant differences.

3. Results

3.1. Parasite growth

Due to the extremely high variation in parasite loads found in murine *T. crassiceps* cysticercosis, which is related to several biological factors of the parasite and the host, we decided to plot the individual parasite burdens found in each mouse after each treatment. Fig. 1 shows the individual data of parasite burdens obtained after progesterone treatment in infected mice of both sexes. It is clear that there is always an individual variation in the number of parasites, but statistical analysis demonstrated that differences were significant when treatments were compared. In control mice, female mice are more susceptible to the infection (**$P < 0.01$) than male mice. Progesterone treatment in males tripled the number of parasites (from 35.8 ± 16.3 to 122.1 ± 17.3), while in females the effect was slighter, since it increased parasite load nearly twofold (from 247.1 ± 97.6 to 394.9 ± 84.7) (**$P < 0.01$). Both genders were compared with their respective infected and placebo controls (Fig. 1).

3.2. Serum steroid levels

Because it was previously found that sex steroids are an important biological factor of the host related to infection, we decided to measure individual levels of sex steroids to correlate them with the number of parasites from each mouse. The individual mouse levels of progesterone, estradiol and testosterone obtained after the different treatments are presented in Fig. 2. Progesterone levels decreased by 50% only in female infected mice (2.39 ± 1.07, ***$P < 0.001$) compared to uninfected matching controls (5.20 ± 1.37). There was no change in the levels of progesterone between control uninfected (0.18 ± 0.02 ng/ml) and infected male mice

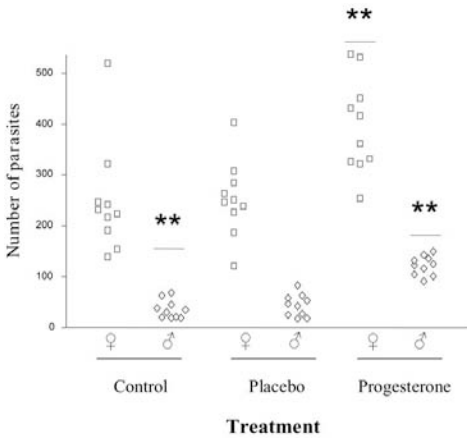

Fig. 1. Number of *T. crassiceps* cysticerci obtained from the peritoneal cavity of BALB/c AnN mice of both genders after different experimental treatments. Each point represents individual parasite loads of a total of 10 mice. *$P < 0.05$ compared with female mice, **$P < 0.01$ compared with both infected and placebo groups, for both genders.

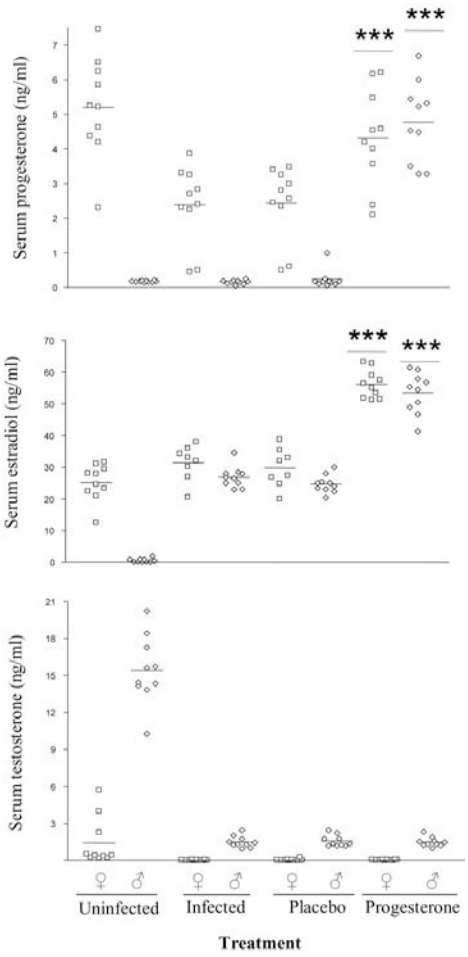

Fig. 2. Serum levels of different sex steroids in individual control, infected, placebo- and progesterone-treated male and female mice infected with *T. crassiceps*. Progesterone, estradiol and testosterone levels throughout the infection course in male mice. Each serum sample was determined in duplicate for each of 15 intact or 15 infected male mice. *$P < 0.05$, **$P < 0.01$, both compared with the placebo-treated group.

(0.16 ± 0.05 ng/ml), both of which had very low circulating levels of progesterone. However, when infected mice of both genders were treated with progesterone, serum levels dramatically increased to reach similar levels in mice of both genders (4.32 ± 1.33 in females and 4.78 ± 1.11 in males). With respect to estradiol, infected male mice had serum estradiol levels as high (26.85 ± 3.18) as normal females (25.16 ± 5.46), while infected females did not show changes in the levels of estradiol compared to uninfected controls. Surprisingly, levels of estradiol in infected progesterone-treated male (53.42 ± 6.14) and progesterone-treated female (56.15 ± 4.21) mice increased two-fold when compared to infected placebo-treated controls (***$P < 0.001$), which suggests that progesterone was probably being metabolized to estradiol in the gonad. With respect to testosterone, it decreased by almost 90% in infected male mice (1.51 ± 0.43, ***$P < 0.001$), compared with control normal male mice (15.42 ± 2.61). Female mice did not show changes in testosterone levels. Progesterone treatment did not affect the levels of testosterone in infected mice of both sexes. Placebo-treated groups showed similar progesterone, estradiol and testosterone levels as control-infected groups.

3.3. IL-4, IL-6 and IL-10 expression

Since *T. crassiceps* infection is under immunological control, and the specific Th1 immune response (IL-2, TNF-α and IFN-γ) in the infected host is protective, while the humoral immune response (IL-4, IL-6 and IL-10) is ineffective, and because the type of immune response is under endocrine control in the infected host, we decided to measure the profile of the Th1 and Th2 immune response in infected animals treated with progesterone. Fig. 3 shows the splenocyte relative expression of IL-4, IL-6 and IL-10 obtained from mice of both genders in response to different treatments. IL-4, IL-6 and IL-10 levels were markedly increased in both sexes in response to the infection, compared with non-infected controls. However, progesterone treatment in infected mice of both sexes returned the expression of these cytokines to levels similar to those found in uninfected controls. Again, placebo-treated groups behaved similarly to the infected control group.

3.4. IL-2, TNF-α and IFN-γ expression

The data obtained for Th1-type immunity, associated with protection against the infection (measured as the relative expression in splenocytes of IL-2, IFN-γ and TNF-α) in response to the different treatments are shown in Fig. 4. First of all, there were no significant differences in the expression of the three cytokines between splenocytes obtained from male and female mice in response to infection, placebo or progesterone treatment. However, IL-2 expression was undetected in parasitized mice of both sexes, while progesterone-treated infected mice showed an increase in the production of both IFN-γ and TNF-α compared to uninfected controls.

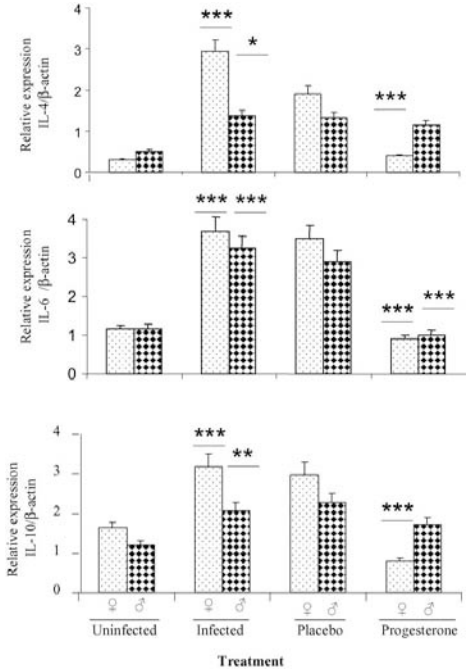

Fig. 3. Effect of chronic infection and progesterone treatment on the expression of IL-4, IL-6 and IL-10 in splenocytes of mice of both genders infected with *T. crassiceps* cysticerci. Data are represented as the mean ± S.D. of two different experiments ($n = 5$). Each splenocyte culture was done in triplicate, after an 8-week infection period. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, both compared with the placebo group.

3.5. PR-A and PR-B expression pattern

Because there is a great deal of controversy regarding the mechanism by which sex steroids could affect immune system function in several infections, we decided to amplify classic nuclear PRs (A and B), as well as both ERs (α and β) in spleen of all experimental animals, and we were able to show that they indeed express both PR isoforms (isoforms A and B), assessed by RT-PCR in splenocytes of normal, infected, placebo and infected progesterone-treated mice of both genders. The expression of β-actin was used as internal control. In all samples, a single product of 22 nt, corresponding to the amplification fragment expected for PR-A, another of 206 nt corresponding to PR-B, and another of 210 nt corresponding to β-actin were obtained. Identity of the amplified genes was verified by sequencing, and found to correspond to that of mouse PR (A and B) and β-actin (100% identity, data not shown). Fig. 5 shows the quantitation by OD of PR-A and PR-B expression in male and female mice exposed to different treatments. Visual inspection of the data in Fig. 5 indicates the variability in expression of both individual genes,

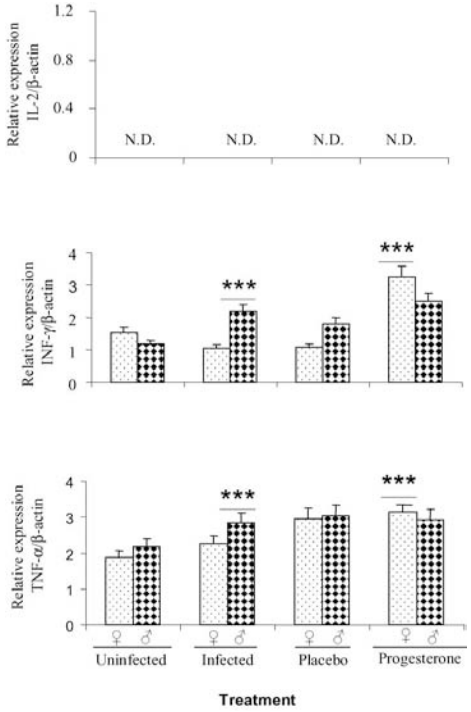

Fig. 4. Effect of chronic infection and progesterone treatment on the expression of IL-2, IFN-γ and TNF-α in splenocytes of mice of both genders infected with cysticerci of *T. crassiceps*. Data are represented as the mean ± S.D. of two different experiments ($n = 5$). Each splenocyte culture was done in triplicate, after an 8-week infection period. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, both compared with the placebo group.

but it is clear that levels of PR-A and B expression with respect to β-actin vary significantly in relation with the infection and the progesterone and infection treatments in splenocytes. The OD quotient for PR-A/B/β-actin in each treatment was calculated for each gender in relation to controls for background variation in PCR amplifications at each different experiment replication. The graphs in Fig. 5 strongly suggest that the quotient PR-A/β-actin is higher in female infected mice treated with progesterone, and this pattern of expression was similar in non-infected, infected control and placebo mice. Three-way analysis of variance strengthened the visual impression, since it revealed statistically significant differences ($P < 0.001$) between infected and infected progesterone-treated mice of both genders. PR-B expression was increased in both genders during infection ($P < 0.01$) compared to their age-matched controls, with no change in the placebo-control group (Fig. 6). In contrast, in the males, PR-B expression showed an increase when female mice were treated with progesterone (Fig. 5).

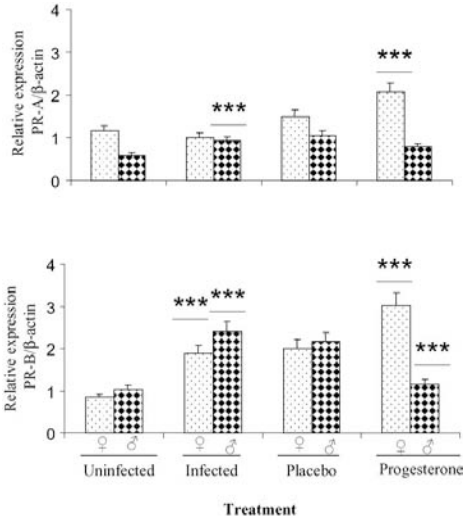

Fig. 5. PR-A and -B gene expression during infection with *T. crassiceps* is induced in splenocytes of mice of both genders. The results of gene expression are reported as densitometric data of the autoradiographic signal. The relative expression was obtained by correcting the expression of PR-A/B to that of β-actin. Data represent a pool of five mice, and each experiment was done in duplicate. Values are mean ± S.D. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, both compared with the placebo group.

3.6. ER-α and -β expression pattern

The spleen, ovary, uterus, and testes were collected from mice at necropsy, and ER-α and ER-β expression was assessed by RT-PCR. The expression of β-actin as internal control was also measured and found to be constant in all mice, in all studied tissues. Densitometric analysis of the RT-PCR is shown in Fig. 6. As shown in Fig. 6, relative expression of both ER-α and ER-β mRNA was increased in splenocytes of infected mice of both genders, while treatment with progesterone did not modify this pattern of expression. It is very interesting to note that the relative expression of ER-α is stronger than that of ER-β, and that there is no sex-associated pattern of expression for a classic nuclear receptor associated with female functions. All of these values were significant at $P < 0.01$ in comparison to the expression of ER-α in uninfected and placebo-infected mice.

3.7. Castration and tamoxifen treatment

Since levels of estradiol were increased above the values obtained for infected mice, and levels of progesterone were not as high as expected in animals treated with this steroid, we concluded that an active metabolism of progesterone to estradiol was taking place in the gonads of infected mice of both sexes, and this was the ultimate reason why the number of parasites increased. We performed two types of experi-

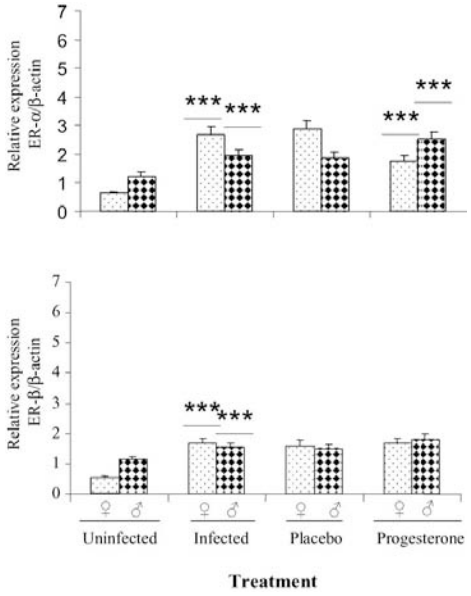

Fig. 6. Induction of ER α and ER β mRNA in the spleen of mice of both genders infected with *T. crassiceps*. Results of gene expression are reported as densitometric data of the autoradiographic signal. Data represent a pool of five mice, and each experiment was done in triplicate. Values are mean ± S.D. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ with respect to the placebo-treated group.

ments: one in which we castrated mice of both sexes (to avoid progesterone metabolism in the gonad) and another in which complete animals were treated with tamoxifen (an anti-estrogen), then a progesterone pellet was implanted, and mice were subsequently infected. Our results showed that gonadectomy (gx) and treatment with progesterone decreased the number of parasites to zero in mice of both sexes, the same pattern as observed with tamoxifen. These results strongly suggest that there was metabolism in the gonad to estradiol, and that the increase in the number of parasites was due to the high estradiol levels rather than to the progesterone treatment itself (Table 2).

4. Discussion

Assuming the reported importance of sex- and pregnancy-associated hormones in the establishment and outcome of parasitic diseases, this is an area of research that is likely to grow. Experiments in which gonadectomy, thymectomy and whole-body irradiation showed that both the endocrine and immune systems of mice are involved in parasite load differences between the host sexes [11,15,18] have confirmed the important role that sex steroids play during murine cysticercosis. Interestingly, orchidectomy in male mice lowered parasite loads, while ovariectomy had the opposite effect, increasing them threefold [28]. The thymus hindered parasite reproduction in both sexes but more so in males than in females, thus tending to equalize the number of parasites in thymectomized mice of both sexes [11].

Table 2
Individual number of *T. crassiceps* cysticerci obtained from the peritoneal cavity of BALB/c AnN mice of both genders treated with progesterone plus tamoxifen, or gonadectomized

Treatment	Number of parasites	
	F	*M*
Control	247.1 ± 102.34	35.8 ± 17.13
Gx	90.8 ± 42.91	61.1 ± 12.96
Control + P_4	394.9 ± 88.82	122.1 ± 18.15
Gx + P_4	0.0 ± 0.00	0.0 ± 0.00
Tamoxifen + P_4	28.0 ± 36.77	0.2 ± 0.40

$^{*}P < 0.05$ compared with female mice, $^{**}P < 0.05$ compared with both control and placebo groups, for both genders.

In the present study, we found that progesterone treatment in host mice of both sexes crucially affects the course of infection with *T. crassiceps* cysticerci. Thus, male and female mice treated with progesterone harbor higher numbers of cysticerci than control-infected and placebo-treated infected mice. Our results are basically in agreement with those by Gill et al. [30], who found that progesterone treatment in guinea pigs induced amoebic liver abscess more frequently than non-progesterone treatment. However, in our model, when progesterone levels were measured, they were not as high as expected and, in contrast, estrogens levels were greatly increased. Thus, it seems that the observed effects were due to the combined action of progesterone metabolism to estradiol and to its own effect.

Although the immune parameters analyzed are purely associative, they are nonetheless correlative to the endocrine changes, which can be directly attributed to the infection, suggesting that estradiol may specifically inhibit the cellular response against *T. crassiceps*, while progesterone metabolism to androgens in the adrenal gland of the infected progesterone-treated mice has the opposite effect: an increase in the specific cellular immune response in charge of eliminating the parasite, thus reducing parasite loads. As a matter of fact, we have previously shown that androgen treatment of the infected host induces protection, associated with an enhanced IL-2 and IFN-γ production in both castrated sexes treated with androgens, which correlated to 75% inhibition of parasite burdens. The fact that IL-6 and IL-10 production do not change suggests that androgens act specifically on the Th1 cell population and do not affect the Th2 response [28].

Previously, we also showed that during murine cysticercosis a remarkable feminization process is produced in the male host, characterized by an increase in serum estradiol levels of 200 times above normal value, roughly similar to values in normal females, while testosterone levels decrease by 90% relative to controls [15,16]. Our present results support and extend these findings. The intriguing question is, how are cysticerci capable of altering the hormonal environment of the

host by limiting T-associated mechanisms that restrict their establishment and growth and promoting a highly permissive estrogen-enriched milieu?

In a previous report, we showed that the expression of the aromatase gene (the limiting step in metabolizing testosterone to estradiol) in the testes of the infected male is as high as that in normal female mice, concomitant to increased expression of IL-6 [18,31]. Then, the enhanced estradiol production observed in chronically infected mice could be the result of increased testicular aromatization caused by increased IL-6 expression. In addition, IL-6 appears to be an important factor in the activation of infected male mice aromatase, since chronically infected male IL-6$^{-/-}$ KO mice do not develop the previously described feminization process [18,31]. The fact that there was an increase in the IL-6 serum levels, and in its production by splenocytes of infected animals, coupled with the fact that IL-6 expression also increased in the testes of infected males, support and extend the notion of the importance of IL-6 in the activation of testicular aromatase [18,31].

These changes in the hormonal milieu of the host equalize the parasite loads between genders. In the same manner, progesterone treatment tends to equalize parasite loads in females and males, which suggests that other gonad-associated factors are involved in the control of parasite growth. Therefore, a more intricate strategy of parasite activity has to be considered. Perhaps, high estrogen levels are the main feature of this intriguing puzzle, since in males, the parasite loads increased more markedly than in females. We suppose that progesterone could be inhibitory due to its metabolism to estradiol, a hypothesis that was tested in this study, where estradiol concentrations were higher in progesterone-treated animals of both sexes. Concomitantly, there was an important decrease in the cellular immune response measured as specific splenocyte proliferation, and IFN-γ and IL-2 production in progesterone-treated mice of both sexes. Although the analyzed immune parameters are purely associative, these changes can be directly attributed to the infection, since the changes were observed using specific parasite antigens, suggesting that progesterone specifically affects the cellular response against *T. crassiceps* in the same way as has been previously reported for estradiol and testosterone [18,28,31]. The fact that IL-6 and IL-10 production do not change suggests that progesterone acts specifically on the Th1 cell population, and does not affect the Th2 response. Our results are in agreement with previously reported data [32], which report an increased production of IL-10 and IL-4 at the site of infection that does not affect parasite growth. Thus, progesterone seems to enhance a systemic specific-Th2 immune response, which is not effective in limiting parasite growth.

That sex steroids are important biological factors of the host that affect the course of infection has also been demonstrated in natural diseases in pigs infected with *T. solium*. Recently, it has been shown that pregnancy doubles the prevalence of naturally acquired *T. solium* pig cysticercosis from about 25% to 50% [27]. This observation points to an important role for sexual hormones in the susceptibility of pigs to *T. solium* infection.

Together, all these findings support our hypothesis that sex steroids act upon the parasite reproduction rate through the immune system of the host: (a) estradiol promotes and testosterone hinders parasite reproduction; (b) estradiol favors the parasite-permissive Th2 immune response that, in turn, down-regulates the parasite-hindering Th1 responses; (c) the immuno-endocrine sexual switch in chronically infected male mice, we had suspected, was performed by the known capacity of IL-6 to induce the expression of P-450 aromatase, which in turn would interrupt the metabolic pathway of testosterone to dihydrotestosterone (DHT) and switch it to estradiol and, hence, to feminization. Since many mechanisms are present by which sex steroids could affect the immune system function, we decided to amplify classic receptors in spleen of all experimental animals, and were able to show that they indeed express both isoforms of the PR (A and B), as well as both of the ERs (α and β). Interestingly, we showed that both isoforms of the classic ER (ER-α and β) and of the PR (A and B) are down- or up-regulated by progesterone treatment in infected mice of both sexes, which suggest that their regulation is the same as in the endocrine system. With this result, we can say that the effects of estradiol and progesterone are due to their binding to the respective nuclear receptors. Binding of the ER or PR to the classic estrogen/progesterone-dependent elements could be responsible for the activation of AP-1 complex genes in the normal metabolism of immune cells, and affect the cytokine expression pattern or cell proliferation/differentiation processes in a specific endocrine environment.

Moreover, another point that should be considered in this immunoendocrine puzzle is the fact that sex steroids could act directly upon *T. crassiceps* cysticerci proliferation and viability, without need of the host's participation: estradiol and progesterone could promote parasite reproduction without affecting their viability, while testosterone and DHT could significantly inhibit parasite proliferation and lead to their destruction. Indeed, we have been able to show that sex steroids directly affect *T. crassiceps* reproduction in vitro, and found that these effects depend on both hormone concentration and on the duration of exposure: DHT was more drastic in its deleterious effects upon cysticerci than testosterone, and estradiol more stimulatory than progesterone [33].

Finally, our data provide some of the first evidence that progesterone can directly enhance the cellular immune response against a metacestode without interfering with the humoral response. Whatever the cysticercosis-relevant "progesterone target" may prove to be, the fact that progesterone and estrogens positively interfere with the development of protective immune mechanisms against *T. crassiceps* cysticerci has important implications for future vaccine development.

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Gonadectomy and progesterone treatment induce protection in murine cysticercosis

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SUMMARY

The effects of progesterone on castrated mice of both sexes infected with Taenia crassiceps *cysticerci were studied. Gonadectomy and treatment with progesterone before infection decreased parasite loads by 100% compared with intact uninfected mice. mRNA levels of IFN-γ and IL-2 (typically associated to Th1-like profiles) were markedly decreased in infected gonadectomized (Gx) mice, whereas progesterone treatment of infected Gx mice did not affected its expression. mRNA levels of IL-4, and IL-10 (typically associated with Th2-like profiles) were reduced by gonadectomy, whereas restitution with progesterone did not affected this pattern in infected Gx progesterone-treated mice. Infection markedly induced expression of progesterone receptor isoform A in splenocytes of Gx mice (5-fold), whereas isoform B had no changes. Progesterone metabolism to dehydroepiandrosterone (DHEA) in Gx animals was increased 3-fold only in infected progesterone-treated uninfecteds of both sexes, but was not detectable in infected Gx progesterone-treated mice. Conversely, DHEA levels increased 100-fold in infected Gx progesterone-treated mice. However, androgen receptor expression in splenocytes of male mice showed a reduction by gonadectomy, and by infection, whereas in females AR expression showed no changes in the different mouse groups. These results suggest that progesterone, through its metabolism to DHEA, negatively affects the establishment, growth, and reproduction of* Taenia crassiceps, *by a mechanism that does not implicate a classic genomic pathway involving a nuclear androgen receptor.*

Keywords *cysticercosis, DHEA, immunoendocrine, metabolism, progesterone*, Taenia

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INTRODUCTION

The reciprocal endocrinological interactions between host and parasite are receiving increased attention (1). Specifically, the course of murine and porcine cysticercosis is shown to be influenced by the function of the neuroendocrine system of the host (2). It was previously thought that the parasite simply defended itself in the face of a hostile host environment. However, new understandings implicate the host–parasite interaction in a more dynamic interplay, wherein the parasite exploits host homeostatic mechanisms for survival, maturity, and transmission. These homeostatic mechanisms involve the complex interaction of the endocrine and immune systems (3).

Experimental intraperitoneal murine cysticercosis is also well known as a source of cross-reacting antigens useful in immunodiagnosis of human cestode disease (4,5,6), and as a practical model to test candidate vaccines against porcine *Taenia solium* cysticercosis (7,8,9). It is also a manageable experimental system designed to explore the role of biological factors involved in host susceptibility (10,11,12). Murine cysticercosis has progressively revealed the complexities of the interactive network between the immunological and endocrinological systems of the host and of the parasite in regulating infection (13,14).

For a brief period, a notably significant sex-associated susceptibility to *T. crassiceps* cysticercosis occurs in mice where females of various strains bear larger parasite loads than males during early infections (15). After 4 weeks of infection, the parasite loads of males increase progressively and approach the massive levels of females in a few months (16). Concomitantly, a feminization process ensues in the chronically infected male mice: serum 17β-estradiol (E_2) levels increase to reach those of females, while testosterone (T) drops to 10–15% of its normal levels (13,16,17).

The feminization process also coincides with a specific shift from TH1 (protective) to TH2 (innocuous) immune responses in the infected host, characterized by a marked decrease of IL-2 and IFN-γ in both sexes, while the secretion of cytokines involved in the specific humoral response is enhanced (IL-10 and IL-4) (18). Castration and treatment

with either testosterone or dihydrotestosterone before infection markedly decreases parasite loads in both genders, whereas treatment with 17β-estradiol increases it in both genders. Specific splenocyte cell proliferation and IL-2 and IFN-γ production are depressed in infected-castrated mice of both genders, whereas treatment with testosterone or dihydrotestosterone produces significant cell proliferation recovery and enhanced production of IL-2 and IFN-γ. The humoral response has an opposite effect. It is unaffected by testosterone or dihydrotestosterone restitution, whereas treatment with estradiol of both genders increases the levels of anti-cysticercus IgG, and of IL-6 and IL-10 production (19,20).

Based upon these studies, it is clear that sex steroids can regulate parasite loads mechanistically through their reciprocal interactions with immune mechanisms, but in addition, they can also act directly upon the cysticercus. Thus, it has been previously reported that estradiol, and progesterone to a lesser extent, stimulate *in vitro T. crassiceps* bud production, DNA synthesis and ^{3}H-thymidine uptake. Conversely, testosterone and dihydrotestosterone (DHT) are slightly inhibitory and even exert a pathogenic effect on the parasites (21).

Most studies, including those in *T. crassiceps*, have examined the effect of sex hormones (oestrogens and androgens) on the establishment of helminths in the host and little information is available regarding other hormonal influences (22). Typically, increased concentrations of progesterone down-regulate immune cell functions, whereas reduced progesterone concentrations up-regulate them (23). It has been demonstrated that progesterone treatment in intact mice, through its metabolism to estradiol, positively affects establishment, growth and reproduction of the helminth parasite *T. crassiceps* (24). However, in this study the effects of progesterone were masked because host gonads bio-converted progesterone to estradiol, and parasite load encumberment was attributed to estradiol, not progesterone.

To investigate the effect of progesterone solely, we castrated animals of both sexes, treated them with progesterone, and investigated how whole parasite counts relate to the host humoral and cellular immune response, to the expression of sex hormone receptors in splenocytes, and to the host's hormonal status in infected gonadectomized mice. Interestingly, although these infected mice have no gonadal tissue, our results point to a positive control of the infection by progesterone metabolism to DHEA in the adrenal glands.

MATERIALS AND METHODS

Mice and experimental infections

Six-week-old BALB/c AnN mice of both sexes were used in this study. They were fed with Purina Diet 5015 and water *ad libitum*, and the light–dark cycle was set at 14 h light: 10 h dark. The fast growing ORF strain of *Taenia crassiceps* isolated by Freeman in 1962 (25) was used to infect the mice of all experiments. Larvae for experimental infection were obtained from female donor mice infected 3–6 months earlier. Ten small (approximately 2 mm diameter) non-budding *Taenia crassiceps* larvae were suspended in 0·3 mL of PBS (0·15 M NaCl, 0·01 M sodium phosphate buffer, pH 7·2) and injected intraperitoneally into 42-day-old mice using a 0·25 gauge needle. After an 8-week period of infection, mice were sacrificed by cervical dislocation after deep pentobarbital anaesthesia, and all cysticerci found inside the peritoneal cavity were counted. A complete parasite count was obtained visually from each mouse after sacrifice and subsequently parasites were collected after thoroughly rinsing with PBS. Parasites were never found outside the peritoneal cavity.

Animal care and experimentation practices at our institute are frequently evaluated by the University Animal Care and Use Committee and by governmental agencies to ensure compliance with established international regulations and guidelines.

Hormonal treatments

Subcutaneous 60-day-release progesterone pellets (5·0 mg) or vehicle (5·0 mg) were introduced using a precision trochar (10 gauge needle; Innovative Research of America, Toledo, Ohio). After a week of steroid treatment, mice were infected as described above. The effects of progesterone upon parasite loads and immunological parameters were measured 8 weeks after infection.

Gonadectomy and hormonal treatments

Four-week-old mice of both sexes were Gx under pentobarbital anaesthesia (100 μL of pentobarbital plus 900 μL of PBS), as previously reported (20). Mice were then allowed a one-week recovery period before progesterone treatment. After another week of steroid treatment, mice were infected as described above. The effects of progesterone upon parasite load were then recorded as described.

Serum steroid levels

Blood for steroid determinations was collected by cardiac puncture, performed in mice under deep anaesthesia. After incubation for 5 h at room temperature, and 18 h at 4°C, the blood clot was centrifuged and serum was obtained. Steroids were ether-extracted and solubilized in buffer and then used for immunoassay. Progesterone, and dihydrotestosterone sulphate (DHEA-S) concentrations were determined by

 Parasite Immunology, **28**, 667–674

liquid-phase kinetics enzyme immunoassay kits (Diagnostics Laboratory Inc., Webster, TX), according to the manufacturer's instructions. After reactions were developed, the samples were read at 450 nm in an ELISA reader.

RNA extraction

Total RNA was isolated from testes, uterus (positive expression uninfected tissues for sex steroid receptors) and splenocytes of uninfected, infected, vehicle Gx uninfecteds, vehicle infected Gx, infected Gx and Gx progesterone-treated *T. crassiceps*-infected mice by the extraction method using TRIzol reagent (Gibco-BRL, NY, USA). Briefly, each tissue was removed and immediately disrupted in TRIzol reagent (1 mL/0·1 g tissue), and 0·2 mL of chloroform were added per ml of TRIzol. The aqueous phase was recovered after 15 min centrifugation at 2500 *g* RNA was precipitated with isopropyl alcohol, washed with 75% ethanol, and re-dissolved in RNAse-free water. RNA concentration was determined by absorbance at 260/280 nm and its purity was verified after electrophoresis in 1·0% denaturing agarose gel in the presence of 2·2 M formaldehyde. Total RNA from all extracted tissues was reverse transcribed followed by specific PCR amplification of the IL-2, IL-4, IL-10, IFN-γ, PR-A, PR-B, AR and β-actin gene sequences.

IL-2, IL-4, IL-10, IFN-γ, PR (A and B) and AR expression in splenocytes

Nucleotide sequences of the primers used for amplification have been previously reported (24). Briefly, 5 µg of total RNA from each tissue was incubated at 37°C for 1 h with 400 units of M-MLV reverse transcriptase (Applied Biosystems, Boston MA) in 1·25 µg of reaction volume containing 50 mM of each dNTP and 0·05 µg oligo (dt) primer (Gibco, NY). Ten µL of the cDNA reaction were subjected to PCR in order to amplify specific sequences of the specified genes. The 50 µL PCR reaction included 5 µL of previously synthesized cDNA, 25 µL of 10x PCR-buffer (Biotecnologías Universitarias, México), 1 mM MgCl, 0·2 mM of each dNTP, 0·05 µM of each primer, and 2·5 units of Taq DNA polymerase (Biotecnologías Universitarias, México). Twenty µL of the total PCR reaction products of each sample were electrophoresed on 2% agarose gel. PCR products were visualized by staining with ethidium bromide. A single band was detected in all cases, as expected. In order to determine if all amplified genes as well as the constitutively expressed uninfected gene (β-actin) were in the exponential phase of amplification, and to make sure that changes in expression were not artifactual (such as β-actin being in the stationary phase), we obtained RNA, cycling and temperature curves for each analysed gene.

Densitometric analysis

Hybridization signals were quantified by densitometric scanning of multiple autoradiograms of various exposures and represented as the ratio of the signal from the analysed gene relative to the expression of β-actin, a constitutively expressed gene used as internal uninfected (relative expression). We used the software 'Scion Imagen for windows' (Scion Corp. Release: Alpha 4·0·3·2)

Experimental design and statistical analysis

The experimental design is a four-factorial experiment. The independent variables were (a) treatment: (two levels: progesterone or vehicle) (b) gender (two levels: male, female) (c) infection (two levels: Yes, No) and (d) gonadectomy (two levels: Yes, No). The dependent variables were the number of parasites, serum sex steroids and the expression of PR-A, PR-B, AR and, IL-2, IL-4, IL-10 and IFN-γ in the tissue sample, as measured by the optical density (OD) of the corresponding gel divided by the OD of β-actin in the same tissue sample in the same gel, used as the uninfected gene for amplification technology. The complete design was repeated twice and the tissues used in each experiment at each time of infection were those pooled from five normal or infected mice. Statistical analysis of variance components was performed in the software Prism 2·01 (GraphPad Software Incorporated). When applied, *post hoc* individual contrasts of group means by the ANOVA test used the sum of the residual and four factor interactions variance to test for significant differences.

RESULTS

Parasite growth

Because of the extremely high variation in parasite loads found in murine *T. crassiceps* cysticercosis, we plotted individual parasite burdens found in each mouse after each treatment. Figure 1 shows the individual data of parasite burdens obtained after progesterone treatment in infected Gx mice of both sexes. Although individual variation was noted in the number of parasites, statistical analysis demonstrated that differences were significant when treatments were compared. In uninfected mice, female mice were more susceptible to the infection (**$P < 0·01$) than male mice. Progesterone treatment in intact males tripled the number of parasites (from 35·8 ± 16·3 to 122·1 ± 17·3), whereas in intact females the effect was slighter, because it increased parasite load nearly two-fold (from 247·1 ± 97·6 to 394·9 ± 84·7) (**$P < 0·01$). Gonadectomy equalized host sex-susceptibility, because both genders harboured a similar number of parasites;

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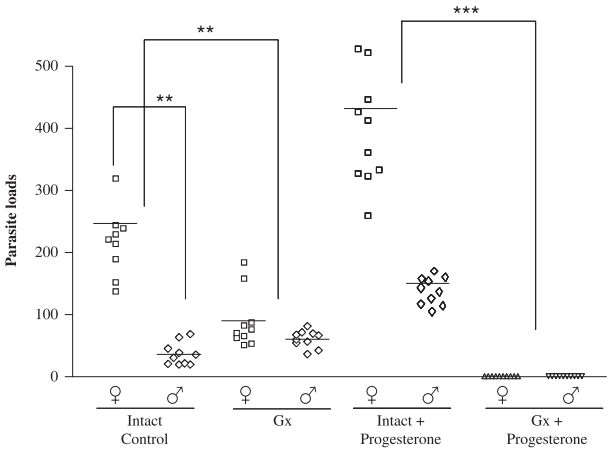

Figure 1 Number of *Taenia crassiceps* cysticerci obtained from the peritoneal cavity of BALB/c AnN mice of both genders after different experimental treatments. Each point represents individual parasite loads of a total of 10 mice. $^{**}P < 0{\cdot}01$ compared with uninfected mice, for both genders. $^{***}P < 0{\cdot}001$ compared with infected and vehicle groups, for both genders.

however, Gx animals treated with progesterone had zero parasites in all animals from either gender (Figure 1).

Serum steroid levels

To create a sex steroid profile, the individual murine levels of progesterone, and DHEA obtained after the different treatments are presented in Figure 2. Progesterone levels decreased by 50% only in female infected mice ($2{\cdot}39 \pm 1{\cdot}07$, $^{***}P < 0{\cdot}001$) compared to uninfected matching uninfecteds ($5{\cdot}20 \pm 1{\cdot}37$). There was no change in the levels of progesterone between uninfected ($0{\cdot}18 \pm 0{\cdot}02$ ng/mL) and infected male mice ($0{\cdot}16 \pm 0{\cdot}05$ ng/mL), both of which had very low circulating levels of progesterone. However, when infected mice of both genders were treated with progesterone, serum levels dramatically increased to reach similar levels in mice of both genders ($4{\cdot}32 \pm 1{\cdot}33$ in females and $4{\cdot}78 \pm 1{\cdot}11$ in males). Interestingly, levels of DHEA in infected Gx progesterone-treated mice also increased three-fold with respect to uninfected Gx and infected Gx mice ($^{***}P < 0{\cdot}001$). Vehicle-treated groups showed similar progesterone and DHEA levels to infected groups (not shown).

IL-4 and IL-10 expression

The profile of the Th1 and Th2 immune responses in infected animals treated with progesterone was measured. Figure 3 shows the splenocyte relative expression of IL-4 and IL-10 obtained from mice of both genders in response to different treatments. IL-4 and IL-10 mRNA levels were markedly decreased in both sexes in response to castration, compared with intact uninfecteds ($^{***}P < 0{\cdot}001$). Progesterone treatment in infected mice of both sexes did not affect the pattern expression of these cytokines. Again, vehicle-treated groups behaved similarly to the infected group.

IL-2 and IFN-γ expression

Figure 4 shows the data obtained for the Th1-type immunity mRNA cytokine profile, associated with protection against infection. Notably, IL-2 mRNA was induced after gonadectomy, because there were no detectable levels of IL-2 in uninfected mice. There were no significant differences in the expression of this cytokine between splenocytes obtained from male and female mice in response to infection, vehicle or progesterone treatment.

Sex steroid receptors expression pattern

In order to amplify classic sex hormone receptors (SHR), we amplified the expression of PR-A and PR-B in splenocytes of normal, Gx, vehicle and infected Gx progesterone-treated mice of both genders. The expression of β-actin was used as internal uninfected. Figure 5 shows the quantification by the OD of PR-A and PR-B expression in male and female mice exposed to different treatments. The OD quotient for PR-A/B/β-actin was calculated for each gender and treatment in relation to uninfecteds for background variation in PCR amplifications at each different experiment replication. The graphs in Figure 5 strongly suggest that the PR-A/β-actin quotient is similar between uninfected, uninfected Gx and progesterone-infected Gx mice of both sexes. However, there

 Parasite Immunology, **28**, 667–674

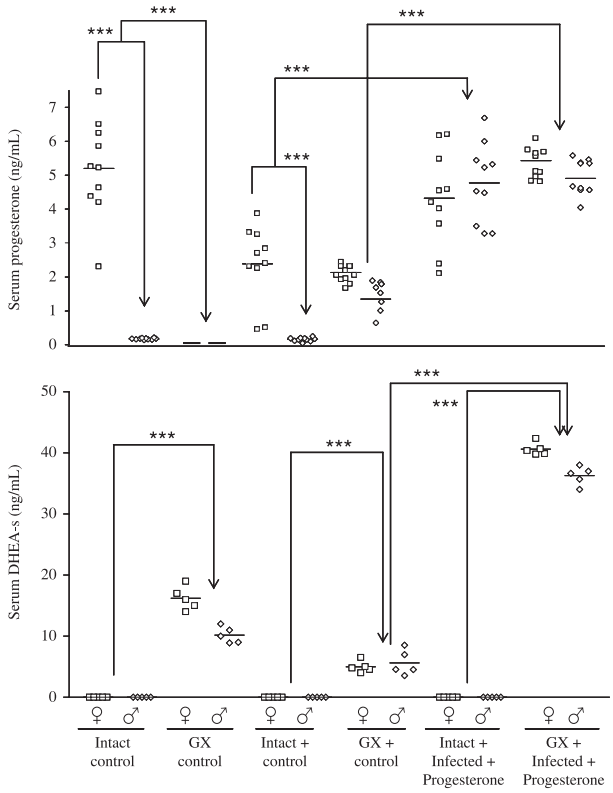

Figure 2 DHEA and progesterone levels throughout the infection course in both genders, compared to differently treated mice. Each serum sample was determined in duplicate of for each mouse. ***$P < 0{\cdot}001$, compared with uninfected mice, for both genders.

is a twofold increase in female and male infected Gx mice compared to all other groups of mice ($P < 0{\cdot}001$). In contrast, in males, PR-B expression showed an increase when both mice genders were gonadectomized, infected and treated with progesterone (Figure 5).

AR expression pattern

The spleen, ovary, uterus, and testes were collected from mice at necropsy, and AR expression was assessed by RT-PCR. The expression of β-actin as internal uninfected was also measured, and found to be constant in all mice, in all studied tissues. Densitometric analysis of the RT-PCR is shown in Figure 6. Relative expression of AR mRNA was higher in splenocytes of male mice ($P < 0{\cdot}01$), but AR mRNA was also expressed in female mice. Castration and infection of both genders decreased the expression of AR in male mice by 50%, while female AR pattern expression remained unaffected. Castrated and infected mice of both genders, as well as infected Gx and progesterone-treated mice of both genders, showed a similar expression pattern for AR mRNA to the Gx-uninfected group. Vehicle-treated groups behaved similarly to the infected group.

DISCUSSION

Experiments involving gonadectomy, thymectomy and whole body irradiation have demonstrated that both the endocrine and the immune systems of mice are involved in parasite load differences between the host sexes (11,13,16). In the present corroborative study, we have found that progesterone treatment in castrated host mice of both sexes protects

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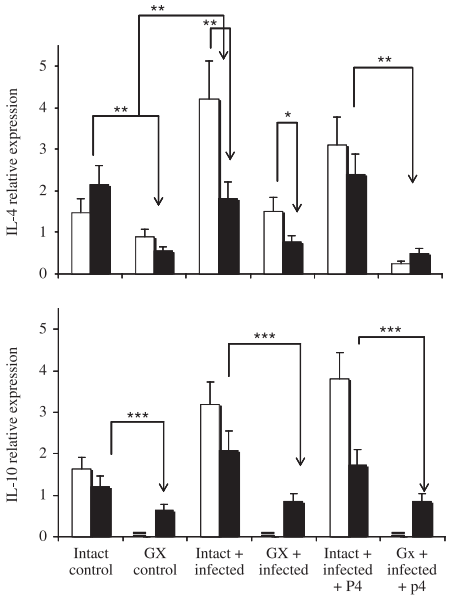

Figure 3 Effect of gonadectomy, infection and progesterone treatment in the expression of IL-4 and IL-10 in splenocytes of mice of both genders infected with *Taenia crassiceps* cysticerci. Data are represented as the mean ± SD of two different experiments ($n = 5$). Each splenocyte culture was performed in triplicate, after an 8-week infection period. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$, all compared to the vehicle group. Open bars represent female mice, while black bars represent male mice.

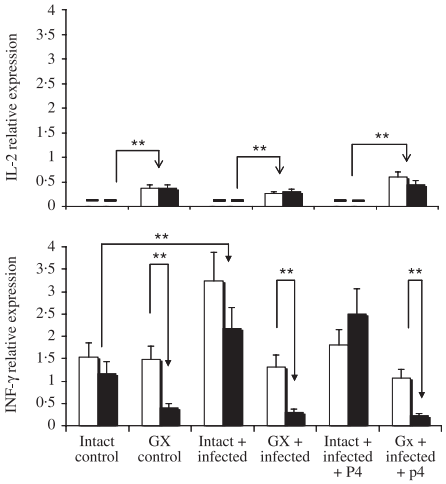

Figure 4 Effect of castration infection and progesterone treatment on the expression of IL-2 and IFN-γ in splenocytes of mice of both genders infected with cysticerci of *Taenia crassiceps*. Data are represented as the mean ± SD of two different experiments ($n = 5$). Each splenocyte culture was performed in triplicate, after an 8-week infection period. $**P < 0{\cdot}01$, both compared to the uninfected group. Open bars represent female mice, while black bars represent male mice.

against infection with *T. crassiceps* cysticerci. Our results demonstrated that castrated male and female mice treated with progesterone were completely protected from parasite burden by comparison to uninfected-infected, infected Gx and vehicle-treated infected mice. These results showed protective levels higher than any yet reported in the literature, including vaccination. Notably, no variation was observed in this experimental system, which otherwise showed large differences in parasite numbers among mice.

The fact that progesterone was being metabolized to DHEA further supports our data that measured progesterone levels were not as high as expected and, by contrast, DHEA levels were greatly increased. Thus, it seems that the observed effects were the result of adrenal conversion of progesterone metabolism to DHEA.

The notion that sex steroids are important biological factors of the host that affect the course of infection has also been previously demonstrated in natural diseases in pigs infected with *Taenia solium*. Recently, it was shown that castration of males doubles the prevalence of naturally acquired *T. solium* pig-cysticercosis from about 25 to 50% (19). This observation suggests an important role for androgens in the susceptibility of pigs to *T. solium* infection. Collectively, these findings support our contention that sex steroids act upon parasite reproduction through an immune interaction with the host. Specifically, androgens (testicular and adrenal) hinder parasite reproduction and estradiol favours the parasite-permissive Th2 immune response which, in turn, down-regulates the parasite-hindering Th1 responses.

The carefully orchestrated events that result in a protective immune response are coordinated to a large extent by cytokines produced by Th1 and Th2 cell subsets. Th1 cells preferentially produce IL-2 and IFN-γ, resulting in a cellular response that helps to eliminate *T. crassiceps* cysticerci. In contrast, Th2 cells produce IL-4 and IL-10, stimulating an Ab response that is not important eliminating this parasite. In cysticercosis, because the influence of gender on immune responsiveness usually becomes apparent after sexual maturity, a crucial role in this process has been attributed to sex

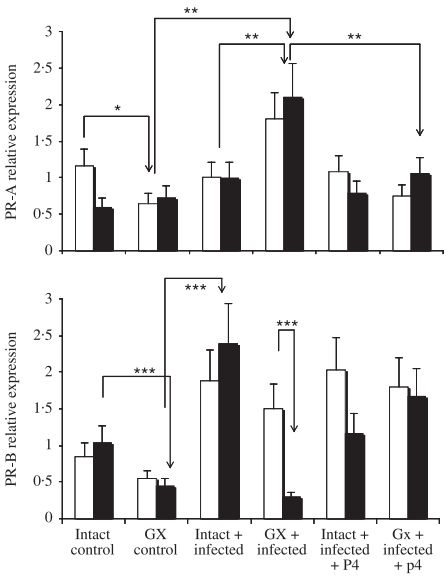

Figure 5 Progesterone receptor A and B gene expression during infection with *T. crassiceps*. The results of gene expression are reported as densitometric data of the autoradiographic signal. The relative expression was obtained by correcting the expression of PR-A/B to that of β-actin. Data represent five mice, and each experiment was performed in duplicate. Values are mean ± SD. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$, compared to the uninfected group. Open bars represent female mice, while black bars represent male mice.

steroid hormones, such as oestrogens and androgens. This fact, may be the reason why the pattern of expression of splenocytes in normal males and females, as well as in gonadectomized males and females, showed a very particular pattern: IFN-γ, IL-4 and IL-10 expression were markedly reduced by gonadectomy only in males, but not females. In contrast, it is very interesting to note that IL-2 was only detectable in Gx mice, irrespective of infection and P4 treatment. Because the other cytokine genes showed no difference when males or females were castrated, this suggests that they may have an imprinting. This is because gonadectomy in both sexes did not alter the pattern of gene expression, whereas IFN-γ, IL-4 and IL-10 may be genes up-modulated by androgens, while the opposite could be for IL-2 genes: because the lack of them induced its expression. The presence of a sex-steroid response element in the promoters of these genes could explain why their expression is affected by castration. To our knowledge, this is the first report that shows a sex-associated pattern of expression of cytokine genes at the transciptional level during a parasitic infection. These results support significant hormonal regulation of the immune system and may have therapeutic implications in several diseases, in addition to cysticercosis.

Because many mechanisms are present by which sex steroids could affect the immune system function, we decided to amplify classic receptors in the spleen of all experimental animals, and were able to show that they expressed both isoforms of the progesterone receptor (A and B), as well as the androgen receptor. Interestingly, we demonstrated that only one isoform of the classic progesterone receptor (B) was down- or up-regulated by progesterone treatment in infected mice of both sexes; however, the androgen receptor showed a marked decrease only in Gx and in infected male mice, whereas in females there were no changes in the differentially treated mice, which suggests that DHEA is probably acting differentially in male or female mice. Perhaps, in female mice, DHEA is acting through other mechanisms that do not involve a classical nuclear receptor.

Because there were no apparent changes in the specific immune response against the parasite, the DHEA inhibitory effect could possibly be acting directly on the parasites' physiology. In fact, we previously showed that androgens can act directly upon *T. crassiceps* cysticerci proliferation and

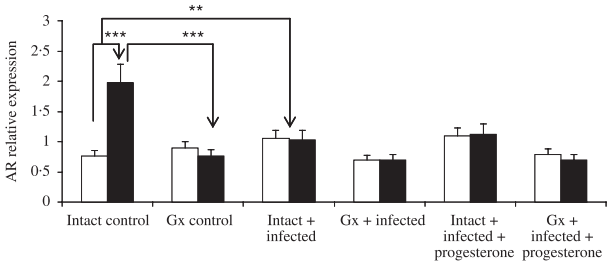

Figure 6 AR mRNA pattern expression in the spleen of mice of both genders castrated, infected with *T. crassiceps* and treated with progesterone. Results of gene expression are reported as densitometric data of the autoradiographic signal. Data represent pools of five mice, and each experiment was performed in triplicate. Values are mean ± SD. $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$with respect to the uninfected group. Open bars represent female mice, while black bars represent male mice.

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viability, without need for the host's participation. DHEA could thus significantly inhibit and/or abrogate parasite proliferation. Indeed, we have illustrated that androgens directly affect *T. crassiceps* reproduction *in vitro*, and found that these effects depend both on hormone concentration and on the exposure period: DHT was more drastic in its deleterious effects on cysticerci than testosterone (21). A similar effect of DHEA has also been supported in the compromising of *Schistosoma mansoni* cercariae, schistosomula, and adult worms viability *in vitro* (26). In this study, we have shown that progesterone negatively interfered with the development of *T. crassiceps* cysticerci, possibly through its conversion to DHEA. These data merit further exploration for future vaccination or chemotherapeutical development initiatives.

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Treatment with dehydroepiandrosterone in vivo and in vitro inhibits reproduction, growth and viability of *Taenia crassiceps* metacestodes

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Abstract

The aim of this work was to explore the effect of dehydroepiandrosterone (DHEA) on the establishment, growth and reproduction of the metacestode stage of the tapeworm *Taenia crassiceps*, both in vivo and in vitro. Administration of DHEA prior to infection in mice of both sexes reduced the parasite load by 50% compared with untreated mice. This protective effect was not associated with the immune response, since there was no effect of DHEA treatment on mRNA levels of IL-2, IFN-γ, IL-4 or IL-10. DHEA treatment of infected mice increased androgen receptor expression in splenocytes of both sexes. Moreover, in vitro treatment of *T. crassiceps* with DHEA reduced reproduction, motility and viability in a dose- and time-dependent fashion. Results indicate that DHEA has strong negative direct modulatory effects on murine cysticercosis. We suggest the use of hormonal-analogues for protective purposes as a therapeutic approach to prevent murine cysticercosis.

Keywords: DHEA; *Taenia crassiceps*; Parasite; Infection; Hormones; Cysticercosis

1. Introduction

The host–parasite interaction in human neurocysticercosis caused by *Taenia solium*, as well as in porcine (*T. solium*) and murine cysticercosis (*Taenia crassiceps*), is extremely complex and the immune response has traditionally been considered paramount in controlling the infection (Sciutto et al., 1991). Paradoxically, the immune response has also been considered a partial cause of the natural disease in humans. The outcome of the infection depends on a balance between the host immune response and the endocrinological environment, which varies among races of pigs or mouse strains (Larralde et al., 1995; Morales-Montor and Larralde, 2005). Many details of this interplay remain to be elucidated.

Recent studies have cited an interaction between the neuroendocrine and the immune systems in the regulation of anti-parasite responses (Klein, 2004). For instance, it has been shown that the hypothalamic–pituitary–adrenal axis modulates the immune response (Morales-Montor et al., 2001a), whereas the hypothalamic–pituitary–gonadal axis is sensitive to it (Morales et al., 1996). Hormones produced by both axes are involved in the regulation of host–parasite interactions, particularly in schistosomiasis (Eloi-Santos et al., 1992), cysticercosis (Morales et al., 1996), trypanosomiasis (do Prado et al., 1998) and amebiasis (Acuna-Soto et al., 2000). In experimental cysticercosis by *T. crassiceps*, it is well known that androgens play a significant role in down-regulating the parasite load in male and female Balbc/AnN mice (Morales-Montor and Larralde, 2005). Nevertheless, studies with other non-gonadal-androgenic-hormones have not been performed.

Dehydroepiandrosterone (DHEA) is a steroid hormone produced from cholesterol by the adrenal glands, the

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gonads, adipose tissue and the brain. It is the most abundant hormone in the human body. In blood, most DHEA is found as DHEA sulphate (DHEAs), with levels of the sulphate form being approximately 300 times higher than free DHEA. In humans, and in mammals generally, DHEA is the dominant steroid hormone and precursor of sex steroids and has proved to be an important molecule in resistance against a variety of infections. These infections include intracellular parasites such as *Plasmodium falciparum* and *Plasmodium berghei* (Freilich et al., 2000), *Cryptosporidium parvum* (Rasmussen and Healey, 1992), as well as extracellular parasites [*Entameba histolytica* (Carrero et al., 2006), *Schistosoma mansoni* (Morales-Montor et al., 2001); *Trypanosoma cruzi* (dos Santos et al., 2005)], although the molecular mechanisms by which DHEA has such widespread parasiticidal effects are still being discussed.

The immunological mechanisms associated with DHEA protection are not well known. On the contrary, DHEA has been shown to have a direct parasiticidal effect. For instance, in vitro, DHEA treatment resulted in a decrease in the growth and viability of *E. hystolitica* trophozoites, and its effect was presumably due to the inhibition of glucose-6-phosphate dehydrogenase (G6PD) enzyme activity (Carrero et al., 2006).

The goal of the present study was to investigate whether DHEA has direct in vitro and in vivo immune-system modulating effects on *T. crassiceps* reproduction, growth, viability and infectivity. Our results suggest that DHEA treatment may be used as a new therapeutic approach against both experimental and natural cysticercosis.

2. Materials and methods

2.1. Reagents

Culture grade human DHEA was purchased from Sigma, St. Louis, MO. DHEA was dissolved in ethanol to the desired stock concentration and sterilised by passage through a 0.2-mm millipore filter. All other reagents were purchased from common commercial sources.

2.2. Parasites and experimental infections

A new (ORF) strain of *T. crassiceps*, donated by R. Kuhn, was used in all experiments. Parasites were maintained in female BALB/c mice by sequential i.p. inoculation of the metacestodes. Metacestodes for experimental infections were obtained from female donor mice infected 3–6 months earlier. Twenty small (approximately 2-mm in diameter) non-budding *T. crassiceps* larvae were suspended in 0.6 ml PBS (0.15 M NaCl, 0.01 M sodium phosphate buffer, pH 7.2) and injected i.p. into 8-week-old mice. Mice were caged in groups of five in a common room under controlled temperature and 14 h dark/10 h light cycle in the animal facility of the Biological Sciences Building at the Institute of Biomedical Research (IIB), and were inspected by the IIB Animal Care and Use Committee and Governmental agencies to ensure compliance with federal regulations and international guidelines. They were fed Purina Diet 5015 (PMI Nutrition International, Brentwood, MO) and water ad libitum, and were sacrificed at 8 weeks of infection by cervical dislocation subsequent to ether anaesthesia. After death, parasites were visually counted in each mouse by collecting the cysts after thoroughly rinsing the peritoneal cavity with PBS. Parasites were never found outside the peritoneal cavity.

2.3. DHEA administration

DHEA was injected sub-dermally (200 μg/25 g), every other day, during the 8 weeks of infection, starting 1 week prior to infection. The vehicle in which the DHEA was diluted (mineral oil) was also administered to another group of infected mice. After 1 week, mice were infected as described above and killed 8 weeks after infection.

2.4. DHEA measurements

Blood for steroid determinations was collected by cardiac puncture performed in deeply anaesthetised mice. Steroids were ether-extracted and solubilised in the buffer used for the immunoassay. The DHEA serum concentration was determined by liquid-phase kinetics enzyme immunoassay kits (D.S. Labs), according to the manufacturer's instructions (www.dslabs.com). After reactions were developed, the samples were read at 450 nm in an ELISA reader.

2.5. IL-2, IFN-γ, IL-4, IL-10 and androgen receptor (AR) expression

Total RNA from spleen and testicle of uninfected, placebo-treated or DHEA-treated mice 8 weeks p.i. was reverse transcribed, followed by specific PCR amplification of IL-2, IFN-γ, IL-4, IL-10, androgen receptor (AR) and β-actin genes. Primers used for amplification have been previously described (Vargas-Villavicencio et al., 2005). Total RNA (5 μg) from each tissue was incubated at 37 °C for 1 h with 400 units of M-MLV reverse transcriptase (Applied Biosystems, Boston, MA) in 1.25 μg of reaction volume containing 50 mM each of dNTP and 0.05 μg oligo (dt) primer (Gibco, NY). cDNA reaction (5 μl) was subjected to PCR in order to amplify specific sequences of the specified genes. The 50 μl PCR included 5 μl of previously synthesised cDNA, 25 μl of 10× PCR-buffer (Biotecnologías Universitarias, México), 1 mM MgCl, 0.2 mM of dNTP, 0.05 μM of each primer and 2.5 units of Taq DNA polymerase (Biotecnologías Universitarias, México). Total PCR (20 μl) products of each sample was electrophoresed on 2% agarose gel. PCR products were visualised by staining with ethidium bromide. A single band was detected in all cases, as expected. In order to determine if all amplified genes as well as the constitutively expressed

uninfected gene (β-actin) were in the exponential phase of amplification, and to make sure that changes in expression were not due to artifact (such as β-actin being in the stationary phase), we obtained the RNA, cycling and temperature curves for each analysed gene.

2.6. Densitometric analysis

Hybridisation signals were quantified by densitometric scanning of multiple autoradiograms of several exposures, and were represented as the relative expression, which is the ratio of the signal of the amplified genes relative to the expression of the β-actin gene, a constitutively expressed gene used as an internal control of expression.

2.7. In vitro DHEA assays

Culture grade DHEA was obtained from Sigma. For in vitro tests, it was dissolved in AIM-V (free of calf serum and other hormones) culture medium to the desired stock concentration and sterilised by passage through a 0.2-mm millipore filter. The experimental design was as follows: using a 24-well culture plate, six wells were used for untreated trials, six wells were supplemented with the vehicle in which DHEA was diluted, six wells were treated with three different concentrations of DHEA. Concentrations of DHEA were randomised across the plates. Control cysticerci were treated with the solvent in which DHEA was diluted, so that a constant volume of solvent (2 ml) was added to each well. Reproduction was measured as the number of buds that each cyst produced in response to treatment and they were counted directly under an inverted microscope (Olympus, MO21) at 10× and 100× magnifications.

2.8. Statistical analysis

We used a three factorial experiment. Independent variables were: (1) treatment: (two levels: DHEA or vehicle); (2) gender (two levels: female, male); (3) infection (two levels: Yes, No). Dependent variables were the number of parasites, serum sex-steroid concentrations and the expression of IL-2, IFN-γ, IL-4, IL-10 and AR in the tissue sample, measured by the OD of the corresponding gel divided by the OD of β-actin in the same tissue sample in the same gel, which was used as the control gene in the amplification technology. Two experiments were performed ($n = 5$ mice in each treatment), and data were analysed using one-way analysis of variance (ANOVA). When performed, post hoc individual contrasts of group means to test for significant differences were carried out using *t*-tests. Hormone dose–response time curves were estimated in three independent experiments performed with freshly isolated *T. crassiceps* cysticerci. DHEA was tested at five different doses; each dose was run in triplicate. The response variable used for statistical analysis was the number of cysticerci for each hormone dose and the time of exposure in each experiment. Differences between groups were estimated using the ANOVA test. Differences were considered significant when $P < 0.01$. The software Prism 2.01 (GraphPad Software Inc.) was used to calculate probability values.

3. Results

3.1. Parasite loads

Due to the extremely variable parasite loads found in murine *T. crassiceps* cysticercosis, linked to a range of biological factors of the parasite and the host, we decided to plot the individual parasite burdens found in each mouse after each treatment. Fig. 1 shows the individual parasite loads recovered from the peritoneal cavity of mice of both sexes. In control mice, females were more susceptible to infection ($P < 0.001$) than male mice. DHEA treatment decreased parasite loads to a similar degree in females, (50%, $P < 0.001$), and males (40%, $P < 0.001$). Treatment with the vehicle did not affect parasite numbers in females or in males; they remained similar to those in control groups (Fig. 1).

3.2. DHEA serum levels

Before starting DHEA treatment in mice, we evaluated DHEA serum levels during the time course of the infection (4, 8, 16 weeks) in uninfected and infected female and male mice (Fig. 2), as previously shown (Morales-Montor et al., 2001). We made an ether extraction of all serum steroids, concentrated those, and used a specific commercial kit to detect DHEA in serum. Fig. 2 shows that, as infection advances, there is a reduction (50%) in DHEA serum levels in 16-week infected mice ($P < 0.001$) compared with uninfected mice of both sexes. Female mice had higher DHEA serum levels than male mice ($P < 0.001$).

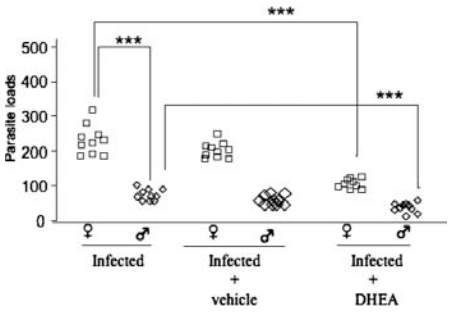

Fig. 1. Effect of dehydroepiandrosterone (DHEA) administration on parasite load. Data show the number of parasites recovered from the peritoneal cavity of 10 female and 10 male BALB/c mice at 8 weeks p.i. Each point represents individual parasite loads. $^{***}P < 0.001$ compared with both sexes mice and treated-group.

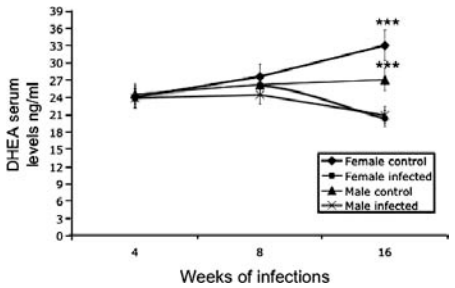

Fig. 2. Dehydroepiandrosterone (DHEA) serum levels throughout the course of the infection in individual mice of both genders with different treatments. Each serum sample was determined in duplicate for each mouse. $^{***}P < 0.001$ compared with uninfected mice of both genders.

3.3. Th-1 and Th-2 immunity

Data obtained for the Th1-type immunity mRNA cytokine profile showed that uninfected (control) male mice expressed higher IL-2 levels (two-fold) than control female mice (Fig. 3). However, DHEA treatment in uninfected female mice changed the pattern of expression of IL-2, which was enhanced 3.5-fold, while in males there was no effect.

DHEA treatment of infected mice of both sexes had no effect on IL-2 mRNA expression. With respect to IFN-γ expression, no statistical differences were found between any of the analysed groups, though there was a clear sex-associated expression that was not affected by DHEA treatment. Vehicle treatment had no effect whatsoever on the cellular immune response of infected animals (Fig. 3). Fig. 3 also shows the relative mRNA expression of IL-4 and IL-10 obtained in splenocytes from mice of both genders in response to different treatments. IL-4 and IL-10 production were not different in any of the groups studied. Vehicle treatment did not affect IL-4 or IL-10 production (Fig. 3).

3.4. AR expression

AR expression was studied to try to explain the lack of effect of DHEA on the measured immune parameters (Fig. 4).

The spleens of infected and DHEA-treated female and male mice showed a two-fold increase in AR mRNA content ($P < 0.001$) compared with uninfected, uninfected DHEA-treated, and vehicle-treated mice. The AR expres-

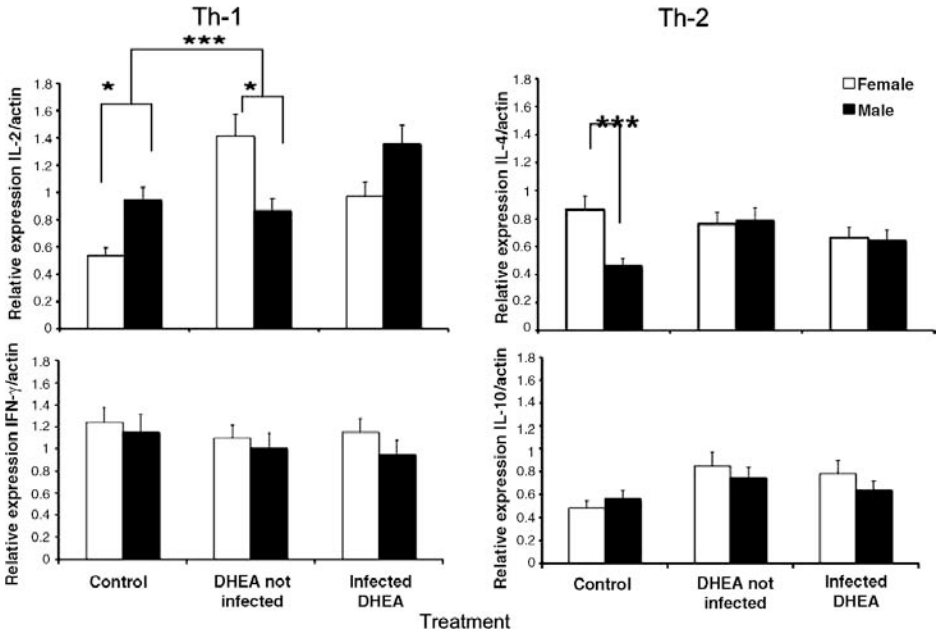

Fig. 3. Effect of chronic infection and dehydroepiandrosterone (DHEA) treatment in the expression of Th-1 (IL-2 and IFN-γ) and Th-2 (IL-4 and IL-10) in splenocytes of mice of both genders infected with *Taenia crassiceps* cysticerci. Data are presented as mean ± SD of two different experiments ($n = 5$). Each splenocyte culture was done in triplicate, after an 8 week infection period. $^{*}P < 0.05$; $^{***}P < 0.001$; both compared with the control group.

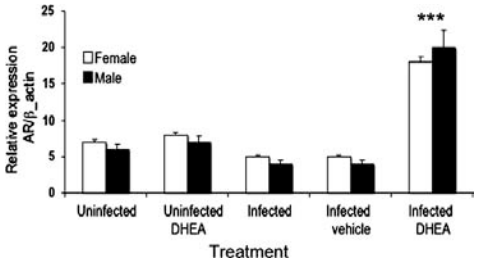

Fig. 4. Androgen receptor (AR) and β gene expression during infection with *Taenia crassiceps*. The results of gene expression are reported as densitometric data of the autoradiographic signal. The relative expression was obtained by correcting the expression of AR to that of β-actin. Data are from five mice, and each experiment was duplicated. Values are mean ± SD. $^{***}P < 0.001$ when compared with the uninfected group. DHEA, dehydroepiandrosterone.

sion level in the spleen of infected mice of both genders was as high as that found in the testicles of uninfected male mice, which was used as positive control tissue of high AR expression (Fig. 4). The expression level of the constitutive β-actin gene was constant in all examined tissues.

3.5. In vitro assays

In vitro DHEA treatment of cysticerci had an inhibitory effect on the parasite, at either physiological or pharmacological doses of DHEA (Fig. 5). DHEA treatment also affected cysticercus motility and survival (data not shown). The effect was also time-dependent, reaching a plateau after 10 days of culture with a sub-lethal dose of DHEA (Fig. 5).

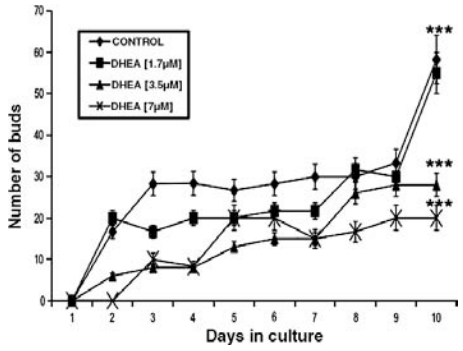

Fig. 5. Dose–response and time curves of *Taenia crassiceps* cysticercus reproduction after dehydroepiandrosterone (DHEA) exposure. Ten cysticerci were incubated for 10 days with different concentrations of DHEA. Each point represents mean (±SD) of five assays counting the number of buds in each parasite and viability in each cultured well. Data were pooled. $^{***}P = 0.01$ with respect to dose-response and time curves after DHEA exposure.

4. Discussion

In another study, exogenous DHEA administration was shown to up-regulate the immune system, specifically the cellular immune response, by increasing the natural killer cell number and function (Loria and Padgett, 1998). Our present findings do not support this notion, since IL-2 mRNA levels do not change in response to DHEA treatment (not shown). The lack of effect of DHEA on cytokine mRNA, but its dramatic effect *in vivo* on parasite load and parasite reproduction, and *in vitro* on survival, support the hypothesis that DHEA exerts its protective properties via direct effects on the parasite. To the best of our knowledge, this effect is consistent with the known effects of DHEA on the survival of other parasites, both metazoan (Fallon et al., 1998; Morales-Montor et al., 2001a) and protozoan (Carrero et al., 2006).

For instance, it has been suggested that in human schistosomiasis, DHEA is the cause of the puberty-associated drop in susceptibility (Fulford et al., 1998). This idea has been reinforced by experiments in which treatment of mice with the bloodstream form of DHEA, DHEA-s, protected them from infection with *S. mansoni* (Fallon et al., 1998). We here extend these findings to the role of DHEA in the protection of mice against *T. crassiceps* infection.

Our findings in mice of a decrease in DHEA levels as infection progresses agree with previous results in a *S. mansoni*-baboon model, in which baboons with primary infections showed decreasing levels of DHEA as the infection progressed, compared with uninfected and re-exposed baboons (Morales-Montor et al., 2001).

The protective effect of DHEA has also been demonstrated in other parasitic infections. Exogenous DHEA administration is able to increase the levels of lytic antibodies and to reduce *T. cruzi* parasitemia in rats (dos Santos et al., 2005). DHEA treatment of mice infected with the protozoan parasite *C. parvum* significantly reduced both the shedding of fecal oocysts and parasite colonisation of the ileum (Rasmussen et al., 1993, 1995). Our results showing that DHEA treatment protects mice against *T. crassiceps* infection support and extend the notion that androgens are an important factor involved in limiting *T. crassiceps* establishment in immunocompetent hosts. Previous immunological experiments have suggested that testosterone and dihydrotestosterone, two potent androgens (such as DHEA), negatively regulate parasite reproduction in mice of both sexes, presumably by interfering with the thymus-dependent cellular immune mechanisms that inhibit parasite growth (Th-2) and enhancing those that facilitate it (Th-1) (Morales et al., 1996; Morales-Montor et al., 2001b), but also by directly affecting parasite motility, survival and reproduction (Escobedo et al., 2004).

Based on the idea that the molecular mechanisms by which gonadal or adrenal steroids (such as DHEA) affect immune system function may be due to their interaction with a specific nuclear receptor, we decided to amplify the classic AR in the spleen of all experimental animals. We showed

that DHEA treatment of infected mice of both genders showed up-regulation of AR expression. These findings suggest that DHEA probably acts through mechanisms that involve a classical nuclear receptor in the immune system, though in our present experiments there was no effect of DHEA on immune stimulation in infected mice.

However, since we did not find a regulatory effect of DHEA on the host immune response, the direct effect of DHEA on parasites was considered. For instance, it has previously been demonstrated that in vitro treatment of cercariae, schistosomula and adult *S. mansoni* with DHEA strongly affect parasite survival (Morales-Montor et al., 2001a). The same study showed that mechanically transformed schistosomula were far more susceptible to the effects of DHEA than schistosomula recovered from mice. Interestingly, adult male worms were considerably less sensitive than females to the lethal action of DHEA, but when adult worms were paired, attrition was markedly reduced. DHEA also significantly inhibited oviposition in vitro (Morales-Montor et al., 2001a).

In vitro, DHEA treatment of *E. histolytica* trophozoites also reduced the growth and viability of this parasite. The effects of DHEA were associated with the inhibition of G6PD activity (Carrero et al., 2006; Di Monaco et al., 1997). Also, DHEA is known to exert anti-malarial protection, via the enhanced opsonisation and phagocytosis of rings, the early forms of this parasite (Ayi et al., 2002; Safeukui et al., 2004). Our results confirm and extend the notion that DHEA is a strong parasiticidal agent, since in vitro DHEA treatment of *T. crassiceps* remarkably reduced the reproduction rate and viability of cysticerci. Also, in our present experiments, the effects of DHEA significantly reduced the parasite burden to a similar degree in males and females. Finally, our results support and extend the notion that DHEA is a potentially useful treatment against a large variety of parasitic diseases. The fact that DHEA interferes with the development of *T. crassiceps* cysticerci may be applied to the development of future therapeutic protocols against other cysticercal infections, particularly those affecting pigs and humans.

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TAMOXIFEN TREATMENT INDUCES PROTECTION IN MURINE CYSTICERCOSIS

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ABSTRACT: Administration of tamoxifen (an antiestrogen) produced an 80% parasite load reduction in female mice, and a weaker effect of 50% in male mice. This protective effect was associated in both sexes, with an increase in the mRNA levels of interleukin (IL)-2 (a cytokine associated with protection against cysticerci) and IL-4 (no effect on infection). tamoxifen treatment modified 17-β estradiol production in females, whereas serum testosterone was not affected. However, the expression of the 2 types of estrogen receptor (ER), i.e., ER-α and ER-β, in the spleen of infected mice of both sexes, was decreased by tamoxifen treatment. In vitro, treatment of *Taenia crassiceps* with tamoxifen reduced reproduction and loss of motility. These results indicate that tamoxifen treatment is a new therapeutic possibility to treat cysticercosis, because it can act at both ends of the host–parasite relationship, i.e., by increasing the cellular immune response protective against the parasite and by directly affecting the parasite's reproduction and survival.

Taenia crassiceps cysticercosis has been a useful model for exploring the physiological host factors associated with porcine cysticercosis, and, to some degree, with human neurocysticercosis (Sciutto et al., 1999). Intraperitoneal *T. crassiceps* cysticercosis of mice (Freeman, 1962; Smith et al., 1972) lends itself well to controlled and reproducible experimentation, generating numerical data for parasite infrapopulation loads in individual mice in a matter of a few weeks after infection. Its general representation of other forms of cysticercosis has been strengthened by similar results in other mouse and parasite strains (Sciutto et al., 1999), by the parasite's extensive sharing of antigens with other taennids and cestodes, and by the DNA homology between *T. crassiceps* and *Taenia solium* (Vega et al., 2003). These characteristics have also made murine cysticercosis the model of choice to test new vaccine candidates (Cruz-Revilla et al., 2000; Toledo et al., 2001) and new drugs or treatments, and to extrapolate findings to porcine cysticercosis.

The role played by sex steroids in mammalian host–parasite infections is a matter of debate regarding causes, mechanisms, and consequences. Moreover, sex steroids do not produce the same effects in all host–parasite systems, nor are these effects always mediated by a single steroid. For example, in experimental intraperitoneal *T. crassiceps* cysticercosis, it is well known that estradiol plays a significant role in regulating the asexual reproduction by cysticerci in male and female BALBc/anN mice (Larralde et al., 1995; Morales-Montor and Larralde, 2005). Thus, immunological experiments have suggested that estradiol (E2) positively regulates parasite reproduction in hosts of both genders, presumably by interfering with the thymus-dependent cellular immune mechanisms that obstruct parasite growth (T-helper [Th]-1) and by enhancing those that facilitate it (Th-2) (Terrazas et al., 1998; Bojalil et al., 1993). Moreover, the strong effect of E2 on the specific immune response to *T. crassiceps* was previously demonstrated by castration and treatment with 17-β estradiol in hosts of both genders, which increased the number of parasites 3-fold, compared with control mice (Morales et al., 2000). This effect was associated with a specific decrease in splenocyte cell proliferation, and interleukin (IL)-2 and interferon (IFN)-γ production (Morales-Montor et al., 2002). Furthermore, it has been shown that cysticercotic mouse splenocytes express both types of estrogen receptors (ERs), i.e., ER-α and ER-β, which suggests that the strong effect of E2 during *T. crassiceps* cysticercosis is due to the binding of the steroid to its specific receptors in immune cells (Vargas-Villavicencio et al., 2005). Together, these results suggest a facilitative role of estradiol during infection, possibly by the inhibition of specific cellular immunity of the host to the parasite (Morales-Montor et al., 2001, 2002). However, it has been demonstrated that E2 not only influences the host immune system but also affects the parasite directly. When added to in vitro cultures, E2 stimulates the parasite's bud production and DNA synthesis level, possibly by binding to an ER-like molecule in the parasite (Escobedo et al., 2005).

Selective estrogen receptor modulators (SERMs) function as both estrogen agonists and antagonists in a variety of tissues (Steele et al., 1987). Tamoxifen, a triphenylethylene derivative, is the most extensively studied first generation SERM. It has been used in the treatment and prevention of breast cancer due to its antagonistic effects on α- and β-ERs in breast tissue (Johnson and Buzdar, 2001). Studies of tamoxifen have also focused on cardiovascular risk factors, such as lipid profile (Bush et al., 2001).

Because E2, through the binding to specific host and parasite ERs, has been shown to be important in facilitating infectivity, growth, and reproduction of *T. crassiceps*, the use of an antiestrogen, such as tamoxifen, should ameliorate the infection, restore Th-1–dependent immune responses, and retard parasite growth and reproduction. This hypothesis was tested by studying the influence of tamoxifen on parasite reproduction both in vitro and in vivo, and its effect on cellular and humoral immune responses, sexual steroid levels (testosterone [T] and E2), and expression of the ER genes in chronically infected mice of both sexes. Our results suggest that tamoxifen treatment is a possible new therapeutic approach to cysticercosis.

MATERIALS AND METHODS

Parasites and experimental infections

A new isolate, the WFU-strain, of *T. crassiceps* was used in all experiments. Parasites were maintained in female BALB/c mice by i.p. sequential inoculation of the metacestodes. Larvae for experimental infections were obtained from donor female mice infected 3–6 mo earlier. Twenty small (approximately 2 mm in diameter), nonbudding *T. crassiceps* larvae were suspended in 0.3 ml of phosphate buffered saline (PBS) (0.15 M NaCl and 0.01 M sodium phosphate buffer, pH 7.2) and injected i.p. in 6-wk-old mice. Mice were caged in groups of 5 in a

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common room under controlled temperature and dark/light cycle in the Biological Sciences Building animal facility at the Institute of Biomedical Research (IIB), and they were frequently evaluated by the IIB Animal Care and Use Committee and governmental agencies to ensure compliance with federal regulations and international guidelines. They were fed Purina Diet 5015 (PMI Nutrition International, Brentwood, Missouri) and water ad libitum, and they were killed at 8 wk postinfection (PI) by cervical dislocation subsequent to ether anesthesia. After death, a complete parasite count was performed visually in each mouse by collecting the cysts after thoroughly rinsing the peritoneal cavity with PBS. Parasites were never found outside the peritoneal cavity.

Tamoxifen administration

Using a 10-gauge needle Trochar (Innovative Research of America, Toledo, Ohio), tamoxifen was administered in the form of subdermal long-term release pellets (0.5 mg/wt/kg; 3-wk release pellets), starting 1 wk before the infection. Three pellets were administered during the study. Placebo pellets were similarly administered to another group of infected mice. After 1 wk, mice were infected as described above and killed 8 wk PI.

T and E2 measurements

Blood for steroid determinations was collected in vivo by cardiac puncture performed in deeply anesthetized mice. Steroids were ether extracted and solubilized in the buffer used for immunoassay. The serum concentrations of E2, and T were determined by liquid-phase kinetics enzyme immunoassay kits (Diagnostic Systems Laboratories, Inc., Los Angeles, California), according to the manufacturer's instructions. After reactions were developed, the samples were read at 450 nm in an ELISA reader (www.dslabs.com).

ER-α, ER-β, IL-2, IFN-γ, IL-4, and IL-10 expression

Total RNA from spleens and ovaries of uninfected, and placebo- or tamoxifen-treated 8-wk-PI mice was reverse transcribed, followed by specific PCR amplification of ER-α, ER-β, IL-2, IFN-γ, IL-4, IL-10, and β-actin genes. Primers used for amplification have been described previously (Morales et al., 2000). Five micrograms of total RNA from each tissue was incubated at 37 C for 1 hr with 400 units of Moloney murine leukemia virus reverse transcriptase (Applied Biosystems, Foster City, California) in 1.25 μg of reaction volume containing 50 mM of each dNTP and 0.05 μg of oligo(dT) primer (Invitrogen, Carlsbad, California). Five microliters of the cDNA reaction was subjected to polymerase chain reaction (PCR) to amplify specific sequences of the specified genes. The 50-μl PCR reaction included 5 μl of previously synthesized cDNA, 25 μl of 10× PCR buffer (Biotecnologías Universitarias, México City, México), 1 mM $MgCl_2$, 0.2 mM of each dNTP, 0.05 μM of each primer, and 2.5 units of *Taq* DNA polymerase (Biotecnologías Universitarias). Twenty microliters of the total PCR reaction products of each sample was electrophoresed on 2% agarose gel. PCR products were visualized by staining with ethidium bromide. A single band was detected in all cases, as expected. To determine whether all amplified genes as well as the constitutively expressed control gene (β-actin) were in the exponential phase of amplification, and to make sure that changes in expression were not artifactual (such as β-actin being in the stationary phase), we obtained RNA cycling and temperature curves for each analyzed gene.

Densitometric analysis

Hybridization signals were quantified by densitometric scanning of multiple autoradiograms of several exposures. Here, they are expressed as the relative expression, which is the ratio of the signal of amplified genes (IL-2, IL-4, IL-10, IFN-γ, ER-α, and ER-β) relative to the expression of β-actin gene (the constitutively expressed gene used as internal control of positive amplification).

In vitro tamoxifen assays

Culture grade tamoxifen was obtained from Sigma-Aldrich (St. Louis, Missouri). For in vitro tests, it was dissolved in medium AIM-V (culture medium that does not need fetal calf serum supplementation) to the desired stock concentration, and sterilized by passage through a 0.2-mm Millipore filter (Millipore, Billerica, Massachusetts). The experimental design was as follows. Six wells of a 24-well culture plate were used as untreated controls, 6 wells were supplemented with the vehicle in which tamoxifen was diluted, and 6 wells were treated with different concentrations of tamoxifen. Concentrations of tamoxifen were randomized across the plates. Tamoxifen was prepared to a final volume of 100 μl and added to 2 ml of medium in each well. Control cysts were treated with the solvent in which tamoxifen was diluted, so that a constant volume of solvent (100 μl) was added to each well. Reproduction was measured as the number of buds that each cyst produced in response to treatment, and they were counted directly under an inverted microscope (model MO21; Olympus, Tokyo, Japan) using ×10 and ×100 magnification. All viability observations were determined microscopically, and cysts were considered dead based on complete loss of motility of the anterior and posterior regions. Motility was defined by the number of times that cysts relaxed or contracted.

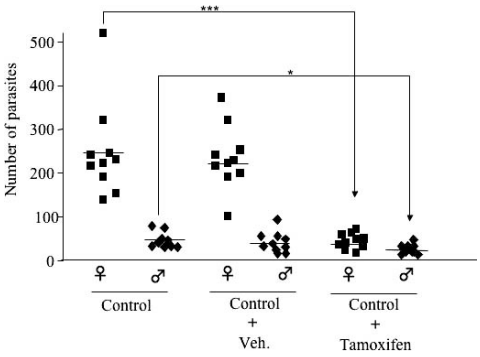

FIGURE 1. Effect of tamoxifen administration on parasite loads. Data show the number of parasites recovered from the peritoneal cavity of 10 female and 10 male BALB/c mice at 8 wk PI. Each point represents individual parasite loads. ***$P < 0.001$, comparing female and control mice. *$P < 0.5$, comparing male and control mice.

Statistical analysis

The design was considered as a 3-factorial experiment. Independent variables were as follows: (1) treatment (2 levels, tamoxifen or vehicle), (2) gender (2 levels, female or male), and (3) infection (2 levels, yes or no). Dependent variables were the number of parasites, serum sex steroids, and the expression of ER-α, ER-β, IL-2, IL-4, IL-10, and IFN-γ in the tissue sample. The gene expression was measured by quantification of the densitometric analysis of each band belonging to each amplified gene in the gels. The corresponding optical densities (ODs) obtained from the different amplified tested genes were divided by the OD obtained for β-actin (the constitutively expressed control gene) to obtain the relative expression of ER-α, ER-β, IL-2, IL-4, IL-10, and IFN-γ. Two experiments were performed (n = 5 mice each treatment), and data were analyzed using 1-way analysis of variance (ANOVA) of individual differences between means. The software Prism 2.01 (GraphPad Software Inc., San Diego, California) was used to calculate the probability values.

RESULTS

Parasite burden

Tamoxifen showed an important reduction in the parasite load recovered from the peritoneal cavity of males ($P < 0.5$), compared with the infected controls (Fig. 1). In female mice, tamoxifen treatment decreased parasite reproduction radically, by 80% ($P < 0.01$), whereas in males, it inhibited parasite re-

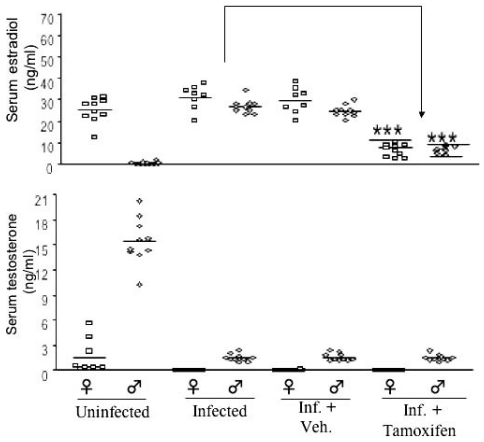

FIGURE 2. T and E2 levels throughout the infection course in individual mice of both genders with different treatments. Each serum sample was determined in duplicate for each mouse. ***$P < 0.001$, compared with control mice of both genders.

production by 50% ($P < 0.5$). Treatment with the placebo did not affect parasite number in females or in males, with numbers produced being similar to those in the infected control group (Fig. 1).

Murine hormone assays

The effects of tamoxifen administration on T and E2 serum levels in both genders are shown in Figure 2. After 8 wk of infection with *T. crassiceps* cysticerci, serum E2 levels had significantly increased in comparison with uninfected animals ($P < 0.01$). In contrast, infected male mice treated with tamoxifen showed serum E2 levels similar to those of uninfected male mice. However, infected female mice treated with tamoxifen exhibited serum E2 levels lower than those of uninfected male mice. The administration of tamoxifen did not alter T serum levels in infected male mice, whose levels where similar to those of uninfected male mice. Placebo-treated infected male mice behaved in the same way as infected nontreated male mice.

Cellular immune response

The cellular immune response was evaluated in terms of IL-2 and IFN-γ mRNA expression. Tamoxifen-treated uninfected mice produced IL-2, whereas nontreated uninfected and infected mice did not. Tamoxifen-treated infected mice restored IL-2 production to values similar to those of tamoxifen-treated uninfected mice (Fig. 3A). Concomitantly, IFN-γ production increased by 100% ($P < 0.01$) in infected male mice, and treatment with tamoxifen reduced these values to almost the same levels as in uninfected mice (Fig. 3B). A placebo had no affect whatsoever on the cellular immune response of infected animals.

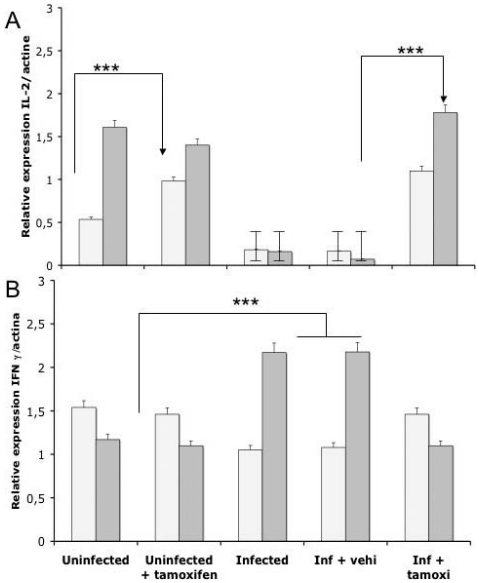

FIGURE 3. Effect of infection and tamoxifen treatment on the expression of IL-2 (**A**) and IFN-γ (**B**) in splenocytes of mice of both genders infected with cysticerci of *Taenia crassiceps*. Data are represented as the mean ± SD of 2 different experiments (n = 5). Each splenocyte culture was done in triplicate, at 8 wk PI. ***$P < 0.001$, both compared with the control group.

Humoral immune response

Figure 4 shows that the humoral immunity trend in response to tamoxifen treatment. IL-4 production was increased at 8 wk of infection, compared with uninfected mice ($P < 0.01$). Treatment with tamoxifen had no significative effect compared with the infected and placebo-treated mice (Fig. 4A). IL-10 showed the same pattern, i.e., a marked increase at 8 wk of infection compared with control mice ($P < 0.1$) and a decrease of almost 35% in IL-10 production by splenocytes of the tamoxifen-treated and infected female mice ($P < 0.01$). Once again, placebo treatment did not affect IL-10 production (Fig. 4B).

ER-α and -β expression

The expression of ERs was studied to show the possible molecular mechanisms of the inhibition (Fig. 5). Spleens of infected females and males exhibited an increase in ER mRNA content ($P < 0.01$) compared with control spleens. The expression level of ER in the spleens of the infected mice of both genders was as high as that of the ovaries in uninfected female mice. Tamoxifen-infected mice showed a decrease to levels observed in control mice. The expression level of the constitutive β-actin gene was constant in all tissues examined.

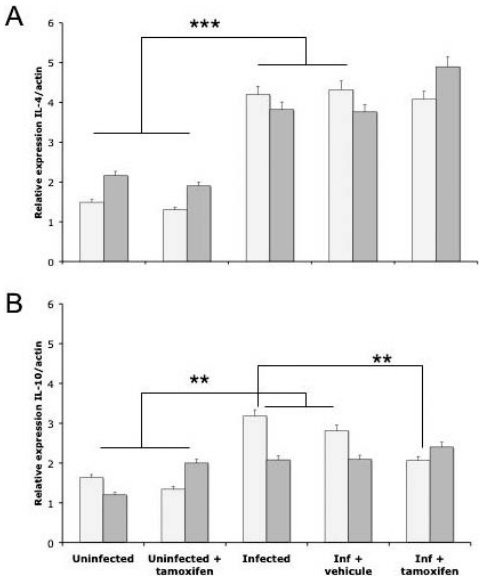

FIGURE 4. Humoral immune cytokine profile in cysticercotic male mice treated with tamoxifen. Levels of IL-4 (**A**) and levels of mRNA gene expression of IL-10 (**B**) measured by reverse transcription-PCR of specific antigen-stimulated splenocyte proliferation, as described in Materials and Methods.$^{**}P < 0.01$, compared with the control and placebo groups; $^{*}P < 0.5$, compared with the infected group.

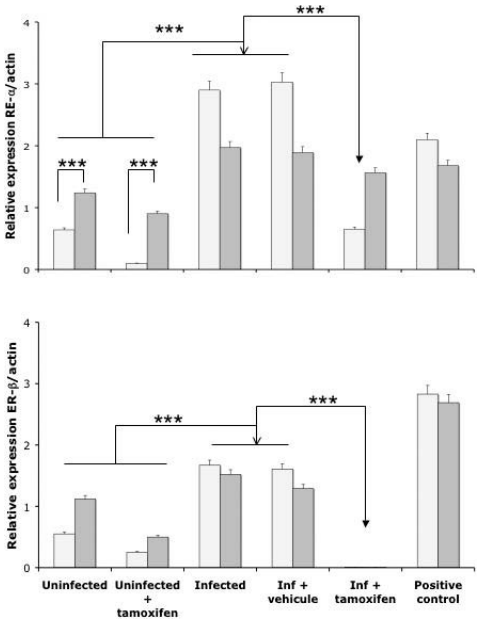

FIGURE 5. ER-α and -β gene expression during infection with *Taenia crassiceps*. The results of gene expression are reported as densitometric data of the autoradiographic signal. The relative expression was obtained by correcting the expression of ER-α and -β to that of β-actin. Data are representative of 5 mice, and each experiment was done in duplicate. Values are mean ± SD. $^{***}P < 0.001$, both compared with the control group.

In vitro assays

In vitro experiments were performed to demonstrate that tamoxifen does affect the parasite directly, in addition to the resulting dominant Th-1 response found after tamoxifen treatment. Tamoxifen in vitro treatment of cysticerci has strong inhibitory effects on motility, survival, and budding of the cysticerci. These effects where time and dose dependent, ranging from physiological to pharmacological doses (Table I).

DISCUSSION

The present study shows that in vivo administration of tamoxifen restricts the growth of *T. crassiceps* cysticerci in mice of both genders. Importantly, tamoxifen has an active metabolite, 4-OH-tamoxifen, which is a minor by-product in humans but which has much higher affinity for the ER. Some of the pharmacological profile of tamoxifen in humans is due to this metabolite. Although active metabolites make it difficult to compare data obtained from in vitro and in vivo experiments, both set of experiments point to tamoxifen as a drug that actually can inhibit parasite reproduction.

The simultaneous down-regulation of the Th-2 and up-regulation of the Th-1 response is hypothesized to be the cause of parasite reproductive arrest, because the cellular immune response is considered critical in the control of this parasite infection (Villa and Kuhn, 1996; Terrazas et al., 1998). The drug blocks the binding of estrogen to its receptors in the spleens of female and feminized male mice and in cells of the parasite, which favors the immune cellular response against cysticerci. In addition, it slows down parasite reproduction directly. Thus, the hormonal changes induced by blocking the estrogen function results in restriction of parasite reproduction. The expression of the ER genes in the spleens of the infected and placebo-infected groups was as high as in normal female mice. These results support and extend our previous findings of differential expression of the estrogen gene in infected male mice (Morales-Montor et al., 1999). The expression of ERs in the spleen of tamoxifen-treated infected mice was diminished, arguing in favor of a specific effect by tamoxifen on their genetic expression.

We had previously found that gender and circulating E2 and T levels crucially affect the dynamics of parasite reproduction in mice infected with cysticerci of *T. crassiceps* (Morales-Montor, Hallal-Calleros et al., 2002). That infection of male mice with *T. crassiceps* leads to striking increases in estrogen levels in the host is consistent with the idea that cysticerci fare better in high-estrogen conditions and somehow induce the host to produce them. However, a more intricate strategy of parasite

TABLE I. Dose–response curve of tamoxifen effect on reproduction of cultured *Taenia crassiceps* cysticerci. Tamoxifen total number cysticerci dose (μg/ml) of bud motility.

Tamoxifen conc (μg/ml)	Total no. of buds*	Cysticerci motility†
0	24 ± 0.8944	*****
5	16 ± 1.0954	****
10	13 ± 0.7527	***
20	6 ± 0.8164	**
40	3 ± 0.7527	*

* Data represent mean ± SD from 3 experiments, with 10 cysticerci per well, 6 wells per dose.

† Refers to a scale used by us to describe the motility of the organism. Injury to cysticerci was recognized microscopically by progressive internal disorganization, by development of whitish opaque areas on the parasite's tegument, and by loss of motility. Dead cysticerci were immobile, opaque, and disorganized structures. *****, very motile and healthy in their appearance; ****, <80% of cysticerci motile, but still 100% alive; ***, <50% of cysticerci motile, but still 100% alive; **, no motility in 80% of the cysts, with a reduction of 50% in survival; and *, no motility in 100% of the cysts, with 100% mortality reached.

activity must also be considered. It is suggested that, perhaps, low androgen levels are also necessary for the parasite, because they are so stunted by them. Because there is a great deal of conserved sequence homology among most hormone receptors, especially in the ligand and DNA-binding domains (Damian, 1997), we previously were able to show that cysticerci expressed both isoforms of the classic ER (ER-α and -β) (Escobedo et al., 2004). It seems that the effects of estrogens are due to the binding of estradiol to a specific receptor in the parasite. Binding of the ER to the classic estrogen-dependent elements could be responsible for the activation of activator protein-1 complex genes in the normal metabolism of *T. crassiceps*. This is reflected in our in vitro experiments, because tamoxifen directly affected the parasite, possibly by binding to the ER, as reported previously by Escobedo et al. (2004). Similarly, peak blood levels in humans after tamoxifen dosing typically range from 0.05 to 0.2 μg/ml. These levels are somewhat lower than the tamoxifen exposures reported in Table I (≥5 μg/ml). However, it is interesting to note that in vitro, there is no possibility of tamoxifen metabolism by other cells, and the effect may not be masked by binding to other cells that are not the target, or by bioconversion of the drug to noneffective metabolites. Thus, the doses used in humans are higher, and they probably will be useful to treat some tapeworm infections, particularly those in which a strong control of the infection by hormones have been reported.

Recently, the ability of hormones to affect the immunological response directed against pathogenic agents has gained attention. This is evident in a range of various parasitic diseases, including malaria, schistosomiasis, toxoplasmosis, cysticercosis, trypanosomiasis, and leishmaniasis, in which strong hormonal regulation of the immune response has been described (Klein, 2004). Recent experimental evidence suggests that parasites cannot only actively evade immune responses but also exploit the hormonal microenvironment within their host to favor their own establishment, growth, and reproduction (Damian, 1997; Escobedo et al., 2005). The benefit to parasites of hormonal exploitation is such that they have evolved structures similar to the steroid and protein hormone receptors expressed in higher vertebrates that can bind to the hormonal metabolites synthesized by the host (Terrazas et al., 1994; Morales-Montor et al., 1998; Escobedo et al., 2005). Thus, the use of hormones or hormone antagonists as immunoregulators, or as agents to avoid establishment, growth, or reproduction of parasites, may be potentially useful in the treatment of a variety of parasitic diseases, particularly for those in which hormones are known to have a strong influence on the infection (Klein, 2004). Antiparasitic drug discovery is a very expensive process that has resulted in few drugs being commercialized for a long period. Because new drug–target interactions must interfere with parasite survival, be selective, and not be cross-resistant with known resistance mechanisms, the knowledge gained by examining the physiological regulation of the host–parasite interaction could be a more rapid and less expensive strategy in the development of new antiparasitic drugs. Discovering the specific genes that are important for parasite survival and that are regulated by hormones could provide the rationale for using hormone antagonists. Tamoxifen is currently available in the market, and it is very well-characterized drug. Moreover, its side effects and tolerated doses in humans are well known. Therefore, it is reasonable to look for new applications for this old drug, instead of undertaking the expensive process of trying to develop new anticysticidal drugs. Recent advances in genomic technology will offer the opportunity to identify, validate, and develop constructs of key cysticercus molecules that could be regulated by hormones for testing drugs, such as RU-486, fadrozole, or flutamide to name a few. This could result in identification of anticysticercotic drug targets. Finally, that tamoxifen interferes with the development of *T. crassiceps* cysticerci could find important applications in the development of future vaccines and therapeutic protocols in other cysticercosis infections affecting humans and cattle.

ACKNOWLEDGMENTS

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RIC

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Embarazo inmunidad adquirida y enfermedades parasitarias: principales mecanismos asociados a la resistencia o susceptibilidad

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Pregnancy, acquired immunity and parasitic diseases: main mechanisms associated to resistance or susceptibility

ABSTRACT

During pregnancy in mammals, the endocrine system plays a protagonic role, characterized by variation of different hormonal serum levels, such as estradiol, progesterone and some gonadotrophic hormones. Furthermore, the immunological system also participates during pregnancy, self-regulation for to avoid not rejecting the fetus. The characteristic immunity during the pregnancy is the humoral type: which is characterized by an increase in the levels of the Th-2 type cytokines IL-4, IL-6, IL-10, concomitant to a diminution in the levels of IL-2, INF-γ, and TNF-α. The type of immunological response present during the pregnancy is mainly regulated by mechanisms associated to sexual hormones. This particular immunological response during the pregnancy, has individual importance if an infectious disease appears, since, depending on the parasite, a susceptibility or a resistance to the infection can exist. The proposed mechanisms to explain this resistance or susceptibility can be one of the following: 1) the hormones are influencing the immunological system of the host (by means of specific nuclear receptors); 2) the hormones acting directly on the parasite, preventing or promoting their reproduction and 3) a combination of both. These mechanisms support the idea of a complex immunoendocrine network (mediated by hormonal receptors, citokynes, antibodies) in host and parasite, interacting in a bidirectional way. The final outcome of this interaction is the death or survival of the host, or the parasite. In this review, we evaluate the information about the more frequent parasitic infections during pregnancy, and discuss the implied molecular mechanisms that affects the establishment, growth, reproduction or elimination of the parasite.

Key words. Pregnancy. Helmints. Immune response. Parasite infections.

RESUMEN

Durante la gestación, el sistema endocrino de los mamíferos tiene un papel protagónico, caracterizado por los incrementos de diversas hormonas, tales como la progesterona, el estradiol y algunas hormonas gonadotrópicas. Adicionalmente, el sistema inmunológico también participa durante el embarazo autorregulándose para evitar el rechazo del feto. El tipo de inmunidad típica durante la gestación es la inmunidad del tipo humoral; caracterizada por un aumento en los niveles séricos de interleucina (IL)-4, IL-6, IL-10, así como una disminución en los niveles de IL-2, INF-γ, y TNF-α. Cabe resaltar que los esteroides sexuales y factores asociados a éstos pueden regular a dicha respuesta inmunológica durante esta etapa. De esta manera, los factores endocrinos e inmunológicos, asociados al embarazo, poseen una repercusión en el desarrollo de la susceptibilidad o resistencia a una enfermedad infecciosa, en muchos de los casos propiciada por un parásito. Los mecanismos propuestos aquí para explicar esta resistencia o susceptibilidad son los siguientes: 1) los esteroides sexuales influyendo en el sistema inmunológico del hospedero (por medio de receptores nucleares específicos principalmente); 2) dichas hormonas actuando directamente sobre el parásito, impidiendo o promoviendo su reproducción o 3) que ocurra al mismo tiempo ambos efectos, en el escenario de una compleja red de interacciones inmunoendocrina hospedero-parásito mediada por receptores hormonales, citocinas, anticuerpos etc., mismos que interactúan de manera directa y bidireccional afectando decisivamente el curso de la infección. El objetivo de esta revisión es discutir la información bibliográfica más reciente acerca de las infecciones parasitarias con mayor recurrencia durante la gestación, proponiendo los mecanismos moleculares implicados en el establecimiento, crecimiento, reproducción o eliminación del parásito.

Palabras clave. Embarazo. Helmintos. Respuesta inmune. Infecciones parasitarias.

INTRODUCCIÓN

El embarazo en los mamíferos se caracteriza por fuertes cambios a nivel hormonal principalmente, tanto de esteroides sexuales (particularmente el estradiol y la progesterona), así como de otras hormonas proteicas (las gonadotropinas, oxitocina y prolactina, entre otras). Estos cambios hormonales en su conjunto, regulan un gran número de eventos genómicos y otros mecanismos no genómicos mediados por cascadas de fosforilación inducidas por la transducción de señales del exterior al interior de las células.

Estudios recientes demuestran que los niveles hormonales encontrados durante el embarazo, no solamente regulan la gestación, sino también le confieren al hospedero cierta susceptibilidad o resistencia hacia algunas infecciones parasitarias.[1] El mecanismo propuesto para este fenómeno de susceptibilidad o resistencia a la infección involucra una serie de interacciones entre el sistema inmune y el sistema endocrino, del sistema inmune con el parásito y del sistema endocrino con el parásito. A continuación se describirán con más detalle estas interacciones.

SISTEMA INMUNE Y EMBARAZO

En los vertebrados, y particularmente en los mamíferos, existen dos tipos de respuesta inmune adaptativa: la inmunidad celular (respuesta tipo Th1) y la inmunidad humoral (respuesta tipo Th2). El sistema inmune humoral está diseñado para eliminar principalmente a patógenos extracelulares y evitar la diseminación de los patógenos intracelulares aprovechando que estos últimos se transmiten de célula a célula a través de fluidos extracelulares. Esto se consigue mediante la producción de anticuerpos específicos. Los anticuerpos por sí mismos, no suelen eliminar más que a ciertos virus o inactivar toxinas bacterianas. En la mayor parte de los casos, la eliminación efectiva del patógeno suele deberse a la inducción de las funciones efectoras de los anticuerpos, que dependen de la activación del complemento por la ruta clásica, misma que conlleva a lisis del patógeno, quimiotaxis de fagocitos, opsonización por anticuerpos, formación de complejos inmunes (Ab-Ag), o citotoxicidad celular dependiente de inmunoglobulinas (ADCC), en donde este último se une a receptores (Fc) presentes en la superficie de células NK y macrófagos. Por lo tanto, en la respuesta humoral podemos distinguir dos grandes fases: la inducción de la producción de anticuerpos y la fase efectora, en la que estas inmunoglobulinas, directa o indirectamente, eliminan al patógeno.

Por otra parte, en la inmunidad celular, los linfocitos T participan principalmente en la eliminación de parásitos intracelulares. Estas células pueden activar a los fagocitos o a otras células, tales como las células asesinas naturales. En respuesta a un estímulo antigénico, las células T secretan proteínas llamadas citocinas, que tienen la función de estimular la proliferación y diferenciación celular. Dependiendo del tipo de citocina es el tipo de células inmunológicas que se activan y, por consiguiente, el patrón de citocinas que se exprese puede determinar la eliminación o no de la infección. Esta respuesta origina una población de linfocitos citotóxicos, que es fundamental en la defensa de infecciones producidas por microorganismos intracelulares, como bacterias, protozoarios y virus.

El endometrio es uno de los órganos más importantes en el proceso del embarazo, ya que es ahí donde se lleva a cabo la implantación del embrión.[2] Generalmente dicho órgano presenta un ambiente inmunológicamente competente ya que, alrededor del 30% del total de las células presentes, son inmunitarias.[3] En las mujeres no embarazadas, las células inmunes más abundantes presentes en el endometrio son las células "asesinas naturales" (NK), los macrófagos, los linfocitos T y pocos linfocitos B.[2-4] Estas células impiden la implantación del embrión y su desarrollo. Sin embargo, en las mujeres embarazadas, se presenta una supresión específica de la actividad de dichas células en contra del feto.[5,6] Esta es la fase llamada ventana de receptividad, en donde el micro-ambiente inmunológico cambia (disminuyen las poblaciones celulares) permitiendo la implantación del embrión.[7-9] Este cambio celular está regulado, entre otros factores, por el estradiol, la progesterona y el sistema inmune.[7-9]

Existen dos mecanismos propuestos para comprender cómo se lleva a cabo la supresión de la inmunidad Th-1. Éstos incluyen a los esteroides sexuales (como el 17-β estradiol y la progesterona) y otros factores inmunoreguladores.

El primer mecanismo propone que los esteroides sexuales modifican el ciclo celular provocando apoptosis en las células iniciadoras de la inmunidad celular, como los monocitos y los macrófagos, y la disminución del número de estas células produce un aumento de la producción de interleucinas, como la IL-4 e IL-10 (producido por otras células como las células presentadoras de antígeno). Además de la participación de otros factores, como el factor inhibidor de la migración de leucocitos y el factor estimulante

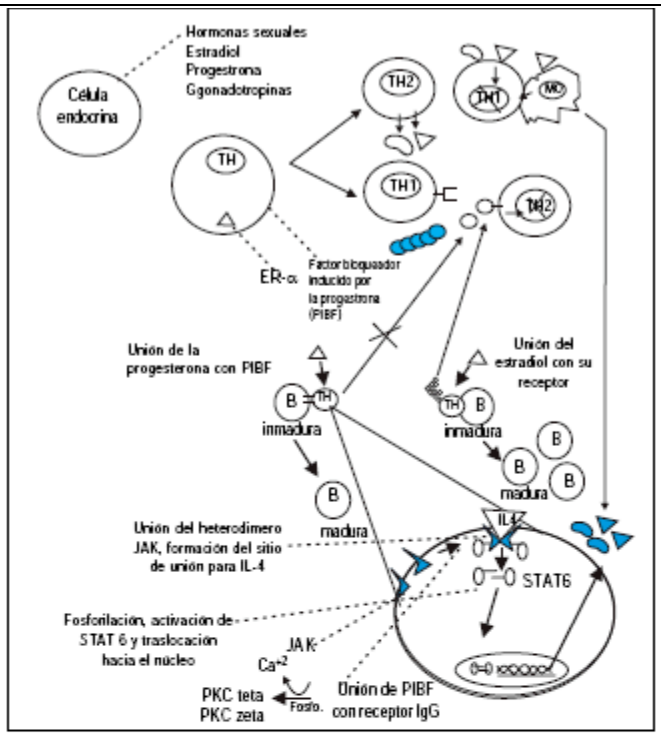

Figura 1. Comunicación celular entre los diferentes sistemas. En esta figura se esquematiza cómo la progesterona puede interactuar con el linfocito T a través de una proteína llamada PIBF. Al unirse con su receptor, se produce una fosforilación por la que se activan receptores Jak/STAT 6, induciendo la transcripción de IL-4, IL-10, TBF-β. Estas citocinas inhiben Th-1 y promueven la respuesta de tipo Th-2.[42-48]

de colonias de monocitos.[5,6] El segundo mecanismo propuesto, sugiere que los esteroides sexuales pueden polarizar la respuesta inmune de tipo Th-1 a uno de tipo Th-2 (es decir de inmunidad celular a inmunidad humoral).[10-12] Estudios recientes muestran que la progesterona se une a un factor bloqueador inducido por la progesterona (PIBF) en los linfocitos T. Esta unión induce la agrupación de los homodímeros JAK, lo que desencadena la activación de la vía de STAT-6. Cuando STAT-6 es activado, éste se transloca hacia el núcleo, promoviendo así la transcripción de IL-4, IL-10, TGF-β. A su vez, estas citocinas inhiben la respuesta Th-1 y promueven la de tipo Th-2, mecanismo que origina la producción de inmunoglobulinas (Figura 1).

Por lo anterior, se deduce cómo es que la progesterona puede inhibir la respuesta tipo Th-1 y exacerbar la de tipo Th-2, caracterizada por un aumento en los niveles de IL-4, IL-5, e IL-10.[5]

Se ha propuesto que, a nivel inmunológico, el estradiol juega un papel dual, ya que, dependiendo de su concentración, inducirá el tipo de inmunidad Th-1 o Th-2. Por ejemplo, al inicio del embarazo las concentraciones de estradiol son menores y el tipo de inmunidad es Th-2; sin embargo, al alcanzar los últimos trimestres del embarazo, existe una respuesta Th-1.[5] Esto debido a la modulación de la expresión de genes específicos, que funcionan como marcadores de cada tipo de inmunidad, por ejemplo el IFN-γ. Aunado a estos, es bien conocido que los niveles de estradiol estimulan la expresión de este interferón, lo que explica parcialmente por qué altas concentraciones de estradiol inducen, preferencialmente, una respuesta Th-1.[5]

Los posibles mecanismos mediados por los esteroides sobre el sistema inmune incluyen la acción por vía genómica o por vía no genómica.[13] En el mecanismo genómico, los esteroides se unen a receptores específicos, los cuales funcionan como factores de transcripción y ejercen efectos positivos o negativos sobre la expresión de genes blanco. Se ha descubierto que los linfocitos T tienen receptores a hormonas es-

teroideas como la progesterona y el estradiol, e inclusive los esteroides sexuales participan en la maduración de los linfocitos T en el timo, modulando la producción de interleucinas. No obstante, también se ha observado que las células presentadoras de antígeno (APC) como los macrófagos, los linfocitos B y las células dendríticas expresan receptores de estrógenos indicando que las hormonas sexuales pudieran estar involucradas en la función de las APC alterando la respuesta inmunológica y confiriendo una susceptibilidad y/o resistencia al hospedero.[14]

Los efectos no genómicos, sobre la función celular inmunitaria, implican la generación de las cascadas de fosforilación a través de segundos mensajeros, aunque esto no siempre ocurre. Por ejemplo, la unión del estradiol a receptores membranales inespecíficos (GABAérgicos acoplados a proteínas G), induce la activación de fosfatidil inositol trifosfato (IP3), lo que provoca fosforilación entrecruzada de la familia Src misma que lleva a las cinasas MEK y a la expresión de factores de transcripción como ciclina D1, y otros activadores del ciclo celular (factor inhibidor de p53), que modulan diversos procesos celulares linfocitarios, como proliferación, diferenciación o expresión de citocinas Th1 o Th2.[13]

La inmunidad tipo Th-2 está presente durante todo el embarazo desde la fase de la pre-implantación, hasta la formación y mantenimiento del feto.[15,16] Estudios recientes llegaron a la conclusión de que la inmunidad tipo Th-1 podría estar involucrada en la pérdida del feto, ya que en mujeres embarazadas se observó que el 99% de las mujeres afectadas se presentaba un aumento en la producción de INF-γ e IL-2 (marcadores de inmunidad tipo Th-1) y abortos espontáneos dentro del primer trimestre de la gestación.[17,18]

La polarización inmunológica a Th-2 durante el embarazo, además, garantiza una reproducción exitosa. No obstante, dicho cambio de inmunidad (de Th-1 a Th-2) puede ocasionar mayor susceptibilidad y/o resistencia al contraer infecciones, particularmente parasitarias (Cuadro 1).

GENES Y EMBARAZO: MECANISMOS DE INVASIÓN PARASITARIA

Diversos genes se han examinado en las distintas fases de los procesos celulares, incluyendo la regulación del ciclo celular, la reparación del ADN, la apoptosis, el mantenimiento de la segregación cromosómica y la construcción del citoesqueleto.[19] Estos diferentes tipos de genes participan además en el fenómeno de la pre-implantación embrionaria, el evento previo a la implantación que comienza desde

Cuadro 1. Comparativo que muestra las diferentes enfermedades parasitarias y si éstas están involucradas en la susceptibilidad o resistencia en el periodo del embarazo.

Parásito	Enfermedad	Efecto de la infección durante el embarazo	Mecanismos asociados	Referencia
Coccidioides immitis	Coccidiosis	↑ S	↓ T ayudador ↓ T supresor	38
Schistosoma japonicum	Esquistosomosis	↑ S	Variaciones hormonales que pueden afectar al SI o aumentar la capacidad parasitaria para reproducirse	35
Leishmania major	Leishmaniosis	↑ S	↑ IL-4 sérica ↑ IL-10 sérica	1
			↑ INF-γ sérico	39
Plasmodium falciparum	Malaria	↑ S	↑ Prolactina	40
			↑ Cortisol	27
			↑ Carga parasitaria	28
Trypanosoma cruzi	Chagas	↑ S	No descritos	41

S: Susceptibilidad. ↓: Disminuye. ↑: Aumenta. SI: Sistema inmunológico.

la fecundación del óvulo (en su recorrido por el cuello uterino) hasta la fase de adhesión en el endometrio y su división como blastocito.

Los genes involucrados en la implantación (como por ejemplo: BRCA1, BRCA2, ATM, TP53, ERB1, MAD2, BUB1, AP1)[20] controlan varias vías de señalización. Una de estas vías es la de mitógenos activadores de cascada de proteínas cinasas (MAPK).[21] Otra vía de señalización es la vía ERK, la vía JNK y la vía p38.[21] La mayoría de estos genes actúan en la formación y el mantenimiento de la cavidad donde se implantará el embrión (decidua), pero también en la activación del engrosamiento del endometrio.[22] Aún se desconoce el mecanismo por el cual aumenta la cavidad endotelial; sin embargo, algunos experimentos sugieren que este efecto es mediado por la migración quimiotáctica de las células sanguíneas hacia el sitio de acción formando nuevos capilares desarrollando un proceso conocido como angiogénesis.[22]

La mayoría de las expresiones génicas que controlan el embarazo están presentes principalmente en la fase de la pre-implantación dentro del útero, ya que esta regulación induce la migración de células sanguíneas hacia el sitio de acción (endometrio) y es en este sitio en donde existe mayor susceptibilidad a las invasiones por parásitos, debido a que la migración de los nutrientes, por vía sanguínea hacia el endometrio, puede acarrear consigo diversos patógenos.[23] Un ejemplo de ellos es *Plasmodium falciparum*, agente etiológico de la malaria. Este parásito se adhiere a la membrana del eritrocito y circula libre por todo el torrente sanguíneo hasta la muerte del mismo. Si el eritrocito migra hacia el endometrio (a través de la sangre) existen mecanismos moleculares que liberan al parásito y lo adhieren a la zona de la decidua. La molécula que regula la adhesión del parásito con el eritrocito es la proteína 1 de membrana del eritrocito del *Plasmodium falciparum* (PfEMP1), la cual es codificada por una familia de genes polimórficos llamados genes var. La proteína PfEMP1 controla la adhesión del plasmodio a través de la unión con el receptor CD36[24] (un receptor que le permite a la célula reconocer y fagocitar células apoptóticas). El parásito puede alojarse en la placenta durante todo el embarazo y provocar la migración de anticuerpos hacia el feto.[25]

EMBARAZO E INFECCIONES PARASITARIAS

Existen algunas evidencias que respaldan el concepto de que la susceptibilidad a ciertas infecciones parasitarias presentada durante el embarazo, puede estar modulada por diferentes factores biológicos, principalmente las interconexiones entre los diferentes sistemas que regulan la homeostasis del organismo (inmunológico, nervioso y endocrino) (Figura 2). Sin embargo, en la actualidad se cuenta con evidencias que apoyan el concepto de que los parásitos también son capaces de utilizar el ambiente endocrinológico de su entorno y utilizarlo en su beneficio. Los factores involucrados en la susceptibilidad o resistencia a infecciones parasitarias durante el embarazo, pueden agruparse en tres principales:

- *Parasitemia*. El número de parásitos o parasitemia es determinante en una infección. Las mujeres embarazadas son, por lo menos, cinco veces más susceptibles a infectarse que las mujeres no embarazadas. Estas enfermedades incluyen desde parásitos protozoarios (como en el caso de la malaria o la toxoplasmosis), hasta parásitos metazoarios, como las filarias y los esquistosomas (Cuadro 1). La parasitemia afecta no sólo a la madre sino también al embrión, ya que estudios recientes muestran que ciertas proteínas que liberan algunos parásitos (como el *Schistosoma mansoni* o *Toxoplasma gondii*) pueden atravesar el saco vitelino.[26]
  También se ha observado que los parásitos pueden migrar hacia órganos blanco como tejido endocrino, feto, placenta, órganos sexuales y otros, lo que le propicia una susceptibilidad mayor al hospedero. Un ejemplo de esto es la migra-

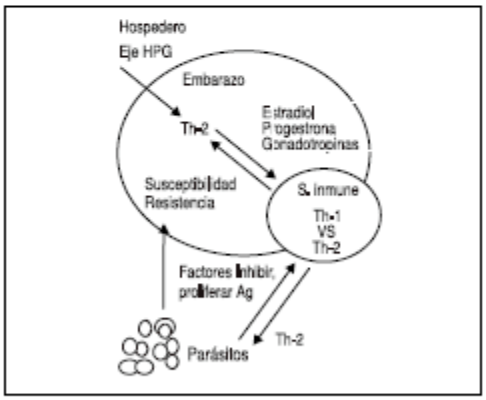

Figura 2. Interacciones inmuno-endocrinas. Esta figura representa los diferentes sistemas interactuando entre sí de manera compleja y ordenada. Cada sistema tiene una respuesta local y una respuesta a distancia, su interacción modula la respuesta definitiva como lo puede ser en este caso "una susceptibilidad o resistencia" a las infecciones parasitarias durante el embarazo.

ción de *Plasmodium falciparum* hacia los órganos reproductores femeninos, en particular hacia el endometrio, la decidua e inclusive la placenta y el cordón umbilical.[25]

Por otro lado, algunas infecciones son capaces de dañar ciertos órganos específicos como el endometrio abriendo canales en donde puede haber infiltrados celulares hacia el interior. Se ha demostrado previamente que en ratonas gestantes e infectadas con *Trypanosoma cruzi* existe migración de células mononucleadas hacia el intersticio del útero.[27] Otro estudio en mujeres embarazadas e infectadas con el mismo parásito mostró la existencia de células polinucleadas en zonas de la decidua, en la placenta y en los fetos abortados.[28,29]

- *Factores inmunológicos*. El perfil de citocinas presentes en el hospedero también determina la susceptibilidad a diversas infecciones parasitarias, ya que en ciertos casos, la inmunidad Th-1 elimina a ciertos parásitos y la inmunidad Th-2 exacerba la enfermedad. En las mujeres embarazadas la inmunidad Th-2 garantiza la sobrevivencia del feto. Sin embargo, esta inmunidad puede implicar mayor susceptibilidad hacia infecciones parasitarias dependientes de inmunidad Th-1, como por ejemplo las provocadas por *Leishmania major* o *Taenia crassiceps* en hospederos murinos.[1,30] En estas infecciones, no solamente se presenta un aumento en la inmunidad Th-2 sino también una disminución en la inmunidad Th-1. En el caso de las infecciones causadas por *Neospora caninum* y *Toxoplasma gondii*, se observa que el aumento en el número de parásitos se correlaciona con la disminución de IL-2 e INF-γ (inmunidad Th-1) mientras que la inmunidad Th-2 no tiene efecto en la infección.[31-33]
- *Factores hormonales*. Uno de los aspectos más importantes en el embarazo es la concentración de hormonas esteroideas dependientes de fase. Estas hormonas se producen normalmente en el organismo; no obstante, se ha visto que las infecciones parasitarias pueden alterar las concentraciones hormonales, aumentándolas o disminuyéndolas. Por ejemplo, en la infección por *Schistosoma mansoni*, se observó que, conforme aumenta la carga parasitaria, aumentan también los niveles de testosterona;[34] mientras que los niveles de progesteronas y estradiol disminuyen significativamente.[35]

En el caso de la cisticercosis experimental murina por *Taenia crassiceps*, se ha observado que durante las primeras cuatro semanas de infección (aguda) los machos y las hembras presentan una importante y marcada susceptibilidad asociada al sexo: las hembras presentan cargas parasitarias cuatro veces más que los machos. Sin embargo, durante la infección crónica (más de cuatro semanas) el parásito logra vencer la resistencia inicial que el macho presenta y lo termina por parasitar igual que a las hembras. En los ratones machos, los niveles de estradiol incrementan 200 veces su valor normal, mientras que los de testosterona disminuyen 90%. Las vesículas seminales y testículos también sufren una atrofia considerable.[36] Estos cambios endocrinos coinciden con un importante aumento en la carga parasitaria conforme avanza el período de infección. Por otra parte, se ha encontrado recientemente que en cerdas gestantes, la prevalencia de la infección por *Taenia solium* se incrementa de manera muy marcada comparada con hembras no gestantes.[30] Todos estos datos sugieren que factores biológicos producidos durante el embarazo, regulan la respuesta inmunológica y pueden afectar considerablemente el establecimiento y velocidad de reproducción de los parásitos a través de dos mecanismos: ya sea afectando la respuesta inmune específica del huésped contra el parásito o sirviendo como factores de crecimiento directos y positivos para el parásito.

INTERACCIONES INMUNO-ENDOCRINAS HOSPEDERO-PARÁSITO

En las infecciones parasitarias intervienen múltiples eventos post-infecciosos que facilitan o restringen el mantenimiento y reproducción del parásito. Estos eventos están regulados por una red de interacciones, debido a que todos los sistemas del organismo se encuentran interactuando directa y bi-direccionalmente. Como ya lo mencionamos, los sistemas se encuentran comunicados a través de moléculas que actúan como señales intercelulares o a distancia. Éstas pueden ser hormonas, interleucinas, neuropéptidos, receptores membranales o nucleares, canales de Na^+, material genético (ADN o ARN), azúcares, lípidos antígenos del parásito.

Las hormonas no solamente pueden actuar sobre las células del sistema inmune, sino además, sobre el parásito. En el caso de la cisticercosis murina, causada por el metacéstodo *Taenia crassiceps*, se ha observado que el tratamiento *in vitro* con estradiol y progesterona incrementan la reproducción del parásito, presumiblemente, por la unión del esteroide a un receptor nuclear parasitario.[37] De esta forma es posible que otros parásitos puedan utilizar el mecanismo mediado por los receptores a hormonas y és-

tas modular la sobreviencia del parásito y/o la susceptibilidad del hospedero.

CONCLUSIONES

La comunicación entre el sistema inmunológico y neuro-endocrinológico, así como su interacción directa con el parásito no han sido previamente estudiadas. Sin embargo, se proponen dos mecanismos: 1) las hormonas interactuando con el sistema inmune o 2) dichos esteroides sexuales interviniendo directamente sobre el parásito. El resultado es la susceptibilidad o resistencia del hospedero ante diversas infecciones parasitarias durante el embarazo. Nosotros proponemos que estas interacciones pudieran estar mediadas por receptores clásicos de hormonas presentes en células inmunes (APC, Linfocitos T y B, macrófagos, entre otros) y/o moléculas semejantes a "receptores de hormonas" en los parásitos.

Las interacciones entre el sistema inmune y el endocrino nos sirve como base para el entendimiento de los procesos co-evolutivos entre el parásito y el hospedero, así como su supervivencia en que el parásito ha adquirido la "habilidad" no solamente para sobrevivir en un ambiente inmunológicamente hostil dentro del hospedero, sino utilizar los recursos hormonales existentes en el hospedero para su completo beneficio.

Por otro lado, el hospedero necesita reproducirse y no infectarse. Por lo tanto, es de esperarse que el hospedero haya desarrollado los mecanismos necesarios para defenderse ante las infecciones parasitarias y reproducirse en buenos términos, asegurándose así la continuidad de su especie. Un entendimiento más claro de estos mecanismos nos proporcionará las bases necesarias para el desarrollo de estrategias sociales, biológicas y farmacológicas, en contra de infecciones parasitarias, antes, durante y después del embarazo y la comprensión de las interacciones inmunoendocrinas a niveles moleculares y evolutivos, siempre en el contexto de la relación hospedero-parásito.

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Short communication

Impact of naturally acquired *Taenia solium* cysticercosis on the hormonal levels of free ranging boars

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Abstract

In chronically infected BALBc/AnN male mice, *Taenia crassiceps* cysticercosis induces changes in the host's sex steroids hormone that lead to their estrogenization and deandrogenization, with possible repercussions on their susceptibility to infections. Here reported are the serum steroid levels in free range cysticercotic male boars. Therefore, the possible effects of *Taenia solium* cysticerci over the pig steroid levels were evaluated. Herein are described the sex steroids and cortisol levels of non-cysticercotic ($n = 25$) and cysticercotic ($n = 22$) adult boars, as diagnosed by tongue inspection, all free-ranging in a typical village of an endemic rural area in Mexico. A significant reduction of testosterone ($P = 0.022$) and a likely one of 17β-estradiol ($P = 0.08$) levels were found in the cysticercotic boars in comparison with those non-cysticercotic, whilst no significant differences in the cortisol and DHEA levels were detected. Serum levels of specific antibodies did not correlate with infection nor with the levels of any of the hormones measured. Results suggest that *T. solium* cysticercosis significantly affects the hormonal status of its porcine host independently of their antibody response.

Keywords: Cysticercosis; *Taenia solium*; Porcine cysticercosis; Boars; Sex steroids

1. Introduction

Many host and parasite factors can affect the host–parasite relationships. Indeed, hormones, hormone-like molecules and cytokines can be produced by both parasite and host, promoting or controlling parasite establishment, reproduction and development in the host (Beckage, 1993; Lawrence, 1991). The relevance of sexual factors in host susceptibility has been explored in many parasitic infections (Klein, 2000). In experimental murine *Taenia crassiceps* cysticercosis, female mice were found more susceptible than males in different syngenic and congenic strains of mice (Sciutto et al., 1991). The finding that gonadectomy equalized susceptibility between sexes, by reducing parasite loads in females and increasing it in males, first clearly pointed to the relevance of sexual hormones (Huerta et al., 1992). Later, the roles of sex steroids upon parasite intensity were firmly established: estrogens favored and androgens inhibited parasite asexual reproduction (Morales-Montor and Larralde, 2005). In chronically infected BALBc/AnN male mice, a clear increase in serum estradiol and decrease in testosterone was also observed as the infection progressed, triggering significant changes in sexual behaviors (decreased

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mounting, intromission, and ejaculation responses, decreased exploratory conduct and loss of social hierarchy) (Morales-Montor and Larralde, 2005). The endocrinological changes induced by cysticercosis infection associated with a TH1 to TH2 shift of the infected mouse immune response, may favor the progression of the infection (Terrazas et al., 1998). In *Taenia solium* cysticercosis of pigs there are also some hints pointing to the relevance of sexual factors in susceptibility. Prevalence of naturally acquired cysticercosis in castrated male pigs and in pregnant sows doubles those of not-castrated (boars) and non-pregnant sows living in the same area (23–50% and 28–59%, respectively) (Morales et al., 2002, 2006). There are also some indications that in human neurocysticercosis caused by *T. solium*, women show a more intense inflammatory profile in the cerebrospinal fluid than men do and, likewise, are more frequently develop a severe and generalized encephalitic process (Fleury et al., 2004).

Because pig castration could modify *T. solium* susceptibility and transmission dynamics it was of interest to enquire if the sex steroids were also implicated in naturally acquired porcine cysticercosis. Thus, we moved to explore the hormonal profiles of testosterone, estradiol-17β, cortisol and dehydroepiandrosterone (DHEA) in boars that had naturally acquired cysticercosis while living freely in a rural community of the State of Morelos, Mexico. Cortisol was also measured because of its well established relationship with the management of chronic infections (Besedovsky and del Rey, 2002)

2. Materials and methods

2.1. Study area and sampling

This study was carried out in Cuentepec Morelos, a rural village of central Mexico. A total of 1087 pigs were captured from September to December 2000 and bled to obtain the sera for further studies. Cysticercosis was diagnosed in each of them by tongue inspection (González et al., 1990). From all inspected pigs, 47 boars of 5–36 months of age were selected: 25 had tongue cysticercosis and 22 did not.

2.2. Hormone levels

Testosterone and 17β-estradiol levels were measured in the sera of the 47 boars, and in 16 of them, cortisol and dehydroepiandrosterone (DHEA) levels were also determined. All hormones were measured by ELISA using the kit from Diagnostic Systems Laboratories, Inc., Webster, TX. Antibody levels against total cysticercal antigens in the sera of 16 of them were also measured by ELISA following the procedure previously described (Sciutto et al., 1998).

2.3. Statistical methods

Mann–Whitney *U* non-parametric test was used to measure statistical significance of the differences between the boar groups. $P \leq 0.05$ was considered statistically significant, $0.05 \geq P \leq 0.1$ was considered as likely different and $P > 0.1$ as not significant.

3. Results and discussion

Fig. 1 shows the significant reduction in the testosterone levels ($P < 0.02$) of cysticercotic boars and the likely reduction in the levels of 17β-estradiol albeit not quite significant ($P < 0.080$). These differences are not due to differences in age between infected and not infected boars since no significant age differences were found between them ($P = 0.86$). Indeed, in both groups 68% of the boars were less than 12 months old. In pigs, both sex hormones increase after sexual maturity and critically mediate male behavior. In fact, the reduction of testosterone may modify the boar's behavior in a manner partially resembling what occurs in male mice infected with *T. crassiceps* cysticercosis (Morales et al., 1996). No significant correlation was found between levels of the different sex steroids, pointing that the reduction in testosterone does not necessarily imply increased estradiol synthesis, as observed in cysticercotic mice (Morales-Montor and Larralde, 2005). Thus, the reduction of testosterone and estradiol levels could be mediated by an effect of the infection upstream the testosterone metabolism. It is worth mentioning that the hormonal changes were detected despite the heterogeneous genetic background of the boars included in the study and their large range of ages.

In contrast, no differences were detected in the two adrenal hormones measured (cortisol and DHEA). Thus, it is possible that the gonads are the major target for the parasite's endocrinological strategy while adrenals are unawares of the cysticercotic infection (Besedovsky and del Rey, 2002).

Since all boars were highly exposed to the parasite, no differences in antibody levels ($P = 0.55$) nor in the percent of seropositive cases ($P = 0.69$) were expected nor found between cysticercotic and non-cysticercotic boars. Considering that the reduction in testosterone

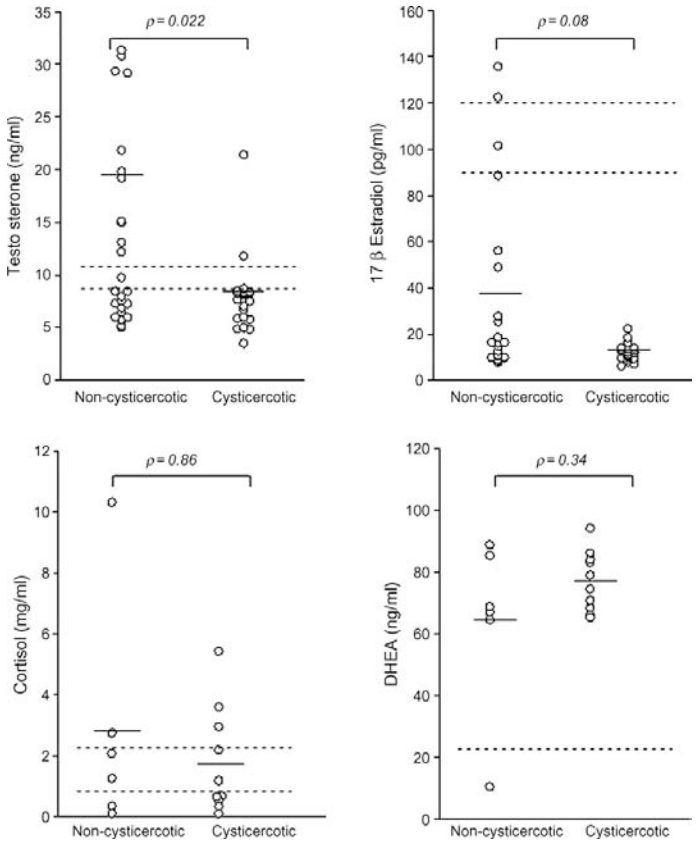

Fig. 1. Level of testosterone, 17β-estradiol, cortisol and DHEA measured by ELISA in sera from non-cysticercotic and cysticercotic boars. Bars indicate the percentile 75 in each group of boars. Medians were compared using the Mann–Whitney non-parametric test. Dotted lines indicate the respective expected values for each hormone (Allrich et al., 1982).

could in theory promote antibody production (Morales-Montor and Larralde, 2005), the correlation of specific antibody levels with hormonal levels was studied but not found to be significantly associated.

In summary, this pilot study shows that important changes take place in the steroid gonadal hormonal status of boars when infected with *T. solium* without apparent consequences for their immune response: very much against the notion of testosterone being a potent immunosuppressive agent (Owens, 2002).

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

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

THE FEMALE HOST SUPREMACY PARADIGM IN PARASITIC INFECTIONS

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

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

EXPERIMENTAL MURINE *TAENIA CRASSICEPS* CYSTICERCOSIS CONFLICTS WITH THE PARADIGM

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

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

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

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

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

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

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

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

FIRST GENERAL SIGNS OF PARADIGM WEAKNESS

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

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

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

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

TABLE I. Continued.

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

TABLE I. Continued.

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

TABLE I. Continued.

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

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

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

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

THE PREVALENCE AND MECHANISMS OF SD IN SPECIFIC PARASITE INFECTIONS

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

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

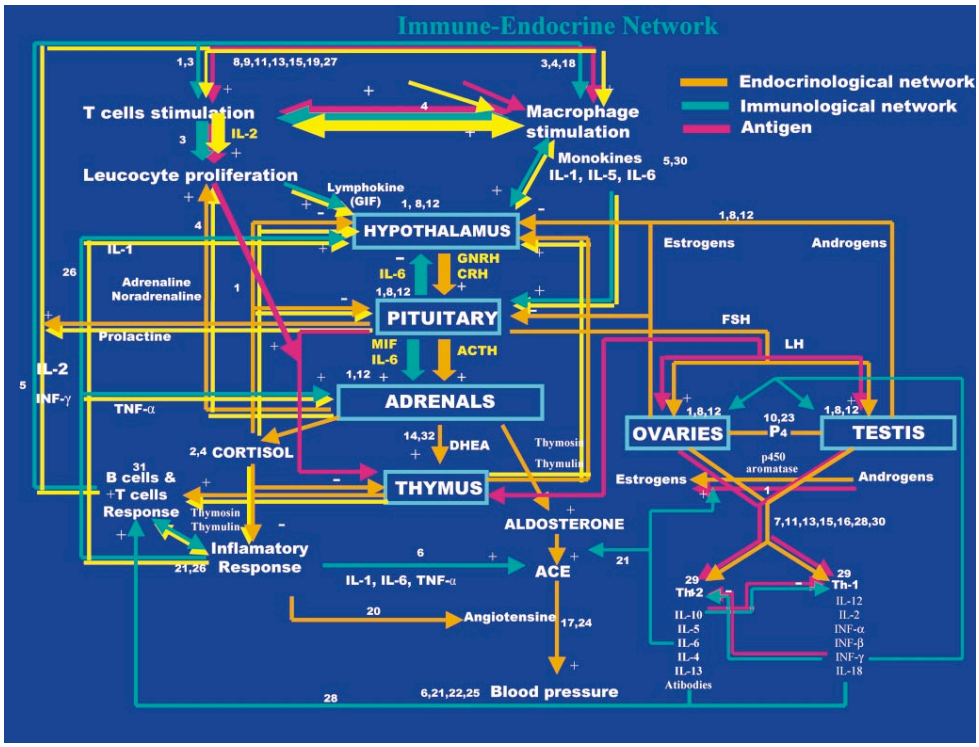

FIGURE 1. The HINEP network circuitry. The magnitude and complexity of the network includes practically all the host's sexual hormones and many of the immunological components described connecting among themselves and with the parasites systems. Arrows (←, ↑, →, ↓) denote connections between nodes; each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

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

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

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

* In acute infection.
† In chronic infection.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

CONCLUSIONS

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

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

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